Amitava Rakshit
Harikesh Bahadur Singh *Editors*

Advances in Seed Priming

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We give credit to God for granting us health, elegance, perception, strength, and act of kindness to bring about this task.

Preface

Seed is an important component of agriculture, contributing significantly to the booming production of food and feed crops across the different agro-ecological regions of the world with constant challenges with reference to production, storage, and quality control. In addition to these challenges, in the era of climate change, different stressors may lead to reduction in seed germination with poor and unsynchronized seedling emergence, poor establishment of crop stand, destruction of the root cell structure, and thus resulting in a significant decrease in the yield of agricultural crops. To conquer these confrontations, a number of seed technologies that augment germination and synchronization of seedling emergence under difficult environmental conditions have been developed. In order to encourage environmental friendly sustainable agriculture across different parts of the universe, priming technology, using inorganic chemicals, plant extract, and beneficial microorganisms, increases the yields of crops while reducing the environmental burden of disproportionate use of chemical fertilizers. As of now, it is a significant tool for hastening seed germination rate, guaranteeing consistent and homogeneous seedling emergence, and improving stand establishment and seedling vigor. The various priming options available are hydropriming, halopriming, osmopriming, thermopriming, solid matrix priming, and biopriming. Priming seeds with these divergent agents provide an innovative, cost-effective, and environmentally sound solution for improving seed quality and crop health and attaining better yields. Further, these collections will provide a much needed platform to discuss the emerging issues and problems in seed priming and will come out with a well-defined strategy to overcome the different stresses within the basic framework of sustainable development goals keeping in mind the challenges of food, environment, and livelihood security. This edited book is a comprehensive one and offers an authoritatively sound and lucid documentation of issues pertaining to priming, molecular biology, and agronomic incentives. The book is intended for use by the students, scientists, extension workers, and policy makers with an in-depth view.

Varanasi, Uttar Pradesh, India

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Acknowledgment

An edited book of this expense does not become possible without contributions of several willing souls. Our sincere gratitude to the colleagues and students who helped us in our endeavor to bring this book to light. We are grateful to Prof. Rattan Lal, Prof. B.D. Singh, and Prof. Panjab Singh for their mammoth impact, unwavering encouragement, and support.

Finally the production team members deserve special appreciation for guiding us through the process of publishing a new work. Last but not the least, we should thank our family, immediate and extended, who always encouraged us to continue the massive task. In spite of the best efforts, it is possible that some errors may have crept into the compilation. Each of the chapter has been the primary responsibility of the invited author/group of authors: We have also read and critiqued all the chapters with extraordinary case. We shall be highly obligated to receive constructive comments and suggestions from the readers for further improvement in the future editions.

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Amitava Rakshit an IIT Kharagpur alumnus, is presently the faculty member in the Department of Soil Science and Agricultural Chemistry at the Institute of Agricultural Sciences, Banaras Hindu University (IAS, BHU). Dr. Rakshit had worked in the Department of Agriculture, Government of West Bengal, in administration and extension roles. He visited Scandinavia, Europe, Africa, and UAE pertaining to his research work and presentation. He was awarded with TWASNxt Fellow (Italy), BioVision.Nxt Fellow (France), Darwin Now Bursary (British Council), Young Achiever Award, and Best Teacher's Award at UG and PG level. He is serving as review college member of British Ecological Society, London, since 2011. He is the fellow of Society of Earth Scientists. He has published 100 research papers, 25 book chapters, 28 popular articles, 1 manual, and coauthored 12 books.

Harikesh Bahadur Singh is presently a Professor of Mycology and Plant Pathology at IAS, BHU. He served state agriculture university, central university, and CSIR institute in teaching, research, and extension roles. In recognition of Prof. Singh's scientific contributions and leadership in the field of plant pathology, he was honored with several prestigious awards, notable being, CSIR Prize for Biological Sciences, Vigyan Bharti Award, Prof. V.P. Bhide Memorial Award, BRSI Industrial Medal Award, Bioved Fellowship Award, Prof. Panchanan Maheshwari Award, IPS Plant Pathology Leader Award, CSIR–CAIRD Team Award, Environment Conservation Award, CST Vigyan Ratna Award, and many more. Prof. Singh has been the fellow of National Academy of Agricultural Sciences. Prof. Singh has written 2 books, several training modules and manuals, and more than 160 research publications, and has more than 18 US patents and 3 PCTs to his credit.

Part I

Concepts

Seed Priming: New Vistas and Contemporary Perspectives

1

Puspendu Dutta

Abstract

Seed germination and uniform plant stand in field are of most critical stages of crop growth that determine the final yield. Crop production is very often hampered under suboptimal conditions, and such effect is principally attributed to poor or uneven germination and unsynchronized seedling emergence. Seed priming is an age-old and simple but effective technique to enhance germination percentage and speed and to achieve uniform plant stand and better yield in a wide range of environmental conditions. However, various priming protocols differ in their effectiveness depending on a complex interaction of factors including plant species or genotypes, water potential of priming agents, duration of treatment and environmental features. Basically priming is physiological advancement of seeds, and it involves the initiation of pre-germinative metabolisms through soaking of seeds in water or solution of other conventional priming agents under controlled condition. But, adjusting of the priming protocols by accurate timing to stop the treatment followed by rapid drying is of major importance to overcome the problem of seed storability due to prolonged treatment. The use of hydrotime analysis or digital image technology has been specified to be useful for optimization of priming protocols. As an alternative to the conventional methods and under the context of contemporary issues of agricultural pollution, the use of physical methods or nanoparticles for seed priming has been evidenced to be advantageous in several aspects. Though these latest methods of priming are receiving great attention by researchers in recent times, the detailed physiological and molecular mechanisms of seed priming with physical methods and/or nanoparticles and their impact on crop plants and environment as well as on human health still remain to be fully explored.

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Seed priming · Conventional methods · Physiological basis · Promising techniques · Future needs

1.1 Introduction

Seed germination and thereby emergence of seedling are thought to be the most critical stages in the plant life cycle that ultimately determine field production of any crop plant through optimum plant stand (Gelmond 1978). The performance of a crop largely depends on rapid and synchronous seedling emergence as slow germination often exposes crop plants to adverse environment conditions (Osburn and Schroth 1989). Thus, researchers have been engaged in developing strategies and techniques for improving seed germination, plant establishment and growth of crop species for many years. A simple way to improve seed germination and seedling establishment and consequently field performance of plants is through seed priming (McDonald 2000). Seed priming is an age-old technique for improving germination and achieving high vigour, leading to better stand establishment and yield. The Greek Theophrastus (371–287 BC) first observed quick seed germination when cucumber (*Cucumis sativus* L.) seeds were soaked in water prior to sowing (Theophrastus Enquiry in Plants Book VII, I.6).

Seed priming is a value-added technique performed on a given seed lot, and this method is useful for quick germination and uniform emergence (Taylor et al. 1998). It has been proved to improve seed germination and seedling establishment of many field and horticultural crops (Basra et al. 2003; Sadeghian and Yavari 2004). Several benefits of seed priming have been reported including rapid and synchronized germination, increased nutrient uptake, relieved phytochrome-induced photo- and thermodormancy, increased range of germination temperature, improved water use efficiency and synchronous maturity of crops (Hill et al. 2008). 'Priming' literally means the triggering of stress tolerance particularly to moderate and intermittent stress. Seed priming also has long been known as a potential way to promote crop performance by enhancing tolerance of plant to biotic and abiotic stresses (Bruce et al. 2007). It actually causes to improve germination and also seed vigour which is a complex agronomic trait controlled by multiple genetic and environmental factors (Rajjou et al. 2012; Jisha et al. 2013).

In spite of delivering many benefits and having successful commercial use of seed priming, its popular application is often hindered due to available contradictory reports on deleterious effects of priming on seed longevity during storage (Tarquis and Bradford 1992). Difficulties also arise in standardizing priming protocol due to occurrence of great variations in effects among species, cultivars and even seed lots. Thus, the detailed understanding of physiological basis of priming effect can ease the optimization of this technique. The conventional priming protocols are

relatively time-consuming with high labour costs and sometimes may have adverse impact on environment. So it necessitates the development of fast, effective and more environment-friendly priming protocols. With this background, this chapter describes the current understandings on physiological basis of seed priming and recent advances in priming methods as a tool to combat with present-day challenges.

1.2 Physiological Basis of Seed Priming

Basically priming is a water-based technique that allows controlled seed hydration to trigger 'pre-germinative metabolism' but does not allow the seed for transition towards full germination. Though the interest of seed priming has been demonstrated since long, a comprehensive physiological basis of this fascinating technique remains poorly understood until recently. But a better understanding of pre-germinative metabolism during priming treatment and the subsequent germination will help to use this simple and cheap but unique technique in a more efficient way.

When a dry seed is kept in water, it passes through three distinct phases before it germinates (Bewley 1997). Phase I is imbibition when there is rapid initial water uptake due to low water potential of seed than outside. During this phase, initially, there is water movement in apoplastic spaces, proteins are synthesized from existing mRNAs and DNA and mitochondria are repaired. In phase II, there is activation of metabolic activities and repairing processes along with synthesis of proteins by translation of new mRNAs and synthesis of new mitochondria, whereas phase III is associated with regaining capacity of rapid water uptake and initiation of growing processes linked with cell elongation that leads to radicle protrusion. Priming allows a seed to hydrate up to a seed moisture content involving the entire phase I and before the end of phase II when the germination still remains a reversible process and just short of radicle protrusion (Bray 1995). Thus, priming actually activates 'pre-germinative metabolism' that includes a broad range of physiological functioning. This activates DNA repair pathways and ROS scavenging systems (that impart for seed repair response) and also helps in preserving genome integrity (Paparella et al. 2015). Taylor et al. (1998) has reported that the advanced germination status of primed seeds actually contributes for increased germination under stressful conditions. Besides it also facilitates the initiation of many germination-related activities such as enhanced energy metabolism, early reserve mobilization, embryo expansion and endosperm weakening (Pandita et al. 2007). Priming also enhances the specific stress-responsive systems which include induced accumulation of LEA and heat shock proteins (Catusse et al. 2011), activation of catalase and other antioxidant scavenging enzymes (Chen 2011) and upregulation of genes encoding peroxiredoxin (Li et al. 2005). Figure 1.1 describes a generalized physiology of priming effects in seed.

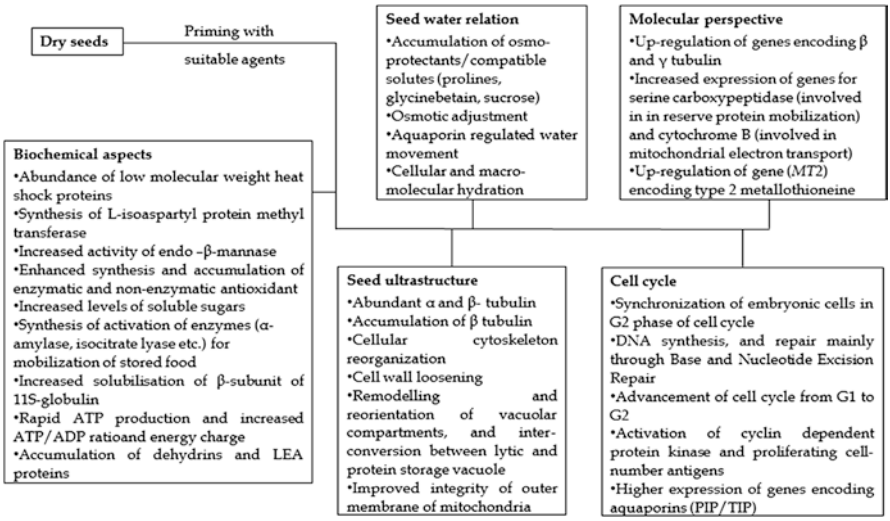

Fig. 1.1 Generalized physiology of priming effects in seed (Adapted from Benamar et al. 2003; Chen et al. 2012; Capron et al. 2000; Zhang et al. 2015; Corbineau et al. 2000; Varier et al. 2010; Paparella et al. 2015)

1.3 Conventional Methods of Seed Priming

Seed priming techniques commonly include several categories based on the choice of priming substances, i.e. hydro-priming, osmo-priming, chemo-priming, hormo-priming, solid matrix-priming, bio-priming, nutrio-priming and thermo-priming (Farooq et al. 2007; Chen 2011). Nonetheless, the efficacy of seed priming technique largely depends on plant species/genotypes, seed morphological and physiological parameters as well as the technique of seed priming. Priming is routinely used to treat many horticultural crops mainly vegetables and flowers, but large-scale use of priming cereals or other field crops is limited (Murungu et al. 2004). Same priming protocol may often have varied effect among species, cultivars and even seed lots (Pill et al. 1994). A summary on effects of different priming agents in crops is given in Table 1.1.

1.4 Latest Approaches of Seed Priming

The use of eco-friendly and cheap methods for enhancing production to cope with the needs of a growing population is a common interest of agricultural scientists. Several proprietary priming agents like (a) EasyPrime/EasyDormex (targeted crops are brinjal, tomato, *Brassica*, melon and lettuce), (b) Advantage®/Xbut®/Emergis® (targeted crops are sugar beet, vegetables and flowers) and (c) Thermocare™/Promoter™ (targeted crops are lettuce, onion and carrot) are available for commercial uses in many crops (Paparella et al. 2015). But researchers are continuously

Table 1.1 Effect of different conventional priming treatments on seed germination and crop performance

Method	Agent	Description	Crop	References
Hydro	Water (hydration/ hydration-dehydration)	Improved growth of nursery seedling, and subsequently crop growth increased panicle number m^{-2}, filled grain $panicle^{-1}$, 1000 grain weight and yield	Rice	Farooq et al. (2007) and Mahajan et al. (2011)
		Improved field stand and plant growth both at vegetative maturity stage and yield	Maize	Nagar et al. (1998) and Murungu et al. (2004)
		Improved endosperm weakening, germination and seedling development	Brinjal	Anese et al. (2011)
		Improved germination under salinity, increased tillers and grain yield	Wheat	Meena et al. (2013)
Osmo	Polyethylene glycol (PEG)	Improved seedling establishment, improved germination, growth and yield	Wheat	Salehzade et al. (2009)
		Improved germination and lowered mean germination time	Rice	Ruan et al. (2002)
		Increased germination, radicle and shoot length	Marigold	Ganji Arjenaki et al. (2011)
		Improved germination under temperature and water stress	Spinach	Chen et al. (2010)
		Increased germination and strengthened antioxidant systems	Sorghum	Zhang et al. (2015)
	Glycine-betaine	Increased seedling emergence and reduced mean emergence time	Rice	Chen et al. (2005)
	Chitosan (large polysaccharides)	Increased germination, lipase activity, GA and IAA levels	Peanut	Zhou et al. (2002)
		Increased resistance to diseases and improved seed quality	Wheat	Reddy et al. (1999)
		Improved germination and seedling growth under low temperature	Maize	Guan et al. (2009)
	Polyamine	Synchronized and enhanced germination and improved seedling length, fresh and dry weight and leaf score	Rice	Farooq et al. (2009)

(continued)

Table 1.1 (continued)

Method	Agent	Description	Crop	References
Halo	KNO_3	Improved germination, early seedling growth, increased plant height, leaf area and yield attributing parameters	Rice	Srivastava and Bose (2012) and Basra et al. (2005)
		Increased germination and emergence percentage, radicle and plumule length, plant height and dry weight	Soybean	Ahmadvand et al. (2012)
Halo	$CaCl_2$	Increased germination percentage, seedling length and weight	Fennel	Hoseini et al. (2013)
		Increased germination percentage, biological yield, harvest index and tassel weight	Maize	Vazirimehr et al. (2014)
		Increased activity of total amylase and protease in germinating seed under salt stress	Sorghum	Kadiri and Hussaini (1999)
		Increased germination speed and seedling vigour	Maize	Kulkarni and Eshanna (1988)
		Improved growth of seedling and stand establishment	Rice	Farooq et al. (2007)
	NaCl	Best germination percentage	Coriander	Fredj et al. (2013)
		Improved germination and germination index	Safflower	Elouaer and Hannachi (2012)
Solid matrix	Calcium-aluminium silicate	Increased seedling vigour, germination percentage and fruit yield	Okra	Sharma et al. (2013)
	Sand	Improved seed emergence and seedling density, increased root number and length	Rice	Hu et al. (2005)
Nutrio	Zn	Improved seed emergence, field performance and grain yield	Hybrid maize	Afzal et al. (2006)
	Mo	Increased plant height, number of pods plant^{-1}, test weight, seed yield, straw yield, water use efficiency	Chickpea	Singh et al. (2014)
	Urea	Increased germination, enhanced antioxidative enzyme activity, soluble sugar and proline	Chinese cabbage	Yan (2015)

(continued)

Table 1.1 (continued)

Method	Agent	Description	Crop	References
Bio	*Trichoderma*	Improved seedling establishment and yield	Rice	Rahman et al. (2015)
		Synchronized seed germination and plant growth	Soybean	Entesari et al. (2013)
		Increased activity of superoxide dismutase, peroxidize, glutathione reductase and glutathione s-transferase	Tomato	Mastouri et al. (2012)
	Azotobacter/*Azospirillum*	Increased crop growth, dry matter accumulation and yield	Maize	Sharifi (2011)
		Increased yield, 1000 grain weight and harvest index	Barley	Mirshekari et al. (2012)
Hormo	Salicylic acid	Improved germination rate, seed stamina index and seedling fresh and dry weight	Fennel	Farahbakhsh (2012)
		Improved germination and antioxidant capacity	Rice	Kata et al. (2014)
	Brassino-steroids	Increased antioxidative response of seedling under salt stress	Alfalfa	Zhang et al. (2007)
	GA_s	Enhanced emergence, germination percentage and speed of germination, stimulation in activity of enzymes related to germination	Soybean	Maske et al. (1997) and Bassi et al. (2011)
		Improved speed of germination, seedling dry weight and vigour index	French bean	Sarika et al. (2013)
		Enhanced germination and emergence of seedling, increased vegetative growth and photosynthetic activity at vegetative stage	Wheat	Bassi (2005) and Sharma et al. (2010)
		Synchronized and enhanced germination, improved root length and leaf score	Rice	Farooq et al. (2007)
		Increased germination and seed vigour	Maize	Kumari et al. (2017)

engaged in finding out some different easy methods for seed priming which can be used as alternative to conventional protocols, and at the same time the latest techniques should be rapid, cost-effective and environmentally safe. Among the latest techniques, use of physical agents and nanoparticles for seed priming is rapidly developing.

1.4.1 Priming with Physical Agents

Physical methods of seed priming have immense potential and advantages over the conventional methods based on chemical substances (Aladjadjiyan 2012). As such, seed priming with physical treatment can be an alternative approach by considering the contemporary issues of agricultural pollution due to injudicious use of chemical compounds (Araujo et al. 2016). These physical methods also are affordable, clean and environmentally safe, and there is every prospect of using these methods to increase seed germination and seedling vigour in a high-throughput scale (Haq et al. 2012). Thus, physical methods for seed priming are being preferred than conventional methods in particular chemical priming since present-day agriculture relies on idea of sustainability with little or no adverse effects on environment (Pretty et al. 2006). Though physical treatments are good alternatives to raise agricultural production along with improvement in plant protection and post-treatment storability (Aladjadjiyan 2012), it is obvious that most of the physical and/or extra-terrestrial environmental factors have genotoxic effects particularly at high levels. But interestingly, stimulatory effects of physical treatments at low doses have been reported by many in recent times (De Micco et al. 2014), and the phenomenon of boosting up of plant characteristic effects by toxic agents at lower doses is known as hormesis. As such, hormesis actually represents an adaptive response of living organisms to moderate or intermittent stress, due to stimulation of cellular defence and repair mechanisms which will otherwise be missed in absence of that stress agents (Mattson 2008). Various physical treatments have been reported for their stimulatory effects when used for seed priming (Araujo et al. 2016) (Fig. 1.2).

Among the physical methods, electromagnetic priming has been described as eco-friendly, cheap and non-invasive technique (Bilalis et al. 2012). Recent researches have shown that seed priming using magnetic field can improve germination rate, vigour and seedling biomass along with tolerance to biotic and abiotic stresses which is possibly attributed to reduced production of superoxide radicles along with increasing activities of antioxidant enzymes (Bhardwaj et al. 2012; Araujo et al. 2016). Another physical agent, ionizing radiation like gamma (γ)-rays is a powerful mutagenic tool in the agricultural sciences, and it can directly interact with cellular components. However, the biological effects of γ-rays are strongly depended on the intensity, dose rate and exposure time. Seed irradiation with γ-rays at doses lower than 100 Gy can remarkably enhance germination percentage and seedling establishment. This γ-ray-induced enhancement particularly at low-dose irradiation can be attributed to favourable changes in the hormonal signalling network in plant cells and trigger the antioxidative capacity which acts as a boost in

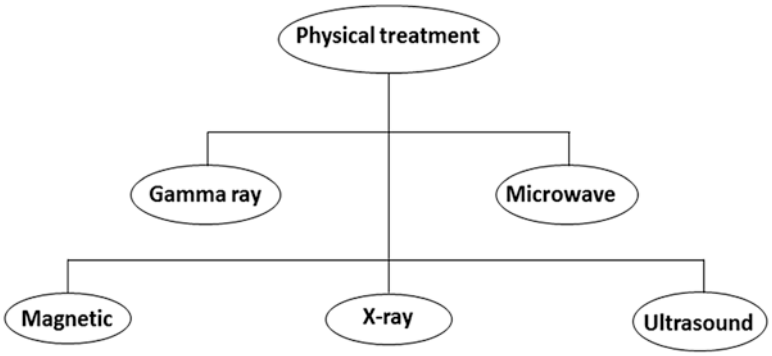

Fig. 1.2 Different types of physical treatments used for seed priming

pre-germination metabolism leading to early dormancy breaking, improved germination and overcome of daily stress factors such as fluctuations of light intensity and temperature in growth conditions (Qi et al. 2015).

Ultraviolet rays are physical agents which fall under the group of nonionizing radiation and are extremely harmful to organisms. It is because UV radiation can penetrate into living tissues and is potential to cause DNA damage and membrane injury. However, few works in recent times have showed that UV radiation can elicit some positive responses like inducing capability to mitigate environmental stresses through increased free radical scavenging activities, increasing total soluble phenols and enhancing the activities of L-phenylalanine ammonia lyase and tyrosine ammonia lyase (Araujo et al. 2016). Microwaves are also known as components of nonionizing electromagnetic spectrum which is able to induce thermal as well as nonthermal effects in biological systems (Banik et al. 2003). The detailed studies to evaluate the impact of microwaves in seed technology are really inadequate to establish it as promising approach for seed priming. But few researches have clear indication of beneficial effects of this physical agent on seedling growth and biomass accumulation (Jakubowski 2010, Talei et al. 2013).

Ultrasound is a novel physical method that involves the application of mechanical waves having frequency in the range between 20 and 100 kHz for seed pretreatment. During the recent years, ultrasonic waves are being immensely applied as an efficient technique for breaking seed dormancy and improving the germination characteristics. This technique is unique among existing seed pretreatment methods in that it is fast, energy-saving and environmentally friendly (Goussous et al. 2010). The mechanisms of action of the ultrasound for seed invigoration are probably clearer than other physical priming methods. Ultrasound priming process enforces the uptake of water and activates enzymatic and other biological reactions in the seed resulting in a faster and more uniform germination. Ultrasound priming processes actually impose a mechanical pressure on the seed coat, and this pressure increases the seed's porosity which is called as acoustic cavitation. This increase in porosity leads to improve water uptake and oxygen availability. Thereby, ultrasonic priming enhances mass transfer of the extra-absorbed water and allows it to react

freely with the cell embryo. As a result gibberellic acid is released and causes an increase in the rate of metabolic processes in aleurone cells due to increasing activities of hydrolyzing enzymes. Then the endosperm nutrients ultimately mobilize due to cell membrane disruption (Miano et al. 2015). All these series of responses finally lead to enhanced germination percentage and speed. Stimulation of seed germination by ultrasound treatment has been reported in various crops (Yaldagard et al. 2008; Goussous et al. 2010).

1.4.2 Priming with Nanoparticles

Application of nanotechnology is almost every field of science owing to the extensively research that being undertaken. Among the latest line of technological innovations, nanotechnology is a versatile field and is a rapidly developing discipline. It is substantially influencing almost all existing fields of science, and introduction of nanomaterials in agriculture has immense possibility of using this unique technology as a solution to several agricultural and environmental challenges. Nanotechnology certainly occupies a prominent position in transforming agriculture and food production (Fraceto et al. 2016) and holds the potential to rejuvenate agriculture. The unique properties of materials at nanoscale make them suitable for the design and development of novel tools in support of sustainable agriculture. Introduction of nanomaterials in agriculture can potentially contribute to address the issue of sustainability under the future challenges of ever-increasing global population and climate change (Parisi et al. 2015). Nanoparticles have been shown to be an attractive alternative for the manufacture of nanofertilizers, which are more effective and efficient than traditional fertilizers. Thus the exploitation of nanotechnology and nanomaterials in agriculture can reduce the application of excess chemical fertilizers to crops and contribute to precision farming as it reduces fertilizer wastage and in turn environmental pollution due to agricultural malpractices (Upadhyaya et al. 2017). Because of their impact on crop nutritional quality and stress tolerance in plants, the application of nanoparticles is increasing. Nanopriming is a new technique of using nanoparticles of less than 100 nm size, and it enhances the germination percentage as well as seedling dry weight and vigour in most of the crops. Seed priming with nanoparticles of micronutrients has been reported as a potential new method for increasing germination percentage and seedling development and vigour (Ghafari and Razmjoo 2013). The increased germination seedling growth rate under nanopriming has been attributed to enhancing water and nutrient uptake due to more penetration through seed coat. Table 1.2 summarizes the effects of different physical and nanopriming methods on seed germination and other plant responses.

Table 1.2 Summary of effect of physical and nanopriming methods on seed germination and plant characteristic

Method	Agent	Description	Crop	References
Physical	Magnetic field	Increased antioxidant potential under soil flooding	Wheat	Balakhnina et al. (2015)
		Increased germination parameters and seedling biomass, increased light harvesting capacity, reduced production of superoxide radicals	Soybean	Baby et al. (2011) and Shine et al. (2011)
		Improved seedling growth, leaf water status and photosynthesis under water stress	Maize	Anand et al. (2012)
		Increased plant height, root length, root biomass and yield	Okra	Haq et al. (2012)
		Increased activities of superoxide dismutase, catalase and glutathione reductase	Cucumber	Bhardwaj et al. (2012)
	UV radiation	Improved tolerance to salinity	Lettuce	Ouhibi et al. (2014)
		Increased germination speed and seedling vigour and reduced susceptibility to root interacting fungi	Mungbean Groundnut	Siddiqui et al. (2011)
	Gamma-radiation	Improved morphological traits like plant height, shoot number, panicle length and seed number panicle^{-1}	Rice	Maity et al. (2005)
		Improved germination, plant growth and pigment contents	Maize	Marcu et al. (2013)
		Improved seed germination, photosynthetic capacity and seed yield	Okra	Hegazi and Hamideldin (2010)
	X-rays	Enhanced leaf growth	Date palm	Al-Enezi et al. (2012)
		No toxic effect in terms of germination and leaf development under increasing irradiation dose	Tomato	De Micco et al. (2014)
	Microwaves	Increased germination percentage and rate, primary shoot and root length	Rice	Talei et al. (2013)
		Enhanced germination and vigour index after exposure during 20s	Barley	Iuliana et al. (2013)
		Highest biomass growth in seed potato germs	Potato	Jakubowski (2010)

(continued)

Table 1.2 (continued)

Method	Agent	Description	Crop	References
Physical	Electro	Increased growth and yield	Rice	Bera and Maity (2003)
		Increased female/male flower ratio, increased length of seedling	Bottle gourd	Rahman and Yasmin (1993)
	Ultrasonic	Improved germination percentage and speed of germination	Chickpea, wheat, watermelon	Goussous et al. (2010)
		Improved germination percentage and increased activities of superoxide dismutase and catalase	Sesame	Shekari et al. (2015)
		Improved water uptake and germination percentage and reduced time for germination	Barley	Yaldagard et al. (2008)
Nano	Calcium-phosphate nanoparticles	Improves seedling growth and stimulates both enzymes and metabolites related with antioxidative responses	Rice	Upadhyaya et al. (2017)
	ZnO nanoparticles	Increased seed germination, growth and yield	Peanut	Prasad et al. (2012)
		Improved germination, reduced time to flowering, increased seed weight per umbel and 100 seed weight	Onion	Laware and Raskar (2014)
	SiO_2 Nanoparticles	Enhanced root length, root volume and seedling dry weight	Rice	Adhikari et al. (2013)
		Increased germination, nitrate reductase activity and nutrient uptake	Soybean	Lu et al. (2002)
	Silver nanoparticles	Increased germination percentage, plumule and radicle length	Wheat	Salehi and Tamaskani (2008)
	Iron nanoparticles	Increased pod number, 100 seed weight and total yield	Chickpea	Valadkhan et al. (2015)

1.5 Seed Priming as a Tool to Combat Contemporary Challenges

Global climate change is inevitable with time, but kind of changes which occurred in the last several decades due to combination of natural and human-made causes are really a matter of great apprehension. As a consequence of rapid climate change, there is a rise in mean temperatures together with more frequent episodes of water deficit and heat waves. Thus, agricultural production is currently under intense challenges of various potentially adverse environmental conditions such as water deficit, high salinity, extreme temperature, submergence, etc. (Jisha et al. 2013) apart from various biotic stress factors. These stresses adversely affect the plant growth leading to substantial and unpredictable loss in crop production. Nevertheless, one of the

biggest challenges of present day's agricultural system is to improve production sustainably to feed up the ever-increasing global population but with minimum impact on environment (Edmondson et al. 2014). It has been reported that one of the major obstacles to high yield and production of crop plants is the lack of synchronized crop establishment due to poor weather and soil conditions (Mwale et al. 2003).

Seed germination and subsequent seedling growth are critically influenced by environmental causes, especially moisture, temperature and salinity (Zahedifar 2013). The poor or uneven germination and seedling growth lead to nonsynchronous maturity and thereby render the possibilities of mechanization and ultimately lead to great financial losses (Ghiyasi et al. 2008). However, it is evident that a previous exposure to moderate stress can affect the subsequent responses and eventually prepare the plants to respond to future stresses in a better way (Li et al. 2014). Priming makes the plants able to respond more rapidly and more effectively for stress protection through developing a higher level of fitness on a given stress. Thus, seed priming is the simple and best solution for higher germination, seedling establishment and yield especially when the crops are grown under stressful and/or unfavourable condition (Singh et al. 2015). Seed priming can effectively attenuate the detrimental effects of stresses during germination and radicle emergence and elicit rapid positive response by triggering efficient plant hormones and antioxidant systems. The improvement in seed quality by priming has been attributed principally to reduced membrane damage or reduced lipid peroxidation through effective scavenging of reactive oxygen species generated during stressful conditions (Chiu et al. 2006). Priming actually leads to promote germination and seed vigour of different plant species through favourable changes in cellular, subcellular and molecular levels under diverse environmental conditions such as salinity, water stress and high- or low-temperature stresses (McDonald 2000). As a consequence, primed seeds result in increased levels of biotic and abiotic stress tolerance and crop yield.

1.6 Limitation and Ways to Overcome

Nonetheless, in spite of exerting many beneficial effects, contradictory reports are available regarding adverse consequences of priming of seed quality. The main drawback of conventional priming techniques is reported as increased deterioration during storage which has been ascribed to loss of seed desiccation tolerance due to prolonged treatments and thereby reduced seed longevity (Tarquis and Bradford 1992) that largely depends on the conditions (temperature, humidity, air composition) in which seeds are kept immediately after priming (Schwember and Bradford 2005). However, several post-priming protocols have been developed for amelioration of negative effect of conventional priming on seed longevity (Gurusinghe and Bradford 2001). Another challenge arises from the internal variability of priming itself, which is that not all priming protocols will lead to an improved seed germination performance and inappropriate priming conditions may render seeds vulnerable to stresses by inducing the degradation of protective proteins (Capron et al.

2000). Thus, it is always essential to first determine an optimal priming protocol specific to plants with regard to the germination performance and its effect on seed stress tolerance. The priming protocols can be optimized by using 'hydrotime concept' which can also be useful in minimizing this setback of reduced longevity due to prolonged treatment. It is because the 'hydrotime concept' describes the relations of seed water potential to time required for radicle emergence (Bradford and Still 2004). Hydrotime is measured in MPa h^{-1} or MPa day^{-1}, and this analysis can allow prediction of the pattern of germination in terms of speed, uniformity and stress tolerance which can vary with different cultivars, seed lots and treatments (Paparella et al. 2015). Thus, hydrotime analysis is useful for evaluating the physiological status of a seed lot since all the parameters used for hydrotime analysis can reveal any possible abnormal response in terms of germination and seedling establishment under stressful condition (Bradford and Still 2004). Additionally, a non-invasive method, namely, digital image technology (DIT), can extensively be exploited for accurate analysis of vigour as well as seed purity since the said technology has immense potential for precise and automated assessment of seed morphological and physiological features (Dell'Aquila 2009). Thus, the effectiveness of different priming methods on germination and seedling establishment can be analysed by using DIT (Mahajan et al. 2011) which allows high-resolution and accurate analysis of germination parameters on a single-seed basis.

Further the detailed impacts of physical treatments on morpho-structural aspects, pre-germinative metabolism, molecular mechanisms and/or gene expressions are also yet to be fully explored, and this gap of knowledge renders hindrance in their successful application (Araujo et al. 2016). And the effectiveness of physical treatments largely depends on several factors such as type, dose and dose rate of radiation and magnetic field as well as plant characteristics such as species, cultivars, age, ploidy level and complexity of target organ or tissue (De Micco et al. 2014). Despite of considerable advances in identifying possible application nanotechnology in agriculture, many issues like characterization and quantification of nanomaterials in different surrounding substances as well as their impact on environment and on human health remain to be fully resolved till date because there are virtually no studies on the potential environmental impact of nanoparticles when used in agriculture.

Thus, the application of physical methods or nanoparticles for seed priming still requires an extensive research for determining detailed impact of these methods on plants and also to define the optimal treatments in terms of dose, dose rate and exposure time which certainly varies with crop species, genotypes and environmental conditions. Further understanding the physiological and molecular mechanisms underlying the improved performance of physical and nano-primed seeds and thereby implicating the combined application of these methods with environmental factors are very important for developing improved priming protocols.

1.7 Conclusion

In the era of modern and precision agricultural technologies, there is a demand that each and every seed should be healthy and readily germinate to produce a vigorous seedling to ensure better crop yield. Seed priming, a very simple and unique technique, has long been identified as a potential tool to improve seed germination, reduce seedling emergence time, produce vigorous plant, reduce time to flower, improve crop tolerance to abiotic stresses and ultimately improve grain yield. But, optimizing the priming protocol by hydrotime analysis of individual species/cultivar would alleviate the several difficulties that arise during conventional priming methods. Recent developments of seed priming particularly through physical methods and/or by using nanomaterials have been found to be very promising ways over the conventional seed priming techniques for sustainability in agricultural production. The application of physical methods or nanoparticles for seed priming still requires an extensive research for determining the detailed impact of these methods on plants and environment as well as on human health. This is further required to define the optimal treatment in terms of dose, dose-rate and exposure time and their interactive effects on particular species/genotypes under specific environmental conditions before these technologies may make significant contribution to agriculture.

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

Bandana Bose, Mahesh Kumar, Rajesh K. Singhal, and Sananda Mondal

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 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).

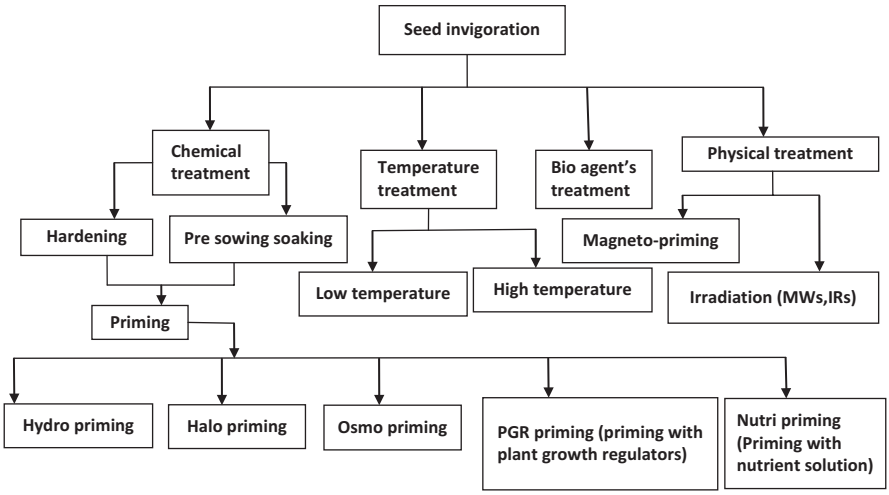

Fig. 2.1 Represent the type of seed invigoration technique

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 osmo-regulators 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

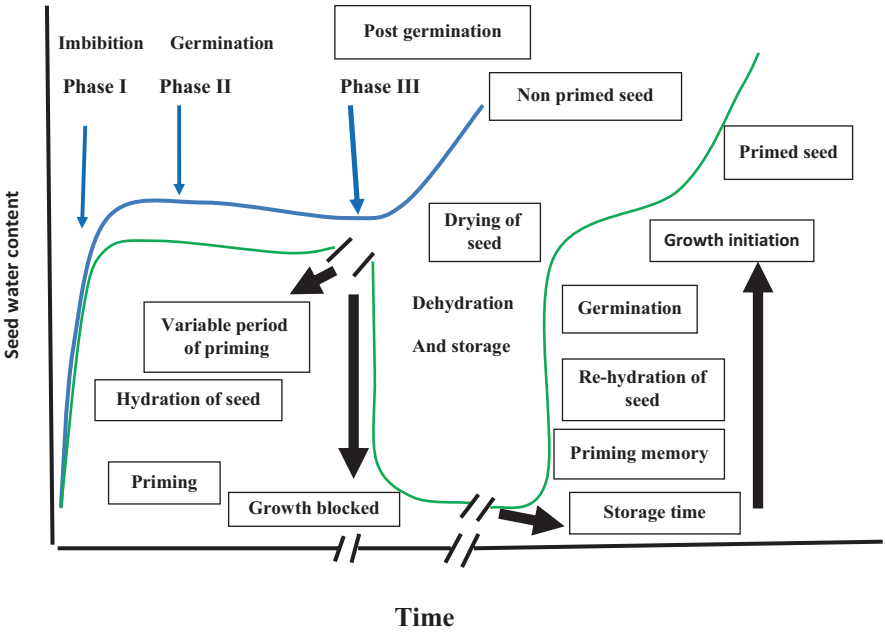

Fig. 2.2 Schematic representation of normal germination and seed priming process (Rajjou et al. 2012) (Phase I, imbibition phase; phase II, germination phase; phase III, post-germination phase)

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

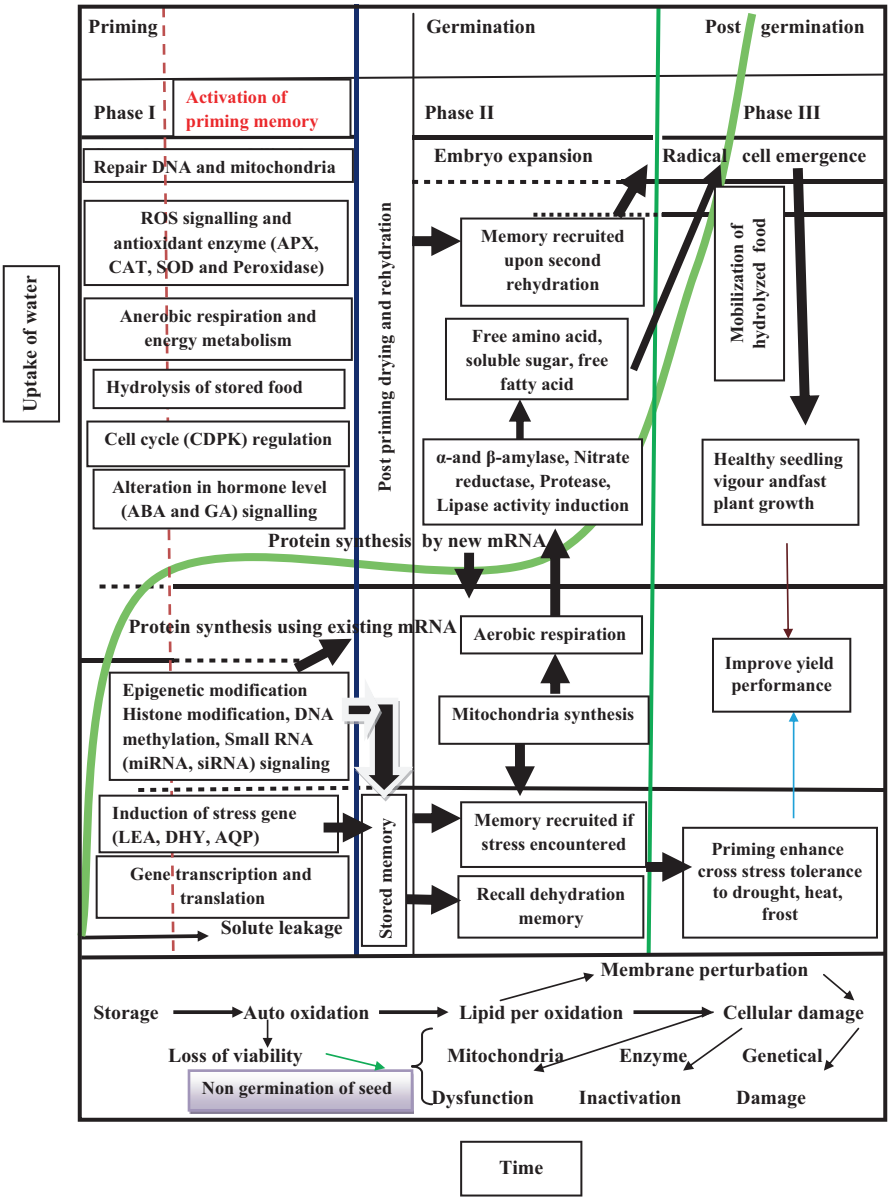

Fig. 2.3 Model for priming memory and its relation to abiotic stress signaling (Chen and Arora 2013)

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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Seed Priming: An Emerging Technology to Impart Abiotic Stress Tolerance in Crop Plants

3

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Abstract

Crop plants encounter a complex set of abiotic and biotic stresses very frequently. Abiotic stresses, being unavoidable, have major negative impact on crop production worldwide. These stresses such as inadequate and inconsistent rainfall, alkalinity, salinity, extreme temperature, and some other factors aren't only limiting crop yield but also seem to be inevitably worsening. Considering present situation, it is imperative to switch to some more sophisticated techniques that shall combat abiotic environmental challenges and improve crop yield efficiently. Among these, seed priming is a commonly utilized technology for enhancing seed vigor and stress tolerance. Seed priming involves the attainment of a specific physiological state by synthetic or natural compounds. Crop plants raised from primed seeds exhibit instant cellular response against abiotic stresses. Primed seed acquire resistance through various cellular and metabolic pathways which involves cascades of signaling networks. Studies, till date, have confirmed that primed seeds have several advantages over traditionally used methods which include uniform germination, reduction in germination and emergence time, and broad range of tolerance against disease and environmental stresses. Seed priming methods are widely used as an emerging technology to produce tolerant crop varieties against abiotic stresses.

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Abiotic stress · Seed priming · Seed germination · Biomarker · Sustainable agriculture

3.1 Introduction

In nature, plants are continuously exposed to number of environmental stresses during different developmental stages. The abiotic stress which adversely affects plant growth includes drought, extreme temperature, salinity, and other crucial factors (Zhao et al. 2007). In agriculture, abiotic stresses on standing crops lead to severe economic yield loss and affect plant health (Jakab et al. 2005). Drought, salinity, and extreme temperature cause osmotic stress on crop plants which create an imbalance at cellular, molecular, and physiological levels which ultimately leads to plant death (Xiong and Zhu 2002). Environmental stresses such as cold, radiation, light, and high temperature are also reported to affect the growth of the plants (Reyes and Cisneros-Zevallos 2007). In natural environment, crops are being exposed to various abiotic and biotic stresses, and to thrive well under such situations, crops have developed intricate mechanism to sense the signals and respond according to external environmental stimulus resulting in modulation of plant metabolites; activation of hormone signaling pathways that are regulated by plant hormones like abscisic acid, salicylic acid, jasmonate, and ethylene; and free radical generation (Fujita et al. 2006). In response to environmental and biotic stress, plant generates numerous volatile organic molecules that play key roles in important defense and signaling processes. Under stress conditions, the amount of emitted volatile organic compounds relies on stress tolerance, time duration, and severity of stresses (Niinemets 2009). Abiotic stresses like drought and salinity are complex traits, governed by a complete set of parameters. The improvement of crop plants through direct selection-based conventional breeding for drought and salinity stress appears to be quite complex (Cushman and Bohnert 2000; Flowers et al. 2000).

In agriculture, other innovative technologies such as plant tissue culture, seed priming, and genetic engineering could play a major role in increasing yield and productivity. Genetic engineering and genome editing technologies, developed for targeted insertion of foreign genes into desired crop cultivars, lead to the creation of designer crops (Gust et al. 2010). The transgenic crop has demerits of pleiotropic effect and gene silencing. Due to above limitation of available technology, we should think of some alternate technologies such as priming, mutagenesis, and tissue culture for combating abiotic stresses. Seed priming is a very simple, cost-effective, and sustainable approach which could be easily carried forward into the field.

3.2 Seed Priming

Priming involves prior exposure to elicitors which brings a cellular state that hinders the harmful effects of abiotic stress, and plants raised after priming are more tolerant of abiotic stress. Priming induces resistance upon pathogen attack and confers enhanced disease protection to the plant (Van Hulten et al. 2006). Seed priming involves soaking of seed in water or other solvents for a certain period that leads to changes in metabolic profile of the seed (Hussain et al. 2016).

Seed priming enhances the germination rate and uptake of nutrients and also controls seed-borne pathogens by influencing the pre-germination metabolic activities (Taylor and Harman 1990). It has the benefit of enhanced and uniform germination along with better yield and high vigor in floriculture and vegetable crops (Bruggink et al. 1999) and field crops (Kaur et al. 2005).

Primed seed exhibits significantly improved germination and greater germination uniformity and plant performance (Basra et al. 2005). Seed priming techniques such as halopriming, chemical priming, osmopriming, hormone priming, hydropriming, and nutrient priming are being used in rice for many environmental stresses (Jisha et al. 2013; Paparella et al. 2015). Seed priming technique could be adopted to enhance germination in soils that are contaminated with heavy metals (Sneideris et al. 2015). The imbibition of pepper seeds in water or other solution containing NaCl resulted in an increased germination percentage (Khan et al. 2009).

The major obstacle for practical application of primed seeds is storage and viability (McDonald 2000). This limitation could be addressed by knowing genes/markers for seed germination, and identified markers could be used for assessing the effect of priming on germination efficiency and seed vigor (Job et al. 2000). Genes/markers involved in rice seed priming were identified by comparing differential proteins between the dry and imbibed seed using two-dimensional electrophoresis (Cheng et al. 2017). Improved germination was reported in polyethylene glycol-6000 (PEG-6000) primed rapeseeds which, on germination, resulted in differential expression of 952 genes and 75 proteins (Kubala et al. 2015).

3.3 Physiological and Subcellular Basis of Seed Priming

Seed priming results in improved germination, vigor, and plant stand. Many previous studies have elucidated the seed priming mechanisms at various physiological, biochemical and cellular conditions using different kinds of spectrophotometric techniques in crop plants. Different proteins specifically appear during seed hydropriming and osmopriming, which are identified by MALDI-TOF in model plant *Arabidopsis thaliana* (Gallardo et al. 2004). There is accumulation of degradation product of B-subunit of 11S globulin and suggested enzyme involved in mobilization of storage protein during seed priming in sugar beet (Job et al. 2000). Activation of reserve mobilization enzymes, viz., carbohydrate and lipid mobilization enzymes, takes place during seed priming. Seed priming drives the synthesis of free radical scavenging enzymes, with expression of catalase and superoxide

dismutase being increased. These enzymes protect the vital cell membrane and organs against oxidative stress induced during priming. Studies on gene expression profiling in osmoprimed seeds revealed genes are being expressed which are responsible for cellular metabolism (Soeda et al. 2005). Damage of DNA occurs inside the seed, which accumulates during seed aging, and is repaired to a great extent by accelerated hydration during early hours of germination (Thornton et al. 1993).

Comparing transcript profiling of rice seedlings under submergence stress reveals genes were differentially expressed in primed and non-primed seed and priming alleviated the harmful effect of submergence stress (Hussain et al. 2016). On constructing and sequencing small RNA libraries from water-soaked seed embryos under salt, drought, and control treatments, there is significant downregulation of six microRNA (miRNA) families and upregulation of one miRNA family, and further analysis revealed miRNA participate in regulation of plant hormone synthesis and play a crucial role in seed germination under environmental stress (Jian et al. 2016).

3.4 Role of Seed Priming Technique in Combating Abiotic Stress

3.4.1 Germination and Growth Promotion

Seed priming exhibited a promotive effect on seedling emergence, seedling growth, and plant performance in sesame (Shabbir et al. 2014). In sunflower priming with pre-optimized levels of priming reagents such as salicylic acid, H_2O_2, thiourea, ascorbic acid, gibberellic acid, sodium chloride, freezing, and thawing induces metabolic changes that lead to improvement in germination, reduced germination time, and promotive effect on seedling growth (Wahid et al. 2008). Hydration treatment and redrying increase germination efficiency and seedling emergence in many species of *Brassica* (Jett et al. 1996). Seed priming improved the germination and vigor of wheat crop that leads to 17% increase in grain yield than non-primed seed (Farooq et al. 2008). Priming with potassium nitrate and hydropriming resulted in better germination and enhanced plant performance in cotton (Rezaee et al. 2015). Seedling emergence involves interaction between soil matrix potential, soil aggregate size, and hydropriming in cotton and maize (Murungu et al. 2003). Seed primed with $CuSO_4$, $ZnSO_4$, and Na_2SO_4 significantly increased the germination in maize (Foti et al. 2008). Osmoriming and hydropriming increased the seedling emergence in sorghum (Moradi and Younesi 2009). Hydropriming increased the yield as a result of better germination and vigorous growth in mung bean (Rashid et al. 2004)

3.4.2 Salinity Stress

Under salinity stress, germination percentage and germination index decrease significantly, and also there is a decrease in chlorophyll a, chlorophyll b, and carotenoid content in maize. During germination, seed priming induces metabolic

changes that help in better acclamation under salinity stress in maize (Sali et al. 2015). Seed primed with $CaCl_2$ followed by KCl induces salt tolerance in rice cultivar that is revealed by enhanced germination efficiency, seedling growth, and dry weight under saline medium (Afzal et al. 2012). High concentration of NaCl reduced the seed germination in wheat cultivars (Akbari et al. 2007). Seed treated with H_2O_2 showed improved salt tolerance in wheat cultivars (Wahid et al. 2007). Mustard seed primed with water, $CaCl_2$, and abscisic acid exhibited higher germination, and crop raised from primed seed contains high dry weight and chlorophyll content under common salt and PEG stress (Srivastava et al. 2010a). Thiourea supplementation in roots of *Brassica juncea* imparts tolerance against salt stress possibly by maintaining water homeostasis (Srivastava et al. 2010b). Hydropriming and KNO_3 treatment significantly improved germination and seedling growth under salinity and water deficit stress (Kaya et al. 2006). Halopriming enhances the growth under salt and drought stress in sugarcane cultivars (Patade et al. 2009). Halopriming counteracts salt stress by increasing the synthesis of antioxidant enzymes, H_2O_2 production, and accumulation of proline in mung bean (Saha et al. 2010). Soaked seed in water exhibited higher grain and straw yield than non-primed seed under saline-sodic condition in barley (Rashid et al. 2006).

3.4.3 Drought Stress

Seed priming with $CaCl_2$ improved seedling emergence, seedling establishment, plant height, tillers number, grain number, grain weight and yield under drought stress in wheat (Hussain et al. 2013). Seed priming with urea, KNO_3 increases germination efficiency, seedling growth, root length, proline and protein content under salinity and drought stress in maize hybrids (Anoshehet et al. 2011). Osmoprimed rice seed with KCl and $CaCl_2$ enhances seed vigour evidenced by improved germination, seedling emergence, kernel yield, number of fertile tiller, straw yield and harvest index. Rapid and uniform germination occurs due to elevated α-amylase activity and enhanced starch hydrolysis there by more sugar are available for embryo and seedling growth that leads to improvement in yield and quality (Farooq et al. 2006). The hydropriming was also used as a seed invigoration technique in maize inbred lines for effective seed germination in the presence of salinity and drought stress (Janmohammadi et al. 2008). The primed and non-primed rice seed performed differently under water deficit condition and the amount of proline and soluble protein are found higher in primed seed (Yuan-Yuan et al. 2010). Seed primed with water, PEG and KCl showed enhanced germination in winter wheat cultivar but priming doesn't benefitted field emergence (Ghana and Schillinger, 2003). Seed primed with water and mannitol showed more seedling growth as compared to non-primed seed under water deficit condition in chickpea (Kaur et al. 2002). Hydropriming increased the germination under water and temperature stress in cotton (Casenave and Toselli 2007).

3.4.4 Temperature Stress

During different reproductive stages of wheat, the heat stress poses a negative impact on final grain yield by affecting multiple traits like grain weight, seed size, tiller size and seed moisture content (Iqbal et al. 2017). Osmopriming in late sown wheat resulted into increased number of tillers, biological yield, and harvest index (Mustafa et al. 2017). Chilling stress or low-temperature stress also has a detrimental effect on normal functioning and biological system of crop plants. Cotton seeds primed with PEG-6000, KNO_3, KH_2PO_4, NaCl, and mannitol were evaluated for the best priming agent under cold stress. Priming with KNO_3 gives the best result for germination rate and 2 h priming with KNO_3 and then drying enables seed for cold tolerance and vigorous seedling (Cokkizgin and Bolek 2015). Maize seed primed with chitosan solutions exhibited enhanced germination, seedling growth, and plant biomass at low temperature (Guan et al. 2009). The water-soaked maize seeds were able to germinate below the optimum temperature and resulted in an improved crop growth and higher yield potential (Finch-Savage et al. 2004). In chickpea, hydropriming and osmopriming with different concentrations of PEG induce faster germination at temperature ranging from 5 to 32 °C (Elkoca et al. 2007) Figure 3.1 Diagramatic dipiction of seed priming induced plant acclimation to various environmental stresses (Tanou et al. 2012).

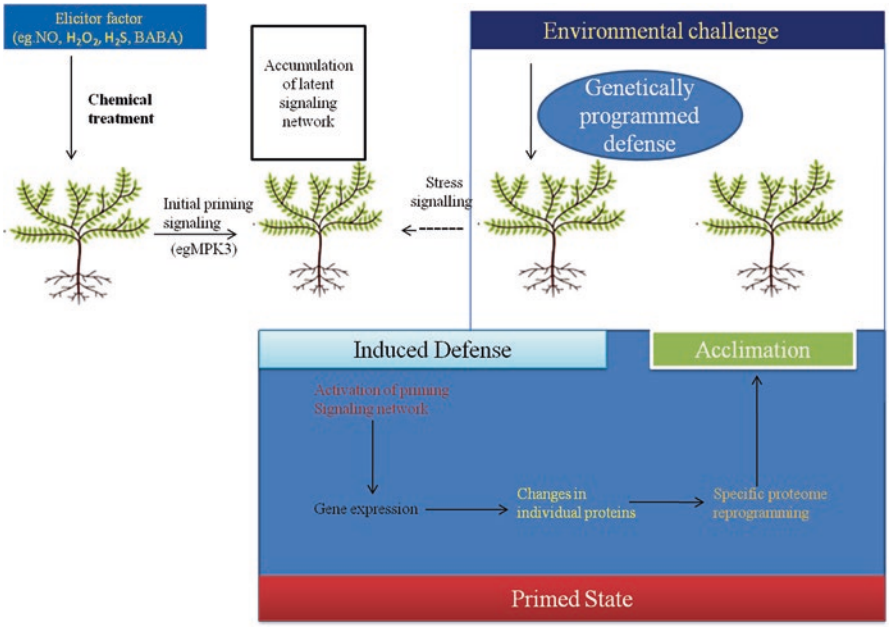

Fig. 3.1 Model depicting the acclimation of plant driven by seed priming in response to environmental stress

3.5 Conclusion

Seed priming emerges as a promising technology for combating abiotic stress in crops and alleviating the detrimental effects of abiotic stress without much affecting its fitness. Drought, salinity, submergence, and extreme temperature (heat, cold) are major challenges in agriculture. These abiotic stresses have similar effects at biochemical, cellular, and molecular levels, and somehow, they are interconnected and activate similar signaling cascades. It is established that seed priming affects signaling pathway at an early stage of growth and development in a crop that results in better tolerance against abiotic and biotic stress. Experimental results revealed that improved germination and vigorous seedling growth occur in primed seed by the mobilization of reserve food and activation of genes responsible for the synthesis of vital enzymes. Priming is also capable of repairing damage which occurs inside the seed. Seed priming has an effect on early stage of germination, and it modulates the DNA replication, transcription, and translation. There is a need to standardize suitable priming methods in different crops to combat abiotic stress in a sustainable manner.

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Recent Advances in Abiotic Stress Tolerance of Plants Through Chemical Priming: An Overview

4

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Abstract

Plants under natural conditions often face multiple environmental constraints in terms of submergence, temperature extremes, salinity, and drought stress. Plant growth and productivity is negatively influenced by these abiotic stresses. Presently, a variety of approaches are being used to overcome abiotic stresses in plants. Recently, seed soaking with various priming agents has emerged as a promising strategy to induce tolerance in plants against abiotic stresses. In seed priming, seeds are treated with synthetic or natural compounds prior to germination so as to initiate specific physiological state in plants. Seed priming could also be defined as physiological state which enables plants to more quickly respond to abiotic stresses. Plants raised from seeds treated with various priming agents tend to show greater abiotic stress tolerance over unprimed seeds. Induction of abiotic stress tolerance through priming is an intricate process that involves various metabolic events. Primed seeds show early and uniform germination and seedling emergence. Seed priming enables plants to survive adverse environmental conditions and gives appropriate yield. In this book chapter, we have discussed a wide range of chemical which are extensively being used for seed priming.

Keyword

Seed preconditioning · Chemical priming · Hormonal priming · Redox priming · Hydropriming

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4.1 Introduction

Stress is called disturbance in equilibrium which produces changes in physiological parameters, and due to stress plant's chemical and physiological changes occur (Gaspar et al. 2002). Plants are growing in continuously under varying environmental conditions which were stressful and unfavorable for plant growth. These stressful circumstances are biotic stress (herbivores, pathogens attack) and abiotic stresses such as cold, heat, heavy metal, nutrient deficiency, salinity, and drought (Fedoroff et al. 2010). The main abiotic strains include heat, cold, salinity, and drought which produced adverse effects on biomass production and yield (Kaur et al. 2008; Thakur et al. 2010), and this creates food insecurity throughout the world. Drought, temperature, and salinity stress produced dehydration and reduced growth of plant and development (Jaleel et al. 2009; Thakur et al. 2010). Abiotic stresses induce excessive (reactive oxygen species) ROS generation. Among ROS, H_2O_2 functions as signal transduction but also reduces photosynthesis and cause cellular damage. The effect of ROS is reduced by antioxidant mechanism, which in turn reduced the effect of stress (Baxter et al. 2013).

4.2 Salinity Stress

Salinity is one of the problems of semiarid and arid regions which reduced the crop yield. Near about one third of the overall word land is affected by salinity (Flowers and Colmer 2008). It is reported that about 65% yield is reduced in wheat because of salinity stress (Ahmad 2012). Excessive buildup of salinity induced osmotic potential lowers the water potential which makes water inaccessible to plants (Munns et al. 2006). Chinnusamy et al. (2005) reported that accumulation of Na^+ and Cl^- cause ion toxicity and ion imbalance which reduce the uptake of other nutrients and reduced growth.

Salinity stress reduces the water potential, and this condition limits plants to uptake water and nutrients from land. Under this situation plants show two types of responses. Firstly, due to excess accumulation of Na^+ in roots, plants face osmotic stress, and second condition involves the increase of ionic stress due to imbalance in essential nutrients (Munns et al. 2006). It is reported that high salt concentration (Na^+ and Cl^-) in soil generate competition between essential nutrients in plants (Munns et al. 2006). High level of Na^+ reduced the influx of K^+ ion which disturbs enzymatic activity, osmotic balance, and stomatal functions. Furthermore, extreme level of Na^+ affects physiological attributes such as fruiting, flowering seed growth, and seed germination (Parihar et al. 2015; Singh et al. 2013). It is reported that ion toxicity in cell increases ROS levels (Parida and Das 2005; Parihar et al. 2015). It is reported by Aldesuquy et al. (2014) that irrigation of wheat with seawater reduced the photosynthetic pigments, light reaction, photosynthesis, and leaf area. In an investigation, it was reported that buildup of salts in barley decreased the photosystem II and photosynthetic activity (Kalaji et al. 2011). Salinity significantly affects plant growth and yield.

4.3 Drought Stress

Drought is termed as duration without substantial rainfall. The existing water reduces, and loss of water occurs through evaporation or transpiration under drought conditions (Jaleel et al. 2007a, b). Shortage of water causes drought that is the most dangerous hazard to food security all over the world. It was the main cause of starvations of past time. Because water supply is limited all over the world, food demand becomes a major problem (Somerville and Briscoe 2001). The harshness of drought stress is unpredictable because it is due to various circumstances like evaporation demand, moisture quality of soil, rainfall distribution, and its occurrence (Farooq et al. 2009). The primary impact of drought on plants is poor establishment and reduced germination (Harris et al. 2002). It was reported by Okçu et al. (2005) that drought stress severely affected the early seedling and germination of five cultivars of pea plants. In another investigation by Kaya et al. (2006), it was studied that drought stress reduced seed stand and its germination. Furthermore, it was examined that under drought stress caused by polyethylene glycol, root length was improved, but it reduced dry and fresh weight of root and shoot, hypocotyl length, and germination potential in alfalfa species (Zeid and Shedeed 2006). It was studied that plant development and growth in vegetative phase was greatly reduced under drought stress in rice (Manickavelu et al. 2006). It was examined by Samarah (2005) that grains and grain weight, spikes per plants, and number of tillers were reduced under drought stress in barley (*Hordeum vulgare*). Nam et al. (2001) reported that drought stress reduced about 40–55% seed yield in pigeon pea. Water relation of plant was disturbed due to disturbance in transpiration rate, canopy temperature, leaf temperature, stomatal resistance, water potential of leaf, and leaf water content. Due to accumulation of dry matter, relative water content of wheat plant leaves was reduced (Siddique et al. 2000). It was reported by Siddique et al. (2000) that relative water content was reduced in stressed plants as compared to nonstressed one.

It was studied by Nerd and Nobel (1991) that total water content was reduced to 57% in cladode (*Opuntia ficus-indica*) under drought stress. Egilla et al. (2005) reported that drought stress reduced the stomatal conductance, turgor potential, transpiration, and relative water content in *Hibiscus rosa-sinensis*. It was concluded that under water stress condition its water use efficiency was greater than nonstressed plants; they correlate it with closure of stomata and reduction of transpiration as reported by Abbate et al. (2004). It was reported that under drought stress the water use efficiency increased under well-watered conditions in lucerne (*Medicago sativa*) as investigated by Lazaridou and Noitsakis (2003). Water availability was reduced due to drought stress which restricts the uptake of nutrient and reduced tissue concentration in plants (McWilliams 2003). Different plant species showed different responses under drought stress; generally moisture stress showed decrease in P and increase in N, but K was unaffected (Garg 2003). McWilliams (2003) reported that K and N uptake was restricted in cotton under drought stress. Similarly, PO_3^{-3} and P uptake was also reduced under drought in plant tissues because moisture accessibility is low as reported by Peuke et al. (2002). It was investigated by Wahid

et al. (2005) that drought stress reduced the efficiency of photosynthesis which in turn decreased food production and senescence of premature leaves and disturbed photosynthetic machinery and leaf expansion. It was also investigated that drought stress affects the photosynthetic machinery (Anjum et al. 2003) which disturbed the production of photosynthetic components and pigments (Fu and Huang 2001) and reduced crop yield by damaging Calvin cycle function (Monakhova and Chernyad'ev 2002). It was investigated by Bota et al. (2004) that drought stress decreased photosynthesis by decreasing the enzymatic activity of Rubisco. Plants undergo changes in different physiological or biochemical (antioxidant defense, cell membrane stability, plant growth regulators, osmotic adjustment, morphological (escape, avoidance, and phenotypic flexibility) and molecular mechanisms (aquaporins, stress protein), and other approaches to tolerate drought and induction of drought resistance through seed priming with plant growth regulators, osmoprotectants, and other important priming agents is also encourgaed (Farooq et al. 2009).

4.4 Heavy Metal Stress

Heavy metal was defined as a metal which have a gravity higher than 4 (Nagajyoti et al. 2010) based on dictionaries and encyclopedia. Nagajyoti et al. (2010) reported that heavy metal phytotoxicity in plants varies with taxonomical groups, and they are mainly derived naturally from geochemical material. Pollution of heavy metals may arise naturally (forest fires, spontaneous combustion, volcanic outgassings, surface mineralization, etc.) or may occur by human action (agriculture and mining). Man produced heavy metal contamination through agricultural activities like use of pesticides, automobiles, municipal incinerators, generator stations, mills, mines, metal smelters, fertilizers, and refineries (El-Ramady et al. 2015). Heavy metals were categorized in to two groups named as nonessential and essential nutrients for plant normal growth and development. The essential micronutrients were Cu, Zn, Ni, Mo, Mn, Fe, and Co which work as electron transfer, redox reaction, and other metabolic procedures, whereas nonessential nutrients were Hg, Cd, Pb, Cr, and As, and these were much toxic for plants (Sebastiani et al. 2004; Rai et al. 2004). Metals related with bioactivity were divided into two groups based on physiochemical property: (I) redox metals, i.e., Mn, Cr, Fe, and Cu, and (II) non-redox metals like Zn, Al, Hg, Ni, and cadmium (Valko et al. 2005; Jozefczak et al. 2012). The first grouped metal members generate oxidative injury in plants through two methods: one was Haber-Weiss method and second was Fenton reaction, and this leads to production of reactive oxygen species (ROS) in plants which produced homeostasis disruption in cell, breakage of DNA strand, protein defragmentation, damage to the photosynthetic apparatus, and ultimately cell death (Schutzendubel and Polle 2002; Flora 2009). Furthermore, second group metal members indirectly influence plant through various mechanisms like depletion of glutathione and sulfhydryl group binding (Valko et al. 2005), inhibiting the activity of antioxidants and produced ROS which damage plant (Bielen et al. 2013).

It is well documented that Cr is a toxic agent for the growth and development of plants (Panda and Choudhury 2005; Mohanty and Patra 2013). It is reported by Singh et al. (2013) that chromium also caused pollution in environment. Chromium is found in two forms, i.e., hexavalent Cr^{6+} and trivalent Cr^{3+}. Chromium metal is not uptaken directly or absorbed by the plants; it enters into cell through specific ion carriers, e.g., iron or sulfate (Singh et al. 2013; Gajalakshmi et al. 2012). Chromium is mostly accumulated in root part instead of other parts of plants (Kumar and Maiti 2013), because it is immobilized in plant cell vacuole so mostly it builds up in roots of the plant (Oliveira 2012; Nematshahi et al. 2012). Chromium mostly showed effect in early stages of plants and reduced the seed dry matter and stem development (Nematshahi et al. 2012). It is reported by Shanker et al. (2005) that chromium stress constrains the elongation and cell division in roots and reduced the length and also shortens the root system. In another report it was investigated that chromium toxicity disturbed cell cycle in roots and decreased shoot length by restricting the nutrient and water absorption process (Srivastava and Jain 2011). It was investigated that seed of cowpea (*Vigna sinensis* L.) showed significant reduction in germination characteristics by reducing total sugar and amylase activity when treated with different amount of chromium (Cr^{6+}) (Nath et al. 2008). It was reported that chromium interfered with other metal ions and buildup of extensive range of various nutrients, i.e., Ca, Mn, P, K, and Fe in root and shoot of plant and reduced plant growth (Samantaray et al. 1998).

Plants have various interrelated and sophisticated mechanisms to combat heavy metal stress. First line of defense against heavy metal toxicity was physical barriers; i.e., trichome tissues, thick cuticle, mycorrhizal association, and cell wall were activated when plant confronted with heavy metal stress (Hall 2002; Wong et al. 2004; Harada et al. 2010). It was reported that trichomes worked as heavy metal storage organs to detoxify the effect of metals by secreting secondary metabolites (Lee et al. 2002; Hauser 2014).

Under heavy metal stress, plant produced low-molecular-weight protein known as chelators or metallochaperones, i.e., mugineic acid, spermine, putrescine, nicotianamine, metallothionein, phytochelatin, organic acid, and glutathione or some cellular exudates, i.e., heat shock protein, protons, phenolics, flavonoids, and various amino acids like histidine, proline and hormones, i.e., ethylene, jasmonic acid, and salicylic acid (Viehweger 2014; Dalvi and Bhalerao 2013; Sharma and Dietz 2006). When plant was unable to combat with heavy metal stress with the above-mentioned strategies, then plant produced reactive oxygen species (Mourato et al. 2012). To alleviate the adverse effects of ROS radicals, plants have defensive system in terms of antioxidants such as glutathione (GSH), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), proline, tocopherol, alkaloids, carotenoids, and other phenolic compounds such as lignin, tannin, and flavonoids. These all scavenged ROS radicals (Sharma et al. 2012; Michalak 2006; Rastgoo et al. 2011)

4.5 Temperature Stress

When temperature was raised from the threshold level which damaged plant growth and its development, then it is called as heat stress. When this temperature raised up to 10–15 °C above ambient temperature, then heat shock occurs (Wahid et al. 2007a, b). High-temperature stress is the cause of decline in crop yield all over the world (Hall 2001). High-temperature stress directly damages the crop by denaturing and aggregating the protein, and indirect injury includes protein degradation and inhibition and degradation of enzymes of mitochondria and chloroplast, and its membrane integrity was also lost (Howarth 2005). These damages produced reactive oxygen species and toxic compounds and reduced ionic influx which leads to growth reduction (Howarth 2005). High-temperature stress causes diverse and adverse effects on plant development, growth, yield, and physiological processes (Hasanuzzaman et al. 2012, 2013). Under high temperature physiological attributes were badly disturbed such as fruit damage, root and shoot inhibition, leaf scrolling and senescence, and abscission which in turn become a cause of decrease in crop productivity (Vollenweider and Günthardt-Goerg 2005). High stress exposure induced molecular level disturbance in plants such as changing gene expression and transcripts accumulation, which lead to synthesis of stress-related genes (Iba 2002). High temperature reduces the dry weight of crop by decreasing plant growth net assimilation rate of shoot as investigated by Wahid et al. (2007a, b). Barnabás et al. (2008) reported that due to high temperature respiration and photosynthesis of plant were disturbed which reduced plant productivity. The initial impact of heat stress on plant was the degradation of enzymes and altered chloroplast protein structure as examined by Ahmad et al. (2010). It was reported that heat stress decreased respiration and photosynthetic activity as well as deterioration of chlorophyll (Todorov et al. 2003; Zhang et al. 2010). It was reported by Adams et al. (2001) that plant induced different mechanisms to tolerate heat stress, such as induction of long- and short-term changes in morphological and molecular changes like cooling, transpiration, leaf orientation, and changing of lipid membrane composition.

4.6 Priming Agents

There are a number of priming agents and/or methods including solid matric priming, redox priming, biological priming, hormonal priming, chemical priming, osmopriming, hydropriming, etc. Seed priming increases germination and growth especially under environmental constraints (Jisha et al. 2013). However, the degree of efficacy of different priming agents varies with plant species and diverse environmental conditions (Iqbal and Ashraf 2005).

4.7 Seed Priming with Nutrients

Seed priming with nutrients is an innovative practice that holds positive influences in terms of enhanced nutrient supply (Al-Mudaris and Jutzi 1999). Seeds are soaked in solutions with limited nutrients, whereas for comparison seeds are also soaked in water (Arif et al. 2008).There exists evidence in the literature that plants with better nutrient supply possess greater potential to tolerate abiotic stress (Marschner 2011). Among different mineral nutrients, potassium is more prominent because it enables plants to survive under different environmental stresses (Cakmak 2005). In this context, seed soaking in Zn^{2+} improved yield in wheat and chickpea (Arif et al. 2007). Ascorbic acid is vitamin C and acts as an important antioxidant. Ascorbic acid is also a promising priming agent (Jisha et al. 2013). A compelling evidence is available in the literature that advocated the requirement of greater endogenous levels of ascorbate for maintaining antioxidant defense system so as to protect plants from oxidative damage (Zhou et al. 2009). For instance, pretreatment with ascorbic acid increased germination in *Agropyron elongatum* when subjected to salinity stress (Tavili et al. 2009). Likewise, salt tolerance in maize has been induced through seed soaking in distilled water or $CaCl_2$, KCl, and NaCl (200 meq·L^{-1}). Plants raised from primed and unprimed seeds were subjected to varying concentrations of NaCl salinity (0, 100, and 200 mM) for 14 days. The results indicated the positive effects of all priming agents on salt tolerance of maize. However, seed priming with $CaCl_2$ was more effective in the induction of salinity tolerance in maize in terms of improved germination percentage and plant biomass. Plants raised from primed seeds also had elevated cellular levels of Ca^{2+}, K^+, and Na^+. Chloride contents were greater in maize plants raised from seeds soaked in NaCl and KCl (Ashraf and Rauf 2001). In a study, seed soaking with KCl, $CaCl_2$, and NaCl (100 mM) alleviated the negative effects of salt stress in wheat under field conditions. Of different priming agents, priming with $CaCl_2$ was found to be more effective because of greater plant biomass and grain yield in two wheat cultivars (Inqlab-91 and MH-97). Seed soaking in $CaCl_2$ also mitigated the inhibitory effects of salinity on hormonal balance in wheat cultivars. In wheat cultivar, namely, MH-97, seed pretreatment with $CaCl_2$ enhanced endogenous levels of salicylic acid (SA) and decreased leaf ABA (abscisic acid) contents under saline or nonsaline conditions. Conversely, $CaCl_2$ priming in cv. Inqlab-91 increased IBA (indolebutyric acid) and (indoleacetic acid) contents. Furthermore, seed-soaking treatment did not cause any change in cellular levels of polyamines. However, plants from seeds treated with $CaCl_2$ had lower levels of spermidine in both wheat cultivars under salinity stress. In addition, seed priming with KCl improved plant growth in Inqlab-91, whereas plants of MH-97 wheat cultivar remained unaffected under salinity stress. Interestingly, NaCl priming mitigated salt-induced decline in auxins (IBA and IAA) in wheat plants under salinity stress. NaCl priming decreased putrescine and SA and increased leaf ABA contents

in wheat cultivar Inqlab-91 under salinity stress. The results suggested that all priming agents were effective in improving plant growth, but the influence of seed priming on hormonal balance was not the same in two wheat cultivars (Iqbal et al. 2006). Iqbal and Ashraf (2007) studied the influence of seed soaking in $CaCl_2$, KCl, and NaCl (100 mM) in salt tolerance of wheat cultivars, namely, MH-97 and Inqlab-91. Both unprimed and primed seeds were grown in field with 15 dS $m^{-1}$of salinity. Seed soaking in $CaCl_2$ was better followed by KCl and NaCl in mitigating the inhibitory effects of salinity on photosynthesis, plant growth, and grain yield. Endogenous levels of Na^+ were significantly lower in plants raised from seeds treated with different priming agents. Despite the positive effect of priming agents on concentrations of ions and photosynthesis, the influence of priming agents was variable in two wheat cultivars.

A study by Anosheh et al. (2011) studied the effect of seed priming with KNO_3 and urea on seedling growth, germination, and proline and protein content in four maize hybrids under salt and drought stress. Plants were subjected to severe and moderate salt and drought stress and control (without stress). Seed priming with urea improved germination percentage, germination rate, and seedling length, whereas seed soaking with KNO_3 increased root length. Seed priming with either of priming agents had no influence on proline contents. Authors concluded that priming with urea and KNO_3 improved abiotic stress tolerance of maize hybrids and recommended that priming could increase plant growth of maize under environmental constraints. Likewise, Jafar et al. (2012) studied the performance of wheat cultivars (MH-97 and SARC-1) under salinity stress. These authors soaked seeds of two wheat cultivars in solutions containing $CaCl_2$, ascorbate, kinetin, and salicylic acid (50 mg L^{-1}) for 12 h. Authors also soaked seeds in distilled water for comparison. In this experiment, plants from unsoaked seeds acted as control plants. Among different priming agents, plants raised from seeds treated with $CaCl_2$ were more effective in terms of stand establishment, harvest index, 1000-grain weight, grains per spike, and number of fertile tillers. These results with $CaCl_2$ seed priming were closely followed by ascorbate in both wheat cultivars. There was decrease in Na^+ and increase in K^+ contents in wheat plants raised from seeds treated with priming agents, and this effect was more pronounced with $CaCl_2$ priming. Plants raised from seeds soaked in $CaCl_2$ had greater values for activities of protease and α-amylase, total soluble protein, and phenolic contents, and this trend was closely followed by seed priming with ascorbate. Authors concluded that priming with $CaCl_2$ is more economical from benefit-to-cost ratio viewpoint. Furthermore, authors found greater yield in wheat plants raised from seeds treated with $CaCl_2$, whereas kinetin was inferior in this context. Likewise, seed priming with potassium nitrate significantly influenced germination percentage and emergence in two soybean cultivars (Gorgan-3 and Sahar) under salinity stress. Cultivar Gorgan-3 performed better over cv. Sahar. Seed priming with KNO_3 increased emergence, germination percentage, plumule and radical length, plant dry weight, leaf area, plant height, and seedling dry weight under salinity stress as compared to plants raised from unprimed seeds (Ahmadvand et al. 2012).

4.8 Osmoconditioning

Osmopriming or osmoconditioning is the seed soaking in solutions with low water potential (Jisha et al. 2013). In osmopriming, degree and rate of imbibition is restricted through the exposure of seeds to low external water potential. Osmopriming is analogous to lengthy initial imbibition of seeds that induces metabolic activities even before germination. Osmopriming enables researchers to study alteration in seeds from physiologically inactive dry state to physiologically active hydrated state (Chen and Arora 2011). Researchers employ a wide range of chemicals in order to generate solutions with low water potential. In this context, PEG (polyethylene glycol) is nontoxic with large molecular size compound that decreases water potential of the solution without influencing seeds during soaking (Thomas et al. 2000). Furthermore, $MgSO_4$, KH_2PO_4, K_3PO_4, KCl, KNO_3, mannitol, NaCl, $CaCl_2$, etc. are other chemicals used to create solutions with low water potential (Jisha et al. 2013). Osmopriming financially, technically, and methodologically is more challenging as compared to hydropriming (Moradi and Younesi 2009) as osmopriming yields easier and faster results and economical than water conservation systems. In addition, osmopriming provides farmers with very smart substitute to improve crop yield (Foti et al. 2008).Osmopriming can maintain the integrity of plasma membrane and gives better germination percentage over hydropriming. This effect of osmopriming is attributed to prolonged precise seed hydration (Jett et al. 1996). Osmopriming of rice seeds increased starch hydrolysis to improve sugar availability of embryo and produced strong seedling growth. Furthermore, osmopriming also improved yield and yield quality parameters (Jisha et al. 2013).

In Bromus seeds, osmoconditioning with PEG increased germination percentage under drought and salt stress (Tavili et al. 2011). PEG osmopriming of *Trifolium alexandrinum* seeds increased seedling growth and germination percentage (Rouhi et al. 2010). PEG osmopriming also produced positive results in soybean (Khalil et al. 2001). Osmopriming of spinach seeds with PEG increased germination percentage, stimulated antioxidant defense system, and thereby induced tolerance to spinach plants (Chen and Arora 2011). Similarly, greater germination percentage was noted in plants raised from seed soaked in water for 12 h or osmoprimed with PEG for 24 h under cold soil environment (Elkoca et al. 2007). Likewise, osmopriming in adequate concentration of PEG improved seedling growth and germination as compared to hydropriming in rice (Sun et al. 2010). In another study, germination varied differentially in *Festuca ovina*, *Agropyron elongatum*, *osmopriming*, *Festuca arundinacea*, and *Bromus inermis* due to seed priming with PEG or hydropriming. Authors concluded that osmopriming with PEG was superior to hydropriming (Rouhi et al. 2011).

Plants produced from seeds osmoprimed with NaCl increase osmotic adjustment ability of plants due to greater endogenous Na^+ and Cl^- contents in roots of primed seeds. In addition, plants raised from primed seeds had greater organic acids and sugar contents of leaves as compared to non-primed seeds (Cayuela et al. 1996). Moreover, seeds soaked in NaCl increase the chances of direct germination from

these seeds when sown under saline conditions (Cuartero and Fernández-Muñoz 1998). The better performance of plants from osmoprimed seeds with NaCl could have been due to accumulation of germination metabolites, metabolic repair courses, and osmotic adjustment during treatments (Haghpanah et al. 2009). Likewise, sugarcane osmoconditioning with NaCl is an attractive pregermination exercise to enable plants to tolerate drought and salinity stress (Patade et al. 2009). Seed osmopriming with NaCl enhanced seedling emergence, seed germination, and plant growth in canola and chickpea (Farhoudi and Sharifzadeh 2006; Sarwar et al. 2006). In a study, NaCl seed priming stimulated antioxidant system and decreased lipid peroxidation in *Momordica charantia* and *Zea mays* (Yeh et al. 2005; Randhir and Shetty 2005). Similarly, salt priming increased salinity tolerance of wheat in terms of improved metabolite reserves, seedling vigor, and K^+ and Ca^{2+} contents along with decrease in Na^+ contents (Afzal et al. 2005). Seed osmopriming with lower dose of NaCl alleviated the inhibitory effects of salinity on mung bean plants in terms of greater values for osmolytes accumulation, chlorophyll contents, and better antioxidant defense system and osmotic adjustment (Saha et al. 2010). Similarly, melon plants produced from seeds treated with salt primed seeds had greater tolerance to salinity stress. Moreover, osmopriming with NaCl also aided in plant establishment in saline medium. Furthermore, salinity tolerance of melon plants is attributed to increased levels of Ca^{2+} and K^+. Plants raised from primed seeds also accumulated more organic solutes that improved osmotic adjustment (Sivritepe et al. 2005). In another study, osmopriming with NaCl also improved salinity tolerance of sunflower through increasing cellular levels of Ca^{2+} and K^+. Plants also showed better osmotic adjustment in terms of greater proline accumulation (Bajehbaj 2010). In a medicinal plant, *Silybum marianum* priming with GA3 and NaCl increased germination percentage and plant biomass under salinity stress. Authors recommended seed priming with NaCl to get better yield under environmental constraints (Sedghi et al. 2010).

Osmopriming with mannitol and hydropriming alleviated the inhibitory effects of salinity and drought on plant growth in chickpea. Plants produced from seeds soaked in different concentrations of mannitol (2% and 4%) improved biomass and length of shoot and roots as compared to plants from non-primed seeds under saline conditions (Kaur et al. 2005). Likewise, hydropriming and mannitol osmopriming increased seedling growth and germination in alfalfa under salinity stress. Plants from seeds primed with mannitol had higher activities of antioxidant enzymes (SOD, POD, and CAT) and minimal electrolyte leakage and malondialdehyde (MDA) contents. Authors concluded that mannitol priming could prevent alfalfa plants from salinity stress damages (Amooaghaie 2011).

A number of other chemical are also used for the purpose of osmopriming. For instance, osmopriming with KNO_3 increased germination in water melon (Demir and Mavi 2004). Similarly, seed soaked in KNO_3 enhanced germination index, germination percentage, shoot and root fresh and dry masses, and lengths in tomato (Nawaz et al. 2011). Likewise, priming of rice seeds with $CaCl_2$, KCl, and ascorbate improved germination percentage and seedling growth. Plants obtained from primed

seeds showed higher length and plant fresh and dry masses. Plants from primed seeds had significant alterations in calcium and nitrogen homeostasis of seedlings that was correlated with improved α-amylase activity and decreased levels of reducing sugars (Jisha et al. 2013). Similarly, osmopriming with KNO_3 increased seedling emergence in tomato (Ozbingol et al. 1998), sorghum (Moradi and Younesi 2009), and leek (Brocklehurst et al. 1984). In another study, seed osmopriming with KNO_3 in sunflower increased seedling vigor and seedling growth and increased germination. Seed soaking with KNO_3 also improved seedling growth in cucumber (Singh and Rao 1993; Ghassemi-Golezani and Esmaeilpour 2008).

4.9 Hydropriming

Hydropriming is a nontoxic, economical, and simple technique that improved plant growth and production under environmental constraints in terms of improved seedling establishment and osmotic adjustment (Kaur et al. 2002). In hydropriming, seeds are soaked in distilled water at room temperature, and seed imbibition determines length of hydropriming (Kaya et al. 2006). It is evident from the literature that soaked seeds must be properly dried because inadequate drying is harmful (Thomas et al. 2000). Soaked seeds are brought to their original weight through proper drying under shade (Jisha et al. 2013). Hydropiming initiates physiochemical alterations in seeds prior to germination (Basra et al. 2003). In addition, protoplast in hydroprimed seeds has greater permeability to nutrients and water, lesser viscosity, and greater resistance to dehydration (Jisha et al. 2013). Plants produced from hydroprimed seeds had greater water uptake which is positively associated with seedling growth (Yagmur and Kaydan 2008). Evidence is available in the literature which indicates that hydropriming is advantageous over non-primed seeds. Plants from seeds soaked in water showed three- to fourfold increase in plant growth as compared to plants from untreated seeds under drought conditions (Kaur et al. 2002). Hydropriming is inexpensive and simple method to improve abiotic stress tolerance of plants (Jisha et al. 2013). Some researchers have also found hydropriming as an excellent method to produce better germination and seedling establishment in different grain crops (Abebe and Modi 2009).

Seed soaking in water improved germination, seedling establishment, and yield in different crops under contrasting environmental conditions (Rashid et al. 2006). Likewise, hydropriming improved seed germination in onion (Caseiro et al. 2004). Hydropriming also improved germination percentage in cauliflower (Jisha et al. 2013). Hydropriming was most effective among various priming agents for mustard (Srivastava et al. 2010a). Similarly, hydropriming in barley is recommended to the farmers in NWFP of Pakistan and in other regions of the world with same environmental conditions. Hydropriming improved seedling emergence and growth in melon (Sung and Chiu 1995). Furthermore, hydropriming also increased germination percentage in coriander (Rithichai et al. 2009), pyrethrum (Li et al. 2011), *Allium porrum* (Ashraf and Bray 1993), and desert cacti (Dubrovsky 1996).

Hydropriming resulted in increased seedling establishment and germination percentage in mung bean plants as compared to that in non-primed seeds. Plants produced from hydroprimed seeds yielded 80% greater above ground plant biomass, 415% additional grain yield, and 264% additional pod yield, whereas non-primed seeds were inferior in this regard (Rashid et al. 2004). Likewise, hydropriming coupled with proline increased germination percentage and plant growth in *Vigna radiata* under temperature stress (Posmyk and Janas 2007). Authors concluded that plants pretreated with proline had minimal lipid peroxidation that in turn maintained the integrity of membranes under chilling stress. Moreover, proline also acts as carbon and nitrogen source and thereby seedling regeneration and growth (Posmyk and Janas 2007).

4.10 Hormonal Priming

The use of plant hormones and other plant growth regulators as seed presowing treatment can improve plant growth under stressful conditions (Jisha et al. 2013). Of various plant hormones, ABA (abscisic acid) mediates plant responses to a number of abiotic stresses such as osmotic, low-temperature, and drought stress (Fujita et al. 2006). Apart from the involvement of ABA in the upregulation in the expression of stress-related genes, exogenous application of ABA improves salinity tolerance of plants (Xiong and Zhu 2002). Evidence is present in the literature that indicates the wide range of genes induced by ABA (Jisha et al. 2013). Among ABA-induced genes, some genes encode different signal transduction pathways including transcription factors, protein kinases/phosphatase, and putative receptors that could improve salinity tolerance of plants (Xiong and Zhu 2002). Mustard plants produced from ABA-primed seeds showed higher rate of seed germination as compared to non-primed seeds (Srivastava et al. 2010a). ABA-primed seeds of canola showed greater germination percentage as compared to non-primed seeds under low-temperature, drought, or salt stress (Gao et al. 2002).

There are a number of reports in the literature where seed soaking in GA3 (gibberellic acid) has been shown to improve germination (Khan et al. 2002; Jisha et al. 2013). In a study, on cotton and faba beans, GA3 enhanced Ca and K when applied as presowing seed treatment (Harb 1992). Akbari et al. (2007) studied seed priming with auxin in wheat crop where auxin priming increased hypocotyl dry weight, hypocotyl length, and seedling fresh and dry weight. Similarly, priming with plant growth regulator GA3 and IAA improved germination in pyrethrum under nonsaline regime (Bisht et al. 2009). Ascorbic acid priming gave better results in wheat over other priming agents such as GA3, kinetin, ascorbic acid, and salicylic acid (Khan et al. 2011). Likewise, seed priming with salicylic acid enhanced germination in fennel under low water potential (Farahbakhsh 2012). Seed soaking with different growth regulators including ethephon, kinetin, thiourea, and fusicoccin mitigated the negative effects of salt stress on germination in a halophyte, namely, *Salicornia utahensis*, while nitrate, betaine, and proline priming did not

significantly influence germination at varying salinity levels (Gul and Khan 2004). Likewise, priming with 3 μM methyl jasmonate and 3% KNO_3 enhanced germination in dormant seeds of *Amaranthus cruentus* L. (Tiryaki et al. 2005). In another study, seed priming with ABA and GA increased activities of antioxidant enzymes over plants from unprimed seeds under drought conditions (Eisv and et al. 2010). Seed priming with vitamin and hormone improved root architecture (Shakirova et al. 2003). Priming with growth regulators might increase cell division in apical meristem of root and thereby increase plant growth. Furthermore, hormonal priming cause increase in tissue levels of cytokinin and IAA which in turn improve plant growth (Shakirova et al. 2003).

4.11 Chemical Priming

Literature indicates the use of diverse chemicals in different crop species. Tolerance in crop plants has been reported in response to seed priming with synthetic or natural compounds such as chitosan, choline, paclobutrazol, putrescine, ethanol, KH_2PO_4, $ZnSO_4$, $CuSO_4$, selenium, and butenolide (Jisha et al. 2013; Demir et al. 2012).

4.12 Seed Priming with Chitosan

Chitosan, a large polysaccharide cation, is extracted from seafood waste materials. Seed preconditioning with chitosan increased tolerance in plants against a number of diseases, and moreover, increased yield quality, IAA and GA3 contents, lipase activity, and germination percentage (Shao et al. 2005; Jisha et al. 2013). For instance, chitosan treatment in wheat improved tolerance against various diseases and improved seed quality (Bhaskara Reddy et al. 1999). Likewise, Ruan and Xue (2002) reported increase in seed germination and stress tolerance in rice plants in response to chitosan. Similarly, chitosan priming improved seedling vigor in maize (Shao et al. 2005). In another study, Guan et al. (2009) reported that seed priming with chitosan increased germination and plant growth in maize under low-temperature stress.

4.13 Seed Priming with Choline

Supplementation of choline induces tolerance in plants against salinity stress by improving cellular levels of glycine betaine (Su et al. 2006). Choline is an integral component in the biosynthesis of glycine betaine in plants (Sakamoto and Murata 2002). In addition, choline is an important component of phosphatidylcholine which is a conspicuous part of membrane phospholipids in plants (Rathinasabapathi 2000). In another study, seed priming with choline chloride improved salinity tolerance ability in wheat plants (Salama et al. 2011).

4.14 Paclobutrazol, Putrescine, and Ethanol

A study by Farooq et al. (2006) reported better germination and leaf score as a result of seed priming with 1% or 5% ethanol. Similarly, seed priming with putrescine improved seed germination and growth as a result of better antioxidant system in tobacco under chilling stress (Xu et al. 2011). Likewise, seed priming with paclobutrazol in *Catharanthus roseus* resulted in better salt tolerance in terms of stimulated antioxidant system (Jaleel et al. 2007a, b). In another study, seed preconditioning with paclobutrazol improved modulated growth and key physiochemical processes in turf grass under drought stress. However, this effect of paclobutrazol is associated with plant species and levels of paclobutrazol (Shahrokhi et al. 2011).

According to Shahrokhi et al. (2011), paclobutrazol seed priming in *F. arundinacea* L. (turf grass) affected growth and physiological traits of plants during drought stress, but it related to concentration of paclobutrazol and the nature of the cultivar.

4.15 Seed Preconditioning with $CuSO_4$, KH_2PO_4, $ZnSO_4$, and Selenium

Selenium improves plant growth and stress tolerance potential of plants when applied in lower concentrations (Mirza et al. 2010). Likewise, priming with selenium protected plants from temperature-induced oxidative injury by stimulating the antioxidant defense system of plants (Chen and Sung 2001). Seed preconditioning with $ZnSO_4$ and $CuSO_4$ enhanced seed germination as well as seed emergence in maize plants (Foti et al. 2008). Likewise, seed priming with KH_2PO_4 improved germination and seedling growth in wheat (Korkmaz and Pill 2003). It has been reported that seed priming with lesser levels of KNO_3 and K_2HPO_4 gave better results as compared to greater levels of these priming agents (Sarwar et al. 2006). Likewise, seed priming with KH_2PO_4 enhanced seedling growth and germination percentage in triticale (Yagmur and Kaydan 2008).

4.16 Butenolide

Butenolide is another important chemical priming agent that improves seedling vigor and growth in diverse plant species (Demir et al. 2012). Butenolide is extracted from burnt cellulose and plant-based smoke. Plants raised from seeds treated with butenolide had lower chances of pathogen attack mainly due to rapid emergence of seedling. Moreover, seed treatment with smoke also protects seeds from pathogen attack in seedbed (Kulkarni et al. 2006). Similarly, treatment of this compound produced better results in tomato grown under low-temperature conditions (Jain et al. 2006).

4.17 Redox Priming

Cellular redox state regulates important processes that mediate growth and development under stressful environment. Plants undergo change in redox status due to external stimuli, and the degree of change in redox status of plants depends upon the concentration, duration, and nature of stimulus (Miller et al. 2009). Plants decrease the intensity of stress damage through the maintenance of reduced redox state (Mittler 2002). It has been reported in the literature that foliar application and seed soaking in a number of thiol compounds especially thiourea improved stress tolerance and plant productivity in different plant species, e.g., wheat and mustard (Sahu et al. 2005; Jisha et al. 2013). In another study by Srivastava et al. (2008), seed treatment with thiourea maintained integrity of mitochondria in seedlings under salinity stress that negatively influenced mitochondrial integrity in *Brassica juncea* (Srivastava et al. 2008). Thiourea pretreatment also improved signaling pathways in *Brassica juncea* under salinity stress (Srivastava et al. 2010b).

Reactive nitrogen species like NO and reactive oxygen species (ROS) mediate a large number of cellular processes under stressful conditions including drought, pathogen attack, and heat and cold stress (Hancock et al. 2011). In another study by Yadav et al. (2011), seed soaking in H_2O_2 improved chilling tolerance in capsicum. Seed priming with H_2O_2 enhanced chilling tolerance in *B. juncea* L., and this effect of H_2O_2 priming was further aggravated when combined with 24-EBL (24-epibrassinolide) (Jisha et al. 2013). Similarly, seed treatment with 24-EBL mitigated the inhibitory effects of H_2O_2 by stimulating the antioxidant enzymes such as SOD, CAT, POD, and APX (Kumar et al. 2010). In another study, seed soaking in H_2O_2 mitigated the adverse effects of salinity stress in wheat (Wahid et al. 2007a, b). Similarly, H_2O_2 seed priming enhanced drought tolerance in maize plants (Ashraf et al. 2015b). Likewise, seed priming with NO donors increased germination and seedling growth in pearl millet (Manjunatha et al. 2008). Moreover, H_2O_2 functioned as signaling molecule and thereby improved seed germination through the induction of alterations at hormonal, transcriptomic, and proteomic levels (Barba-Espín et al. 2012). Glutathione, an important compound of the antioxidant pathways in plants, mediates redox signaling networks which directly affect plant growth and defense (Maughan and Foyer 2006). Similarly, seed priming with antioxidant compounds such as tocopherol, glutathione, and ascorbic acid improved seedling vigor in sunflower under low-temperature stress (Draganic and Lekic 2012). Cysteine is not directly involved in redox priming but significantly affects synthesis of GSH (Barba-Espín et al. 2012). Furthermore, seed priming with cysteine alleviated injury due to gamma radiation, and cysteine caused more conspicuous effects in the elongation of primary root (Reddy and Smith 1978). Contrarily, seed priming with L-cysteine failed to prevent methyl methanesulfonate-induced damages to barley seeds except for little protection at higher concentrations (Rubluo 1982).

4.18 Proline As Promising Priming Agent for Plants Under Abiotic Stress

Plants often face multitude of abiotic stresses due to their sessile nature. UV radiation, nutrient deficiency, trace metal toxicity, temperature extremes, water-limited conditions, and salinity are among foremost environmental limitations to crop production worldwide. Plants possess a variety of cellular mechanisms to mitigate negative effects of abiotic stress. For example, a wide range of compatible solutes is accumulated in plants. Of compatible solutes, amino acids such as proline are accumulated in substantial amount. There evidence is still not obvious to explain the relationship of proline accumulation with abiotic stress tolerance of plants. However, as a general consensus among plant researchers, proline accumulation is taken as beneficial for plants particularly after stress recovery (Verbruggen and Hermans 2008; Kaur and Asthir 2015). According to an estimate, plants accumulate 80% proline under abiotic stress in comparison to 5% under normal conditions. This greater accumulation of proline under stress condition is attributed to enhanced biosynthesis and limited degradation of this biomolecule (Szabados and Savouré 2010; Kaur et al. 2017). Proline has been documented as a multifunctional amino acid with diverse functions under stress conditions. Protection of cellular functions through detoxification of ROS, maintenance of membrane integrity, proteins, and subcellular structure are the major functions displayed by this important biomolecule. Functional variation in the metabolism of proline is largely due to compartmentalization of proline degradation and biosynthesis in mitochondria, chloroplast, and cytosol. Proline is involved in the maintenance of redox balance and homeostasis via dissolving additional reduction potential due to saturated electron transport chain under environmental constraint (Kaur and Asthir 2015). Proline plays important role in maintaining plant growth particularly after stress recovery because proline degradation is associated with oxidative respiration where it directs energy to recommence growth. Proline protects plants from damage of trace metals through scavenging ROS, but it may also act as protein-compatible hydrotrope (Busch et al. 2014) thereby mitigating cytoplasmic acidosis and preserving suitable $NADP^+$/NADPH adjusted with the cellular metabolism (Filippou et al. 2014). Proline impacts plant growth and development through its action as metabolic signal mediating metabolite reservoir (Verbruggen and Hermans 2008). Proline also provides protection to plants through the induction of stress-related proteins (Kaur and Asthir 2015; Kaur et al. 2017).

Plants subjected to abiotic stress conditions experience growth retardation. Conversely, supplemented proline improved plant growth through osmoprotection under salinity stress (Roy et al. 1993; Yancey 1994; Savvides et al. 2016). Exogenous application of proline in lower concentration significantly alleviated the inhibitory effects of salt stress in rice (Roy et al. 1993). Similarly, addition of proline to the culture medium increased fresh weight and decreased membrane damage in terms of lipid peroxidation in *Arachis hypogea*. Conversely, when greater levels of proline were added to the medium, it imposed no beneficial effects (Jain et al. 2001). In another study by Ehsanpour and Fatahian (2003), addition of proline to the saline medium improved dry mass and proline levels in *Medicago sativa*. Likewise,

treatment of premature maize embryos with proline induced somatic embryogenesis (Duncan and Widholm 1987). Presowing seed treatment of wheat with proline increased growth and grain yield under drought conditions (Kamran et al. 2009). A study of Ali et al. (2008) reported improved uptake of N, P, Ca^{2+}, and K^{+} in maize plants treated with proline under water-deficit conditions. Likewise, added proline also mitigated negative effects of Cd on cultured tobacco (Islam et al. 2009). In a study by Hua-long et al. (2014), presowing seed treatment with proline increased rice germination under salinity stress. Furthermore, proline priming also increased relative germination energy and relative germination rate under salinity stress. Likewise, proline priming also stimulated amylase activities in rice under salinity stress. These authors concluded that presowing seed treatment with proline effectively alleviated negative effects of salinity on rice germination. In another study, seed priming with proline significantly alleviated the inhibitory effects of drought in wheat under water-deficit conditions (Kamran et al. 2009). Similarly, proline scavenged superoxide radicals by improving the SOD activity in *Solanum nigrum* plants under cadmium toxicity (Xu et al. 2009). In another study, Agami (2014) recorded the influence of presowing seed treatment on growth, leaf anatomy, antioxidant enzyme, electrolyte leakage, relative water content, proline, and chlorophyll in *Hordeum vulgare* L. under salinity stress. Plants grown under saline conditions (100 and 200 mM NaCl) had decrease in growth, soluble sugars, chlorophyll, and relative water content along with significant changes in leaf anatomy. However, proline presowing seed treatment mitigated negative effects of proline and enhanced above stated attributes. Furthermore, salinity stress increased activities of antioxidant enzymes (POX, CAT, and SOD) and endogenous levels of proline and electrolyte leakage. There was a marked variation in leaf anatomy and antioxidant enzymes in plants raised from proline-treated seeds under nonsaline or saline conditions. Likewise, Sadak and Mostafa (2015) studied the impact of seed soaking with proline on growth, key biochemical attributes, and yield in sunflower plants under seawater salinity. Salt stress caused reduction in plant growth, chlorophyll contents, yield, and yield quality. Conversely, seawater salinity increased proline contents, free amino acids, total soluble carbohydrates, and phenolics. Plants raised from proline-treated seeds had greater tolerance to salinity in terms of better growth, chlorophyll content, proline, total soluble carbohydrates, and free amino acids. Authors concluded that priming with proline alleviated the inhibitory effects of salinity on sunflower through improving endogenous levels of chlorophyll and osmoprotectants. Likewise, presowing seed treatment with proline mitigated the negative effects of temperature extremes in wheat plants in terms of improved germination and α-amylase activity (Sultana et al. 2000).

4.19 Glycine Betaine As Potential Priming Agent

A plethora of organisms accumulate GB under abiotic stresses (Chen and Murata 2002; Giri 2011). Plants with natural potential of GB production can grow well under saline and water-limited conditions (Chen and Murata 2008; Giri 2011). Compatible

solutes are small organic molecules highly soluble in water and are not toxic even at higher concentrations (Giri 2011). GB is known to function as compatible solute (Chen and Murata 2008). A number of halotolerant plants accumulate substantial amount of GB in plastids and chloroplasts. GB accumulation is also very high in plants subjected to abiotic stress (Ashraf and Foolad 2007). There existed a positive association between the endogenous levels of GB and degree of abiotic stress tolerance in plants (Rhodes and Hanson 1993). GB protects and stabilizes the quaternary structure of complex proteins and thereby maintains the integrity of cell membranes at high-temperature and salinity stress (Papageorgiou and Murata 1995). The survey of literature showed that plants treated with added GB or plants engineered with GB biosynthesis genes had significant tolerance to environmental constrains (Ashraf and Foolad 2007; Chen and Murata 2008; Giri 2011). Of various compatible solutes, GB provides effective protection to plants against multitude of environmental constraints (Sakamoto and Murata 2000, 2002). Literature showed the effectiveness of this biomolecule in the induction of tolerance against a wide range of stresses during different ontogenic phases (Sulpice et al. 2003; Park et al. 2004).

Literature survey showed that exogenous application of GB markedly mitigated the negative influences of abiotic stresses in plethora of plant species where GB enhanced growth and yield (Chen and Murata 2008). Plants readily take up GB when applied exogenously as foliar spray (Park et al. 2006). Plants can also efficiently take up this biomolecule from roots (Park et al. 2003). Plants normally produce reactive oxygen species (ROS) as products of different metabolic products under nonstress conditions (Chen and Murata 2008). Plants hold an efficient defensive antioxidant system that scavenges ROS and thereby decrease cellular levels of ROS (Chen and Murata 2011). Abiotic stresses such as salinity, drought, and heavy metal stress induce oxidative damage to plants (Ashraf et al. 2015a). Addition of hydroxyl radicals to *Arabidopsis* roots induced concentration-dependent efflux of K^+. However, growth medium with GB (5 mM) significantly reduced efflux of K^+ (Cuin and Shabala 2007). In addition, tomato plants treated with exogenously applied GB markedly decreased the cellular levels of H_2O_2 under chilling stress (Park et al. 2006). GB is not directly involved in the scavenging of ROS, but GB stimulates the activities of antioxidant enzymes or reduces ROS generation through an unknown mechanism (Giri 2011; Chen and Murata 2008). In a study, turfgrass seeds were primed in 5–50 mM GB in order to evaluate the effect of GB on temperature, salinity, and drought stress during germination. It was observed that abiotic stresses more drastically affected daily germination percentage than final germination percentage. *Lolium perenne* L. exhibited better tolerance to temperature, salinity, and drought stress followed by *Festuca arundinacea* Schreb and *Agrostis palustris*. Conversely, Bermuda grass, zoysia grass, and Kentucky bluegrass were found sensitive to abiotic stress. Seed priming with 10 mM GB in Bermuda grass and Kentucky bluegrass enhanced germination percentage under different abiotic stresses. Furthermore, authors concluded that other grasses did not respond significantly to seed-soaking treatment with GB. The results suggested that impact of GB priming is plant, type of abiotic stress, and GB concentration dependent (Zhang et al. 2014).

4.20 Conclusion

Seed priming is an attractive approach to counteract the negative effects of stress in plants against a variety of abiotic stresses such as salinity, drought, heavy metal, and temperature stress. Seed priming is realistic, effective, and smart choice for better plant production. Oxidative stress, temperature extremes, salinity, and drought are associated and often induce similar type of damage. As a result, these multiple abiotic stresses introduce similar kinds of cellular responses and signaling pathways. Seed priming stimulates these signaling pathways earlier and improves plant defense responses. The precise process that governs priming is not well known. However, it is assumed that inactive signaling proteins accumulate in primed cells. When plants from primed seeds face abiotic stresses, such inactive signaling proteins are activated which amplify the signal transduction and result in more intricate response of plants under stress. There is increasing evidence in the literature where seed priming has been proposed as one of the possible solutions to counteract the negative effects of abiotic stresses. However, there are still many gaps where more input is required from researchers around the globe. For instance, whether seed priming also influences changes in physiochemical processes of plants at different growth stages or not. Seed priming agents work the same for different plant species or not. How far important physiochemical processes are linked with seed priming. In nutshell, seed priming acts as important yardstick for the induction of tolerance in plants against wide range of abiotic stresses.

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Seed Priming Technology in the Amelioration of Salinity Stress in Plants

5

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Abstract

A large proportion of the global cultivable land is inflicted by saline conditions. Several popular plants and staple crops cannot be cultivated on these vast stretches of land due to their susceptibility to salt stress. Crops growing under such suboptimal conditions exhibit deteriorated physiological development and compromised yields. Several agro-biotechnology-supported programmes are available to enhance plant salt tolerance. Among them, seed priming or 'pretreatment' is the most acceptable one from the point of biosafety and socio-economic views. Seed priming provides an abiotic stress-like condition to the dormant seed. It partially reprogrammes the seed metabolome so that it experiences such suboptimal condition and can better adapt to salt stress. Partial hydration of the seed during priming weakens the endosperm, channelizes the energy reserves, makes the seed ready for radicle protrusion (germination) and recharges the entire antioxidant machinery. This chapter provides an insight into the multiple mechanisms via which seed priming with various inorganic as well as endogenous agents can ameliorate salinity stress-related damages across multiple plant species.

Keywords

Antioxidative machinery · Protective agents · Seed priming · Salinity stress · Salt tolerance

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5.1 Introduction

Abiotic stresses like salinity, drought, heavy metal toxicity, irradiation, etc. lead to large-scale crop losses throughout the world. Among these environmental challenges, salt stress is most prevalent in arid, semiarid and coastal regions and spreads easily in the irrigated lands (Munns and Tester 2008). The FAO report (2011) highlights that 60–80 million hectares of land are inflicted by salt. This ultimately will lead to the loss of about 50% of the cultivable lands by the twenty-first century. High salt content in the soil or the irrigated water directly interferes with seed germination and seedling growth, thus making most plants susceptible to this kind of abiotic stress (Hubbard et al. 2012). Salt stress delays the advent of germination in susceptible plant seeds (Thiam et al. 2013). An interesting contradiction has been noted in the development of plants in response to low and high salt concentrations (Khan and Weber 2008). It was seen that whereas low salt levels promote seed dormancy, high salt concentrations directly inhibit seed germination. However, both these stress inductions ultimately decrease the germination rate and thus lead to phenotypically retarded development (Khan and Weber 2008). Several crops like *Oryza sativa*, *Zea mays*, *Brassica oleracea*, *Abelmoschus esculentus*, *Vigna unguiculata*, *Apium graveolens*, *Foeniculum vulgare*, *Petroselinum crispum*, *Raphanus sativus*, *Ipomoea aquatica*, *Silybum marianum*, *Lactuca sativa*, *Glycine max*, etc. are reportedly sensitive to a gradient of salt concentrations (Banerjee and Roychoudhury 2016a; Basu and Roychoudhury 2014; Ibrahim 2016).

Esechie (1995) showed that the top 10 cm layer of the soil accumulates higher salt levels than the lower layers. Seeds of cultivated crops are usually sown in this top layer. High evapotranspiration in plants growing in the arid environments results in water loss and accumulation of salt around the roots. This retards translocation and crucial physiological processes (Bernstein and Hayward 1958). Hence, novel strategies are required to ameliorate salt stress in developing crop plants. Transgenic technology has often been adopted to generate genetically modified (GM) plants overexpressing a target gene which confers stress tolerance. However, this technology faces several biosafety issues across multiple countries, and hence such GM plants cannot be popularly marketed. Thus researchers have designed a novel technology called ‘seed priming’ where an inorganic chemical solution or an endogenous osmoprotectant or ‘eliciting factor’ is purified and used as the pretreating agent to make the seeds tolerant to future stress exposures (Tanou et al. 2012). In this technology, the seeds are hydrated in a prescribed solution containing the optimum concentration of the ‘eliciting factor’ and then dried. This improves germination, triggers multiple epigenetic alterations and up-regulates genes encoding stress-responsive transcription factors (TFs) (Farooq et al. 2009; Bruce et al. 2007). The treated seeds reportedly exhibit higher germination and seedling emergence rates under stress conditions in comparison to the non-treated seeds (Sharma et al. 2014). Studies show that seed priming can even improve crop productivity under optimum conditions (Jisha et al. 2013). The popularity of seed priming lies in its easy usage, low cost and lesser environmental risk (Ibrahim 2016).

5.2 Salinity and Seed Germination

Salt stress primarily increases the soil osmotic potential which results in constrained water and solvent uptake via roots (Daszkowska-Golec 2011). The osmotic balance in the plant gets disrupted due to generation of reactive oxygen species (ROS) like hydroxyl radicals, superoxides and hydrogen peroxides (Das and Roychoudhury 2014). Massive oxidative stress caused by Na^+ and Cl^- toxicity jeopardizes macromolecular structures and membrane integrity and even affects embryo development. Physiological processes like photosynthesis, growth, respiration and flowering are severely inhibited by salt stress (Roychoudhury and Chakraborty 2013). The overall systemic deterioration leads to cellular apoptosis coupled with the degeneration of membrane lipids, enzymes and nucleic acids (Banerjee and Roychoudhury 2017a). Peroxidation of membrane lipids produces malondialdehyde (MDA), an important stress marker in plants. Such MDA levels sharply increase in salt-sensitive plants exposed to stress (Das and Roychoudhury 2014).

Salinity-induced ROS accumulation triggers the up-regulation of *osmotic stress responsive* (*OR*) genes and their upstream transcription factors (TFs) in a cultivar-dependent fashion (Roychoudhury et al. 2013; Banerjee and Roychoudhury 2017b). The *OR* gene products confer tolerance in specific cultivars of the crops exposed to salt stress. The salt-tolerant cultivars exhibit higher expression of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), etc. Most of these enzymes restore the cellular oxidative equilibrium by operating through the ascorbate-glutathione cycle (Anjum et al. 2015). Recent studies have also highlighted the massive histone modifications, DNA methylation and chromatin remodelling occurring in signature genomic regions of the plants exposed to salinity (Banerjee and Roychoudhury 2017c). The transposon-associated differentially methylated regions (DMRs) in IR-64 (stress susceptible), Pokkali (salt tolerant) and Nagina 22 (drought tolerant) rice cultivars were closely related to the transcript abundance of the protein-coding genes (Garg et al. 2015). However, close association of the hypermethylated silenced heterochromatin with the small RNAs (smRNAs) was noted (Banerjee et al. 2016). This clarified the existence of a crosstalk among the chromatin methylation status, gene expression and smRNA abundance during salt stress response in rice.

5.3 Seed Physiology and Priming

Seed hydration triggers germination via three stages: imbibition, lag phase and radicle protrusion through the testa (Ibrahim 2016). Priming promotes partial hydration of seeds. This effectively accelerates pregermination metabolism but is not enough to facilitate the transition of a dormant seed towards complete germination (Paparella et al. 2015). Hence priming converts a metabolically naive seed into a quasi-metabolically active unit. However, such quasi-metabolic state does not support the complete emergence of the radicle.

Table 5.1 The different applied seed priming techniques

Seed priming technique	Nature of the treatment
Hydropriming	Water treated
Osmopriming	Aqueous solution of osmolytes like polyamines, etc.
Halopriming	Inorganic salt solution
Hormone priming	Phytohormones like abscisic acid (ABA), salicylic acid (SA), etc.
Hardening	Hydrated seeds are redried
Solid matrix	Solid materials like ground Leonardite Shale (Agro-Lig), etc. mixed with water in known proportions
Humidification and stratification	Seed processing like cold and moist treatments to promote faster germination after sowing
Physical	Irradiation, heat, etc.

The next crucial phase is the post-priming redrying (or drying back) of the seeds to restore their relative moisture content back to the initial control levels. Redrying of the primed seeds at the correct stage is extremely important for seed storage, preserving seed longevity and tolerance towards abiotic stresses (Ratikanta 2011). It has been reported that the partially hydrated seeds in the imbibition or lag phase tolerate redrying without significant physiological deteriorations (Rajjou et al. 2012). However, seeds with already emerged radicles if redried usually exhibit compromised seed vigour (Rajjou et al. 2012). The rate of redrying also regulates seed viability in due course (Gurusinghe and Bradford 2001). Bruggink et al. (1999) stated that the drying back of the primed seeds should be performed slowly as this improves seed longevity and tolerance to desiccation.

5.4 Seed Priming Techniques

The classification of the priming techniques varies with the chemical nature of the priming agent. Eight different priming techniques are usually reported (Ibrahim 2016). They have been highlighted in Table 5.1. Out of the different priming strategies, hydro-, osmo-, halo- and hormone priming are the most popular (Paparella et al. 2015; Maiti and Pramanik 2013). Depending on the technique to be used, other variable parameters also require standardization to gain optimum ameliorative results. These variables include water potential, priming duration, temperature, seed vigour, cultivar and post-priming storage conditions (Maiti and Pramanik 2013).

5.5 Priming-Induced Alterations Which Ameliorate Salt Stress in Susceptible Plants

Priming promotes embryo swelling and accelerates the development of immature embryos. The partial hydration state reduces the physical resistance of the endosperm, improves physiological parameters and leaches out the chemical inhibitors of germination (Bewley et al. 2013). Sadeghi et al. (2011) reported that priming

modifies the seed metabolic balance as a result of which germination and seedling development is more rapid even under suboptimal saline conditions. Such stress tolerance is facilitated by metabolome reprogramming and generation of 'priming memory' in seeds (Pastor et al. 2013). 'Priming memory' is supposedly epigenetic signatures etched within the seed genome during the stress-like conditions created as a result of seed priming (Banerjee and Roychoudhury 2017c). Such epigenetic alterations in the chromatin architecture lead to the overexpression of several stress-responsive genes like *late embryogenesis abundant* (*LEA*), whose protein products confer tolerance towards salt stress (Banerjee and Roychoudhury 2016a; Roychoudhury et al. 2007).

Sharma et al. (2015) showed accelerated germination in the primed seeds. Such improvements could be attributed to specific germination-associated genes which get up-regulated in the primed seeds (Sharma et al. 2015). Several antioxidant genes also exhibit increased expression as the entire metabolic equilibrium of the seed is altered after optimum priming (Sadeghi et al. 2011). Such antioxidants promote seed germination and seedling development by scavenging the toxic ROS and lowering oxidative stress under saline conditions (Kubala et al. 2015). Salt stress imposes large-scale oxidative stress in the plant. If uncontrolled, this can lead to chromosomal damages, protein degradation and metabolite leakage (Netondo et al. 2004). Oxidative stress-induced membrane peroxidation triggers the accumulation of MDA which inhibits the activities of crucial enzymes (Younesi and Moradi 2015). Priming reportedly reverses these degenerative effects of salt stress and facilitates early replication, transcription and chromosomal repair (Roychoudhury and Chakraborty 2013).

The abiotic stress tolerance generated by seed priming is conferred via the synchronization of several physiological, biochemical, systemic, cellular and molecular modulations (Siri et al. 2013). The metabolome reprogramming enables mobilization of energy reserves via endosperm weakening and promotes the expansion and initial development of the dormant embryo (Chen and Arora 2011). This boosts the germination potential of the seed. The activities of several enzymes which facilitate reserve mobilization are enhanced. These are essentially proteases, lyases and amylases (Varier et al. 2010). Proper seedling development is allowed by inducing cell division, elongation, plasma membrane fluidity and stress-responsive proteins like the heat shock proteins (HSPs) and LEAs. Reports have shown alterations in H^+/ATPase activities and even in the transcriptome and proteome of the primed seeds (Ibrahim 2016). Stress tolerance in the primed seeds is also mediated by an increased potential in protein synthesis and post-translational modifications and by maintaining the optimum quotient for the translational turnover (Kubala et al. 2015).

Bakht et al. (2011) reported that seed priming efficiently eliminated the harmful Na^+ and Cl^- ions via activating membrane efflux pumps. On the contrary, the active uptake of inorganic ions facilitates the accumulation of K^+ and Ca^{2+} ions which in turn lowers the cellular osmopotential and promotes water uptake under saline conditions. Apart from these beneficial effects, K^+ ions balance membrane potential and turgor, whereas Ca^{2+} ions maintain the cellular morphology and integrity and mask the growth inhibitory effects of Na^+ ions (Summart et al. 2010; Gobinathan et al. 2009).

A large number of inorganic and organic solutes have been isolated from plants which mediate osmotic adjustments and confer salt tolerance. Solutes like proline (Pro), glycine betaine, free amino acids, soluble sugars, etc. undergo accumulation in the seeds and seedlings after osmopriming. These solutes might also be used as the priming agents to ameliorate salt susceptibility in plants (Roychoudhury and Chakraborty 2013). A chronological representation of the significant priming reagents used across several plant species to generate salt tolerance is presented in Table 5.2. Antioxidant enzymes like SOD, CAT and peroxidase (POX) also exhibit increased ROS scavenging upon seed priming (Nawaz et al. 2012). Compatible solutes like polyamines [putrescine (Put^{2+}), spermidine (Spd^{3+}) and spermine (Spm^{4+})] maintain cellular osmolarity and membrane integrity by chelating out the toxic Na^+ ions (Paul and Roychoudhury 2016; Roychoudhury et al. 2008). Similar antioxidative effects are conferred by seed priming using ascorbic acid and glutathione (Roychoudhury et al. 2012). Imbibition with the universal stress hormone, abscisic acid (ABA), generates a 'stress memory' in the seeds and makes them salt tolerant (Roychoudhury et al. 2009). Priming also induces the accumulation of photoprotective pigments like anthocyanin which exhibit ROS scavenging and plant protection (Banerjee and Roychoudhury 2016b). Overall, the priming strategies utilized to generate salt tolerance reduce MDA content and optimize ROS levels via accumulation of multivariant antioxidants and protective proteins (Nawaz et al. 2012).

5.6 Conclusion and Future Perspectives

Priming is a biologically safe and cheap crop expansion technology which modifies the seed metabolome and makes the tissue ready to tolerate suboptimal conditions like salinity. From the mechanism of stress amelioration by several priming agents (Table 5.2), it can be summarized that they recharge the antioxidant machinery and up-regulate multiple stress-responsive genes (Paul and Roychoudhury 2017). This promotes seed development and germination even under adversely saline conditions. Seed priming is also economically cheap since a small volume of priming solution is sufficient for seed imbibition, and this solution can even be reused. In spite of the huge potential of this technology, little information regarding its molecular mechanisms actually exists. One such perspective is the epigenomic basis of 'stress memory', which is required to be unravelled. Precise concentrations of the priming agents are extremely important for agronomic purposes as unusually high concentrations can cause irreversible damages to the developing seeds. Thus, future investigations revolving around the molecular and metabolomic platforms in this field shall bear credible impacts.

Table 5.2 Some seed priming approaches adopted to ameliorate salinity stress across susceptible plant species

Type	Agent	Concentration/dose	Treatment time	Plant Species	Salt tolerance	Mechanism	References
Halopriming	KNO_3	0.25 mM	–	*Silybum marianum*	^	Improved germination indices and peroxidase activity	Zavariyan et al. (2015)
	Silicon priming (Na_2SiO_3)	30 mM	–	*Triticum aestivum*	^	Accumulation of Ca^{2+} and K^+ ions	Azeem et al. (2015)
	KCl	10 mM	36 h	*Capsicum annuum*	^	Increased proline accumulation	Aloui et al. (2014)
	$CaCl_2$	2 mM	24 h	*Cucumis sativus*	^	Increased proline accumulation	Joshi et al. (2013)
	NaCl	300 mM	24 h	*Solanum lycopersicum*	^	Increased gibberellin content promoting endosperm weakening and germination	Nakaune et al. (2012)
	NaCl	100 mM	36 h	*Cucumis melo*	^	Increased proline, soluble carbohydrates and antioxidants	Farhoudi et al. (2011)
	KNO_3	−1.0 MPa	24 h	*Helianthus annuus*	^	Better osmoregulation	Bajehbaj (2010)
	NaCl	1 mM	–	*Capsicum annuum*	^	Generated 'stress memory'	Khan et al. (2009)
	KCl, KNO_3, $CaCl_2.2H_2O$, $Ca(NO_3)_2.4H_2O$	Varying concentration	12 h	*Triticum aestivum*	No change	–	Ashraf and Iram (2002)
	Mixed salt	–	–	*Oryza sativa*	^	Increased activities of amylases, root dehydrogenase and shoot catalase	Chang-Zheng et al. (2002)
	$CaCl_2$	10–60 mmol L^{-1}	12 h	*Gossypium hirsutum*	˅	Species-specific effect; low germination	Xiao-Fang et al. (2000)
	KNO_3	–	–	*Cajanus cajan*	^	Accumulation of proteins, free amino acids and soluble sugars	Jyotsna and Srivastava (1998)
	$CaCl_2$						

(continued)

Table 5.2 (continued)

Type	Agent	Concentration/dose	Treatment time	Plant Species	Salt tolerance	Mechanism	References
Osmopriming	Spermidine	5 mM	8 h	*Oryza sativa*	^	Up-regulation of several stress-responsive genes and transcription factors; enhanced expression of membrane Na^+ efflux pumps	Paul et al. (2017)
	Glycine betaine	10 mM	24 h	*Capsicum annuum*	^	Decreased MDA levels due to higher accumulation of proline	Roychoudhury and Banerjee (2016) and Korkmaz and Şirikçi (2011)
	Nitric oxide	75 µM (Na-nitroprusside)	24 h	*Jatropha curcas*	^	Increased accumulation of glutathione and ascorbate in the endosperm-embryo axis; higher activities of catalase 1, catalase 2 and glutathione reductases (GR1 and GR2)	Gadelha et al. (2017)
	H_2O_2	–	–	*Cakile maritima, Eutrema salsugineum*	^	Redox balance was restored	Ellouzi et al. (2017)
	Spermine	2.5 mM	8 h	*Oryza sativa*	^	Osmo-protection	Paul and Roy choudhury (2016)
	β-amino butyric acid	1 mM	6 h	*Vigna radiata*	^	Increased accumulation of proline, total carbohydrates, total protein; enhanced activities of nitrate reductase, superoxide dismutase and guaiacol peroxidase	Jisha and Puthur (2016)
	Trehalose	10 mM	48 h	*Oryza sativa*	^	Enhanced the activities of multiple antioxidant enzymes	Mostofa et al. (2015)
	Ascorbic acid	Variable	–	*Triticum durum*	^	Proteins associated with metabolism, energy, disease, defence and storage showed increased abundance	Fercha et al. (2014)
	Ascorbic acid	0.5 mM	24 h	*Cucurbita pepo*	^	Increased activities of catalase and peroxidase	Fazlali et al. (2013)
	Choline	5 mM	24 h	*Triticum aestivum*	^	Increased glycine betaine accumulation and maintenance of osmotic potential	Salama et al. (2011)

Physical priming	UV-C	0.85 KJ m^{-2}	1–4 min	*Lactuca sativa*	^	Increased levels of phenolics and flavonoids which efficiently scavenged the diphenyl-picrylhydrazyl radicals	Ouhibi et al. (2014)
Hormone priming	Salicylic acid (SA) + fish flour	0.1 mM SA	Overnight	*Triticum durum*	^	Enhanced activities of phenylalanine ammonia lyase, peroxidase; accumulation of phenolics and flavonoids	Karadag and Yucel (2017)
	Methyl jasmonate	25 µmol L^{-1}	–	*Brassica oleracea*	^	The contents of indolic glucosinolates, glucobrassicin, neo-glucobrassicin, anthocyanins and chlorogenic acid derivatives increased	Hassini et al. (2017)
	Salicylic acid	0.5 mM	Overnight shaking at 150 rpm	*Triticum durum*	^	Accumulation of total phenols, flavonoids and carotenoids; increased activities of phenylalanine ammonia lyase and ascorbic acid oxidase	Yucel and Heybet (2016)
	Melatonin	–	–	*Vicia faba*	^	Increased photosynthetic efficiency, total carbohydrates, total phenols, indole acetic acid, K^+ and Ca^{2+} levels	Dawood and El-Awadi (2015)
	ABA	30 ppm	8 h	*Phaseolus vulgaris*	^	Increased phosphatidylcholine/ phosphatidylethanolamine ratio and confers root cell membrane protection	Salama et al. (2015)
	Melatonin	1 µM	24 h	*Cucumis sativus*	^	ABA catabolism and gibberellic acid biosynthesis were promoted; the antioxidant machinery recharged	Zhang et al. (2014)
	Gibberellic acid (GA_3)	4.5 mM	12 h	*Lactuca sativa*	^	ABA biosynthesis is suppressed which triggers efficient germination	Hela et al. (2012)
	Gibberellic acid (GA_3)	20 mg L^{-1}	24 h	*Foeniculum vulgare*	^	ABA biosynthesis is suppressed which triggers efficient germination	Sedghi et al. (2010)

^ Enhanced
˅ Decreased

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Seed Priming with Plant Growth Regulators to Improve Crop Abiotic Stress Tolerance

6

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Abstract

Plants are frequently subjected to abiotic stress such as drought, salinity, heat, and cold which constitutes a major limitation to agricultural production worldwide. The unfavorable environmental conditions that plants encounter in vegetative cycle perturb their metabolic reactions and negatively affect growth at cellular and biochemical plant levels. Preventing crop losses and generating more food to meet the demands of growing human populations have gained importance. Identifying plant mechanisms to neutralize abiotic stresses and sustain their growth and survival under unfavorable conditions holds huge importance. Research studies have revealed that plant growth regulators (PGR) confirm their significance as metabolic engineering targets for producing abiotic stress-tolerant crop plants. In addition, seed priming has shown its importance as a powerful technique to improve germination, growth, and yield of crops under unfavorable environment conditions. The combination of the two effects, seed priming with PGR, could have very prevailing results. In this context, during this chapter, we evaluate the effect of seed priming with PGR in plant growth development and abiotic stress tolerance.

Keywords

Abiotic stress · Seed priming · Plant growth regulators · Growth · Germination

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6.1 Introduction

The human population is growing rapidly and requires a considerable increase in agricultural productivity worldwide. However, diverse abiotic stresses are limiting crop productivity (Wani and Sah 2014). In order to nourish the rising world population, crop productivity must be amplified in the near future. That's why plant breeders and biotechnologists should improve crop tolerance to abiotic stress by recognizing these mechanisms. However, plant machineries leading to environmental stress response and tolerance are complex (Qin et al. 2011). Since the complication of stress tolerance, conventional breeding trials have met little success. That's why original and effective approaches should be developed. Seed priming is a revolutionary technique used to improve seed germination and seedling growth in abiotic stress conditions. During this process, a series of physiological and biochemical processes are triggered leading to improving plant growth under stress conditions (Eisvand et al. 2010). Seeds can be soaked in solutions containing exogenous molecules such as salts (Khan et al. 2009a, b) or plant growth regulators (PGR) (Nakaune et al. 2012). Seed priming with PGR pretreatment is a commonly used strategy to improve seed germination and seedling growth in unfavorable conditions (Masood et al. 2012; Hu et al. 2013). Seeds presoaked with optimal concentration of PGR enhance germination, growth, and yield of crops under stress condition by rising nutrient reserves through improved physiological activities and root profusion (Afzal et al. 2002; Akbari et al. 2007). PGR are organic compounds produced in extremely small amount and play a vital function in growth, expansion, and yield of crops. They regulate, as chemical messengers, a range of cellular processes in higher plants and coordinate diverse signal transduction pathways during abiotic stress response (Vob et al. 2014; Kazan 2015). For instance, seeds of rye (*Secale montanum*) pretreated with gibberellic acid (GA3) increased germination under water deficit (Ansari et al. 2013). Khan et al. (2009a, b) confirmed that pepper seeds (*Capscum annum* L.) pretreated with salicylic acid resulted in better germination and seedling growth under salt stress. Furthermore, ethylene reduces high temperature effect on seed germination of lettuce (Nascimento et al. 2004).

6.2 Abiotic Stresses: World Agricultural Challenge

Exploring how abiotic stresses can influence plant growth at the physiological, biochemical, and molecular levels is decisive to advance crop production, since stresses cause crop losses (Kazan 2015). Abiotic stresses, including drought, salinity, chilling, freezing, heat, and UV radiation, are the main environmental factors restraining crop production. They negatively influence growth, biomass production, and yields of food crops threatening consequently food security worldwide (Kaur et al. 2008; Thakur et al. 2010). Among these stresses, drought, salinity, and temperature severity are the most common abiotic stresses limiting crop productivity in the world (Jaleel et al. 2009; Thakur et al. 2010). They affect plant survival, pigment content, membrane integrity, water relations, osmotic adjustments, and photosynthetic

activity (Sanghera et al. 2011; Pathak et al. 2014). Drought and salinity affect together more than 55% of the world's agricultural land (Dos Reis et al. 2012). Since abiotic stress tolerance is multigenic in nature (Collins et al. 2008), an enormous challenge was undertaken to comprehend key mechanisms to go forward in selective breeding purposes. Understanding the machinery of plants' environmental stress tolerance is of critical importance for the development of stress-tolerant and high-yielding food crop cultivars.

6.2.1 Plant Drought Tolerance

Drought tolerance is the ability to survive and produce stable yields under water scarcity. Drought stress decreases plants' photosynthetic rate, decreasing consequently the amount of assimilates available for export to the sink organs (Kim et al. 2000). Abscisic acid (ABA) is a key plant growth regulator in the response and adaptation of plants to water scarcity. It is involved in stomatal closure, accumulation of osmoprotectants, and changes in gene expression (Umezawa et al. 2010). The biosynthesis of osmoprotectants such as amino acid, amines, and carbohydrates is another indispensable strategy for plant resistance to water stress. The most common osmoprotectants are proline, glycine betaine, fructans, starch, and mono- and disaccharides.

6.2.2 Plant Heat Tolerance

Heat stress perturbs cellular homeostasis and causes protein denaturation and dysfunction in plant cells, leading to brutal growth retardation. During this stress, electron transport is altered affecting electron flow from oxygen-evolving complex (OEC) toward the acceptor side of photosystem II (PSII). These alterations affect the generation of ATP, Rubisco for carbon fixation, and starch (Asthir 2015). Drought stress generates the accumulation of ROS leading to a severe damage in DNA and peroxidation of membrane lipids and pigments. Other changes include a decrease in photosynthetic pigment ratio and inhibitions of stomatal conductance and photosynthesis rate. These alterations ultimately reduce the partitioning of photosynthates, which manifest by reduced growth and economic yield. Other morphological damages associated with heat stress comprise scorching of leaves, branches, and stems, leaf senescence, fruit discoloration, and damage (Hasanuzzaman et al. 2013).

6.2.3 Plant Cold Tolerance

Cold stress occurs at temperatures less than 20 °C. Chilling (<20 °C) or freezing (<0 °C) temperatures can trigger the formation of ice in plant tissues, cause cellular dehydration and leakage of intracellular solutes, and reduce plasma membrane integrity (Chinnusamy et al. 2007). Consequently, cold stress severely affects plant

growth and leads to substantial crop losses (Sanghera et al. 2011). To cope with this unfavorable condition, plants adopt several strategies such as activating primary metabolisms, raising the level of antioxidants, and maintaining osmotic balance (Miura and Furumoto 2013). During cold stress, membrane rigidification occurs as opposed to heat stress. This process is the upstream trigger for the induction of cytosolic Ca^{2+} signatures leading to a transient increase in cytosolic Ca^{2+} levels (Knight et al. 1991).

6.3 Plant Growth Regulators: Key Mediators of Plant Responses to Environmental Stresses

Plants have to adjust their development to respond to various abiotic stresses. Plant growth regulators (PGR) are cells signaling molecules acting in very small quantities that mediate these responses. Their crucial functions are advancing plant adaptation to an altering environment by mediating growth, development, and nutrient allocation (Fahad et al. 2015a, b). PGR are endogenous substances responsible in adjusting physiological and molecular responses for plant survival. They include gibberellins (GAs), salicylic acid (SA), auxin (IAA), ethylene (ET), cytokinins (CKs), brassinosteroids (BRs), abscisic acid (ABA), and jasmonates (JAs).

6.3.1 Abscisic Acid (ABA), the Abiotic Stress Hormone

Abscisic acid (ABA) is the most studied PGR. It plays an important role throughout numerous plant physiological processes and developmental stages including stomatal closure, embryo morphogenesis, seed dormancy, and synthesis of storage proteins and lipids (Sreenivasulu et al. 2010). ABA is a vital messenger in the adaptive response of plants to abiotic stress. During this response, endogenous ABA levels increase rapidly, activating specific signaling pathways and altering gene expression levels (O'Brien and Benkova 2013). Zhang et al. (2006) and Hossain et al. (2010) stated a substantial increase in ABA concentration upon exposure of plants to salinity and drought. It regulates the expression of different stress-responsive genes implicated in the accumulation of compatible osmolytes and the synthesis of proteins and antioxidant enzymes (Chaves et al. 2003; Verslues et al. 2006).

6.3.2 Auxins (IAA)

IAA (indole-3-acetic acid) is a multifunctional PGR and is vital for plant growth under stress conditions (Kazan 2013). IAA boosts plant root and shoot growth and plays consequently a fundamental part in plant adaptation to salt stress (Egamberdieva 2009; Iqbal et al. 2014; Fahad et al. 2015a, b). Auxin stimulates the transcription of primary auxin response genes identified and characterized in several plant species (Javid et al. 2011).

6.3.3 Gibberellins (GAs)

GAs improve seed germination, leaf expansion, stem elongation, fruit development, and abiotic stress response and adaptation (Yamaguchi 2008; Colebrook et al. 2014). It interacts with other PGR in many stimulus-response processes (Munteanu et al. 2014).

6.3.4 Salicylic Acid (SA)

SA plays a vital role in the regulation of plant growth, ripening, and responses to abiotic stresses (Khodary 2004; Miura et al. 2013; Miura and Tada 2014). Gharib and Hegazi (2010) showed that SA stimulated growth of bean seedlings and reduced the adverse effect of cold and chilling stresses.

6.3.5 Cytokinins (CKs)

CKs are involved in many plant growth processes and abiotic stress tolerance (Nishiyama et al. 2011; Kang et al. 2012; O'Brien and Benkova 2013). CKs are often considered ABA antagonists (Pospíšilová 2003). It has been linked to different abiotic stress tolerance like cold stress and freezing stress (Jeon et al. 2010). Salinity or osmotic stress shows an effect in the expression levels of CK receptors and metabolism, respectively, in *Arabidopsis* and maize (Zalabák et al. 2013).

6.3.6 Jasmonates (JAs)

Jasmonates are involved in plant development including reproductive processes, secondary metabolism, and plant responses to environmental stresses (Pauwels et al. 2009; Seo et al. 2001; Fahad et al. 2015a, b). Exogenous application of JA significantly reduced salinity and heavy metal stress symptoms in plants by activating the antioxidant machinery (Yoon et al. 2009; Yan et al. 2013). Wang et al. (2010) have reported a significant increase in endogenous levels of JA in rice roots under salinity stress. In addition, JA confers tolerance to metal stress in plants via the accumulation of phytochelatins (Maksymiec et al. 2007).

6.3.7 Ethylene (ET)

ET, a gaseous PGR, is involved in plant growth and development, notably fruit ripening, flower senescence, leaf and petal abscission, and stress response regulation (Gamalero and Glick 2012; Groen and Whiteman 2014). Enhanced abiotic tolerance was achieved with higher endogenous ET concentrations in plants (Shi et al. 2012; Groen and Whiteman 2014). ET also induces plants' defense response to heat

stress (Larkindale et al. 2005). Yin et al. (2015) have shown that ET and ABA act in synergy or in antagonism to control plant growth.

6.4 Seed Priming as a Strategy to Improve Abiotic Stress Tolerance

Recently, diverse strategies have been employed to induce abiotic stress tolerance in plants. Seed priming is an effective, practical, and low-cost technique to obtain rapid emergence, high seedling vigor, and better crop yields under unfavorable environmental conditions (Jisha et al. 2013; Paparella et al. 2015). It is a controlled hydration technique triggering metabolic processes during early phase of germination before radicle protrusion (Hussain et al. 2015). Higher and synchronized germination of primed seeds is due to reduction in the lag time of imbibition (Brocklehurst and Dearman 2008), enzyme activation (Lee and Kim 2000), buildup of germination-enhancing metabolites (Hussain et al. 2015), metabolic repair during imbibition (Farooq et al. 2006), and osmotic adjustment (Bradford 1986). Primed plants exhibit activation of cellular defense responses, which imparts abiotic stress tolerance (Jisha et al. 2013). Various seed priming techniques have been employed under different environmental stresses including hydropriming, osmopriming, chemical priming, nutrient priming, and hormonal priming (Jisha et al. 2013; Paparella et al. 2015). During seed priming, germination process is induced by soaking seeds in solutions containing exogenous molecules such as salts (Khan et al. 2009a, b), metals (Mirshekari et al. 2012), or hormones (Nakaune et al. 2012). Varier et al. (2010) and Eisvand et al. (2010) suggest that seed priming activates a series of physiological processes that improve plant growth under stressful conditions, including the induction of antioxidant systems.

6.4.1 Seed Priming with Plant Growth Regulators

PGR pretreatment is a commonly used priming approach to improve seed germination under stressful conditions (Jisha et al. 2013; Hu et al. 2013). It can be used to advance germination, seedling growth, and yield under drought, salinity, metal, cold, and heat stresses.

6.4.1.1 Seed Priming with PGR Under Water Deficit

Seeds of rye (*Secale montanum*) primed with gibberellic acid increased germination under water deficit (Ansari et al. 2013). ABA-primed seeds of *Brassica napus* exhibited earlier germination and higher final percent radicle protrusion than non-primed control seeds, under water stress (Gao et al. 2002). Seeds of *Agropyron elongatum* primed with gibberellin and abscisic acid exhibited induced CAT and SOD activities under drought conditions when compared to unprimed seeds (Eisvand et al. 2010). Farooq et al. (2013) have shown that seeds primed with ascorbic acid improve emergence, growth, yield, and water statue of wheat seedlings

under water deficit. Priming with ascorbic acid showed significant effects on germination percentage, shoot length, root length, vigor index, and CAT and POX activity in rapeseed (*Brassica napus* L.) plant under drought condition (Razaji et al. 2014).

6.4.1.2 Seed Priming with PGR Under Salt Stress

In pepper (*Capscum annum* L.), Khan et al. (2009a, b) showed that pretreatment with acetylsalicylic acid and salicylic acid resulted in greater uniformity of germination and establishment of seedlings under high salinity. ABA-primed seeds of *Brassica napus* exhibited earlier germination and higher final percent radicle protrusion than non-primed control seeds, under salt stress (100 mM NaCl) (Gao et al. 2002). In wheat seed germination, auxin pretreatments increased the hypocotyl length, seedling fresh and dry weight, and hypocotyl dry weight under saline conditions (Akbari et al. 2007). Salicylic acid priming in fennel seeds also showed better germination under salt stress (Farahbakhsh 2012). Iqbal et al. (2011) have reported that seed priming with gibberellic acid induced an increase in grain yield of wheat plants, modulation of ion uptake and partitioning, and hormone homeostasis under saline conditions.

6.4.1.3 Seed Priming with PGR Under Heat Stress

Additionally, ethylene was used to minimize the effect of high temperatures on seed germination of lettuce (*Lactuca sativa* L.) (Nascimento et al. 2004). Rehman et al. (2012) have shown that seed priming with salicylic acid improved temperature stress resistance in spring maize through an earlier emergence, increased seedling dry weight and tissue water status, and improved membrane stability. Seed priming with salicylic acid or jasmonic acid improves growth, carbohydrate content, and chilling resistance in sunflower (*Helianthus annuus* L.) (Gornik and Lahuta 2017). Singh and Singh (2016) have shown that seed priming with three levels of salicylic acid (0.25 mM, 0.5 mM, and 0.75 mM) improves growth, flowering, yield, and fruit quality under high-temperature stress conditions.

6.4.1.4 Seed Priming with PGR Under Cold Stress

The incorporation of methyl jasmonate (3 μM) into the priming solution on low temperature improves germination and emergence performance of watermelon (*Citrullus lanatus*) cv. Crimson Sweet (Korkmaz et al. 2004). Gamel et al. (2017) have shown that seed priming with 100 ppm gibberellic acid improves germination, growth, yield, and fruit quality of three tomato cultivars under low temperature. Ansari and Zadeh (2012) have shown that seed priming with gibberellic acid (25 ppm) advances germination and seedling growth of mountain rye (*Secale montanum*) under cold stress.

6.4.1.5 Seed Priming with PGR Under Metal Stress

Seed priming with ethylene (100 μM) improves germination parameters of pigeon pea under cadmium stress (Sneideris et al. 2014). PGR priming using auxin, cytokinin, and gibberellic acid at concentration of 10–100 μM was the most appropriate priming treatment for soybean (*Glycine max*) seeds grown under lead (Pb) stress

conditions (Abu-Muriefah 2017). Seed priming with jasmonate advances growth and activity of SOD and POD and increases significantly the accumulation of chlorophyll and carotenoid and neutralizes the toxic effect of Cu^{2+} on *Cajanus cajan* seedlings under copper stress (Poonam et al. 2013).

6.5 Conclusion and Future Perspectives

It can be concluded that seed priming with PGR has the potential for improving crop abiotic stress tolerance which provides new opportunities to maintain sustainable crop production to feed the growing population under changing environmental conditions. Even though, with rapid development of genomic technology, significant attempts have been done on the way to decoding the plant abiotic stress responses, many challenges still lie ahead to uncover the complexity of stress signal transduction pathways. More hard work will be required at the genetic level of PGR biosynthetic pathway. The success in elucidating roles of PGR in stress tolerance at molecular levels will help in showing positive effects of seed priming with PGR and their substitutes in improving stress tolerance in a wide range of crop species. However, more research will be needed in unraveling the mechanism of PGR, especially with stress-responsive genes.

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Addressing Stresses in Agriculture Through Bio-priming Intervention

7

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Abstract

Concurrent occurrences of different stresses, i.e. biotic and abiotic, are very common in the environment of plants which consequently reduce yield. As cost-effective options are very limited, bio-priming is a suitable tool to address the numerous challenges associated with agriculture. Plant growth benefits are easily attainable through this technique while managing the natural resources and enhancing the environmental sustainability.

Keywords

Bio-priming · Alleviation · Abiotic stress · Biotic stress

7.1 Introduction

Various stress factors are responsible for lowering of the agricultural production, which create the need of identifying these stresses, note the changes occurring in plant system and develop suitable tool which can help plant to easily fight against them. Abiotic stresses mainly include extreme temperatures (heat, cold), drought (limited precipitation, drying winds), heavy metals and salinity (Krasensky and

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Jonak 2012), but the notable biotic stresses are viruses, fungi, bacteria, weeds, insects and other pests and pathogens (Suzuki et al. 2014). Wang et al. (2003) reported that abiotic stresses can reduce average yields by >50% for most major crop plants. Increasing abiotic stress like increase in temperature can further facilitate pathogen spread and weaken the defence mechanisms of plants (Suzuki et al. 2014). Genetic manipulation to generate stress-tolerant crops is one area where many research works are being carried out with the help of breeding programmes. But these practices are time-taking and involve huge investments. Cost-effective options are very limited, indicating the urgent need of solution which is agriculturally economical for the farmers to easily adapt it. Bio-priming is a seed treatment process using microbial agents to accelerate germination and improve seedling establishment under adverse conditions (Bhatt et al. 2015; Singh et al. 2016b).

7.2 How Is Bio-priming Effective?

The beneficial effects of microbe-plant interactions include plant growth promotion and induced disease resistance and tolerance to abiotic stresses (Table 7.1, Fig. 7.1). Bio-primed plants adapt themselves to environmental stresses by metabolic adjustments in their system, leading to the accumulation of several compatible organic solutes like sugars, polyamines, betaines, quaternary ammonium compounds, polyhydric alcohols, proline and other amino acids (Sandhya et al. 2010; Ray et al. 2016). Development of induced resistance in plants treated with microbes help to overcome pathogen infection and control the plant diseases (El-Mohamedy et al. 2015). Microorganisms like *Trichoderma* augment deep root growth, helping the plant in water acquisition and nutrient uptake during adverse conditions (Rawat et al. 2012; Keswani et al. 2016).

7.3 Precautions Needed

Region-specific bio-inoculants must be screened and isolated according to their traits linked with the environment in which they can survive, tolerate and effectively function in adverse conditions (Bisen et al. 2015; Rakshit et al. 2015). Successful inoculation is dependent upon type of soil, optimum temperature, concentration of nutrients and salt, moisture level, etc. Modern screening methods focussing on the microbial traits, including their culturing, storage, transportation and application, should be given special attention. Mechanisms underlying the actions of microbes (root colonization, parasitism, antibiosis, antagonism, etc.) must be studied (du Jardin 2015).

7.4 Present Status

Numerous strains have been identified as seed dressers, potential rhizosphere colonizers and stimulants for plant growth and health beyond the germination and seedling mergence stage of different crop species (Singh et al. 2016a, b; Singh 2016).

Table 7.1 Effect of bio-priming in alleviation of various stresses

	Crop	Stress	Microbial agents	Betterment	References
Pulses	Lentil	Strain stress (*Pythium* spp.)	*Rhizobium leguminosarum* bv. *viceae*	Good seedling emergence and vigour. Yield improvement by 43.11%	Huang and Erickson (2007)
	Pea	Strain stress (*Pythium* spp.)	*Rhizobium leguminosarum* bv. *viceae*	Yield improvement by 41.35%	Huang and Erickson (2006)
	Mung bean	Strain stress (root rot) *Macrophomina* spp.	*T. harzianum*– RA	Root rot incidence decrement by 12.45%	Raguchander et al. (1997)
	Chickpea	Biotic stress (*Sclerotium rolfsii*)	*Mesorhizobium* (RL091), fluorescent *Pseudomonas* (PHU094), *Trichoderma* (THU0816)	Plant mortality (%) decreased by 76 ± 4%	Singh et al. (2014)
Cereals	Wheat	Salinity stress	*Trichoderma harzianum* (Th-19)	Increase in germination % by 0.87 during the salinity stress	Rawat et al. (2011)
	Wheat	Salinity stress	*Trichoderma longibrachiatum*	Superoxide dismutase, catalase, peroxidase increased by 29%, 39% and 19% respectively	Zhang et al. (2016)
	Rice	Nutrient stress	*Azospirillum amazonense*	NUE increased up to 18.5%	Rodrigues et al. (2008)
	Maize	Biotic stress (*Fusarium* spp.)	*Burkholderia cepacia*	Inhibited *F. solani* spp. by 43%, *F. moniliforme* 58%, *F. proliferatum* spp. 66%, *F. subglutinans* ISPV 517 74% and *F. graminearum* spp. 74%	Bevivino et al. (1998)
	Wheat and barley	Biotic stress (*Fusarium culmorum*)	*P. fluorescens* strain MKB 249, *Pseudomonas* sp. strain MKB 158	Level of disease symptoms decreased by 53–91%	Khan et al. (2005)
	Maize	Drought	*Pseudomonas* spp.	Improved plant biomass, relative water content, leaf water potential, root adhering soil/root tissue ratio, aggregate stability and mean weight diameter, decreased leaf water loss	Sandhya et al. (2010)
	Rice	Salinity	*Trichoderma harzianum*	Alleviation of oxidative damage and significant increase in length and fresh weight of shoot and root, number of leaves, leaf area, photosynthetic rate, chlorophyll fluorescence, chlorophyll content	Rawat et al. (2012)
	Baby corn	Nutrient stress with reference to N and P	*Trichoderma harzianum*	Improved nitrogen acquisition efficiency	Meena et al. (2016)

(continued)

Table 7.1 (continued)

	Crop	Stress	Microbial agents	Betterment	References
Fruits and vegetables	Cucumber	Nutrient stress	*T. asperellum* strain T 34	Increase in micronutrient use efficiency	Santiago et al. (2012)
	Tomato	Nutrient stress	*T. harzianum* T969	PUE increase by 65.8%	Azarmi et al. (2011)
	Melon	Strain stress (*Fusarium oxysporum* f.sp. *melonis*)	*Glomus intraradices* and *T. harzianum*	Disease incidence decreased by 50% (*T. harzianum*) and significantly by *Glomus intraradices*	Martínez-Medina et al. (2010)
	Broccoli	Nutrient stress	*P. fluorescens*, AM fungi	Increase in NUE up to 235.42% and PUE up to 163.33%	Tanwar et al. (2013)
	Green bean	Pathogens: *Rhizoctonia solani* and *Fusarium solani*	*Trichoderma harzianum*	Improved plant growth, total yield and its components, green pod nutritional value parameters	El-Mohamedy et al. (2015)
	Tomato	Osmotic	*Enterobacter* spp.	Improved germination and enhanced seedling growth	Bhatt et al. (2015)
	Tomato	Biotic stress (root-knot nematode)	*Meria coniospora*	Galls/root system decreased from 9.5 ± 4 (control) to 1.5 ±	Jansson et al. (1985)
Oilseeds	Soybean	Nutrient stress (Fe)	*Trichoderma virens* As19–1	Fe uptake increased to 77%	Entesari et al. (2013)
	Groundnut	Biotic stress stem rot disease (*Sclerotium rolfsii*)	*Trichoderma harzianum* (ITCC - 4572)	% disease inhibition by 57%	Ganesan et al. (2007)
	Safflower	Biotic stress *Macrophomina phaseolina* (root rot disease)	*Trichoderma harizianum, Pseudomonas fluorescens* and *Bacillus subtilis*	Disease incidence due to *Bacillus subtilis* decreased by 16%and *Trichoderma harizianum* and *Pseudomonas fluorescens* by 18%	Govindappa et al. (2010)
Plantation crops	Sugarcane	Nutrient stress	*P. fluorescens* strain R62+R81	PUE increased by 0.719	Yadav et al. (2013)
	Tea	Nutrient stress	*Azospirillum brasilense*	Increase in NUE up to 65%, PUE up to 25% and KUE by 16%	Thomas et al. (2010)

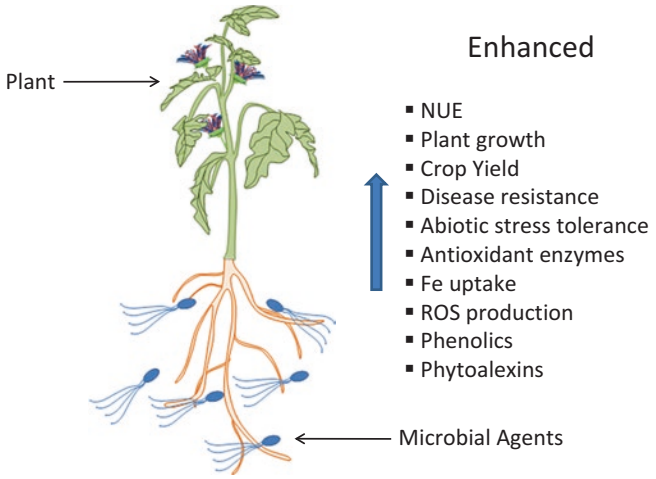

Fig. 7.1 Systemic representation of microbial-mediated growth promotion and defence responses in plants

Bio-priming is an important means of managing many soil and seed-borne diseases (Rao et al. 2009). The technique has enormous potential to have multifunctional impact on soil-plant system which can improve NUE, nutrient uptake, plant growth and plant tolerance to multi-stress by reducing the dependency on chemical inputs and thereby lowering the environmental pollution and increasing the agricultural sustainability (Rakshit et al. 2014).

7.5 Conclusion

Exploration of microbes in bio-priming sector can contribute a lot towards curbing the stress factors that the plants face in their environment. Present understanding of the microbial diversity and their effective functioning along with the mechanisms involved in their actions has contributed in the advancement of technology and commercialisation of inoculants.

7.6 Future Thrust

Bio-priming has a huge scope in practising modern agriculture. Isolation of specific inoculum or development of consortia (crop based) is quite challenging for the researchers. Screening and identification of microbes as a particular stress tolerant and those which can acclimatize themselves in varied adverse conditions with longer shelf lives are of utmost necessary. Further extensive research can help in sustainable management of resources.

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8 Role of Microbial Seed Priming and Microbial Phytohormone in Modulating Growth Promotion and Defense Responses in Plants

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Abstract

Plant growth and development are greatly affected by various biotic and abiotic stresses. Various strategies are utilized to minimize stresses in plants. Seed priming with beneficial microorganisms is one of the most beneficial methods to improve plant growth and development and induce systemic tolerance in plants towards biotic as well as abiotic stresses. Seed priming is a method of conditioning the seeds by plant growth-promoting microbes which provide better abilities to the plant to withstand various environmental challenges beginning from seed germination. Seed priming with beneficial microorganism is also known as bio-priming that enhances seed germination, protects germinating seed from different phytopathogens, and provides suitable conditions for establishment of the plant. Bio-priming has several mechanisms to stimulate morphogenesis and plant immunity, viz., production of phytohormones, induced expression of plant growth-promoting genes, increased nutrient status into the plant, mycoparasitism, antibiosis, induced phenolic production, activation of antioxidant production, and systemic defense activation. Some important microorganisms that synthesize

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phytohormones include *Azotobacter* spp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Rhizobium* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Paenibacillus polymyxa*, *Trichoderma* spp., *Pseudomonas putida*, *Rhizobium phaseoli*, *Bacillus cereus*, and *Acinetobacter calcoaceticus*. The main objective of this chapter is to enlighten the importance of seed priming with microorganisms and the role of different phytohormones produced by them in modulating growth, development, and defense activation in the host plant.

Keywords
Seed biopriming · Microbial phytohormone · Plant growth promotion · Defense responses

8.1 Introduction

Seed bio-priming is the technique of treating seeds with plant growth-promoting microbes (PGPM) that also enhances the basal level of plant defense by inducing the plant immunological memory. It is also defined as priming of seeds with beneficial microorganism followed by seed hydration (Singh et al. 2016a, b; Singh 2016; Rakshit et al. 2014; Rakshit et al. 2015a, b). During bio-priming, the stimulus from beneficial microbes is perceived and leads changes in the physiological, transcriptional, metabolic, and epigenetic behavior of the plant (Meena et al. 2016; Meena et al. 2017). These altered behaviors show higher defense responses resulting in enhanced resistance and stress tolerance (Mauch-Mani et al. 2017). Seed bio-priming process comprises different priming methods such as incubating under moist conditions in a plastic bag (Callan et al. 1991) or in a moistened finely ground coal or lignite materials (solid matrix priming) (Harman and Taylor 1988) and drum priming (Bennett and Whipps 2008). During bio-priming, there is an increase in microbial population on the seed surface. Callan et al. (1990) reported that during bio-priming, the bacterial population increases from 10- to over 10,000-fold depending on the initial level of inoculum. However, it is important to sterilize the seeds to remove or reduce the undesired microflora before priming. If the seeds were infected with the pathogen, their population will also increase during the priming process causing adverse and undesirable effect on emerging plants (Reddy 2013; Singh et al. 2013a, b; Jain et al. 2015). On the other hand, multiplication of undesired indigenous microorganisms may reduce the survivability of beneficial microbes used for seed bio-priming process (Wright et al. 2003). Seed bio-priming ensures better survival of the plants right from seed germination to maturity stage and also plays a role in enhancing nutritional value of the edible parts of the crop plants.

8.2 Seed Priming Regulate Microbe-Plant Interaction

Seed bio-priming provides perfect conditions for the microbial inoculum to colonize the seed surface (McQuilken et al. 1998). It helps the microbes to be established in the rhizosphere zone of the plant and provides long-term protection to

plants from various biotic stresses along with promotion in plant growth (Yadav et al. 2015) (Figs. 8.1 and 8.2). The interaction of beneficial microbes with plant leads to establishment of various types of relationships, in which microbes benefit through utilization of carbon compounds released from the colonized plants. On the other hand, organic compounds from plant root exudates also enhance the microbial biomass and their functional activity in the rhizosphere zone (Ortíz-Castro et al. 2009). Free-living microbes such as saprophytic fungal species of the genus *Trichoderma* and important plant growth-promoting rhizobacteria (PGPR) are able to enhance plant growth and inhibit the soil-borne plant pathogens (Ortíz-Castro et al. 2009; Keswani et al. 2016) (Fig. 8.1). These beneficial microbes overcome the challenges of pathogens by various mechanisms such as mycoparasitism, production of phytohormones, competition for nutrients and space, and several others (Keswani et al. 2014; Bisen et al. 2015). Further, application of microorganisms also helps in decomposition and mineralization of organic matter and increase the availability of important nutrients such as iron and phosphorus (Valencia-Cantero et al. 2007; Ortíz-Castro et al. 2009). Plants have evolved a number of the physical and chemical barrier to protect it from pathogens, while some of the inducible responses are activated after pathogen perception (Hammond-Kosack and Jones 1996). This recognition step was achieved by microbe-associated molecular patterns (MAMPs) that are common to many classes of microbes (Ortíz-Castro et al. 2009).

The ultimate role of seed bio-priming is to provide better health and protection to the plant from any harmful environment during initial plant growth stage (et al. 2016b). The presence of microbial population in rhizosphere plays different important roles as mentioned above and their presence is also directly related to the synthesis of phytohormones. A wide range of microorganisms is capable of producing phytohormones that regulate plant growth and development

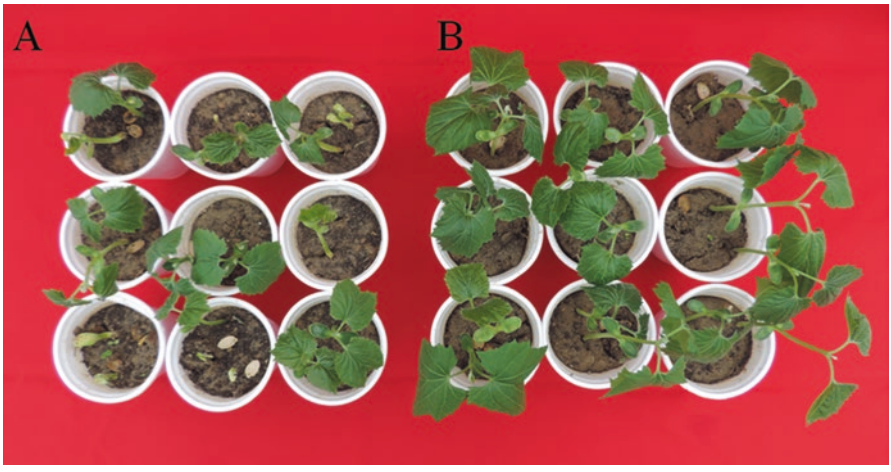

Fig. 8.1 Effect of seed priming with beneficial microorganism on plant growth promotion in bitter gourd; (**a**) control; (**b**) treatment (primed with beneficial microorganism). Microbial production of phytohormones and their role in plant growth

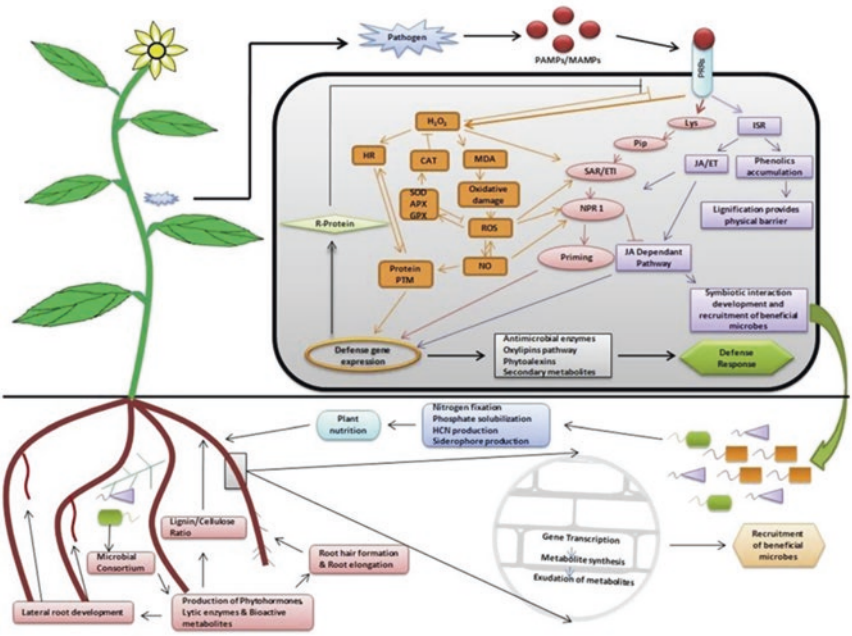

Fig. 8.2 Systemic representation of seed bio-priming with beneficial microbe-mediated growth promotion and defense responses in plants (Sarma et al. 2015)

(Ortíz-Castro et al. 2009; Spaepen and Vanderleyden 2011; Glick 2012). Auxin is one of the most important phytohormones that play a crucial role in plant growth and development. Among various forms of auxins, IAA (indole-3-acetic acid) is the most studied and most common auxin (Spaepen and Vanderleyden 2011; Glick 2012). IAA plays a major role in cell division, extension, and differentiation, enhances xylem and root development, promotes tuber and seed germination. It also helps in initial adventitious and lateral root formation, enhances photosynthesis, pigment formation, and mediate responses to gravity, light, and fluorescence (Tsavkelova et al. 2006; Spaepen and Vanderleyden 2011; Glick 2012). Several bacterial species are reported to synthesize auxins such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), or their precursors (Martínez-Morales et al. 2003; Spaepen et al. 2008). Patten and Glick (2002) reported the IAA synthesis by wild-type *Pseudomonas putida* GR12-2. Likewise, fungal species also synthesizes auxins as found in *Arabidopsis thaliana* and maize (*Zea mays*) that was inoculated with *Trichoderma* leading to changes in the root system architecture and enhanced plant growth. The effects were observed as increased root hair growth and lateral root formation (Bjorkman et al. 1998; Harman et al. 2004; Contreras-Cornejo et al. 2009).

Cytokinins stimulate cell division and differentiation processes in meristems both in plant shoots and roots. They are also involved in the processes including primary root growth, shoot formation, and callus formation. Cytokinins help plants

Table 8.1 Phytohormones synthesized by microorganisms

Phytohormones	Microorganisms	References
Auxins	*Azotobacter vinelandii* and *Azotobacter beijerinckii*	Azcon and Barea (1975)
	Trichoderma virens and *Trichoderma atroviride*	Contreras-Cornejo et al. (2009)
	Azospirillum brasilense	Martínez-Morales et al. (2003)
	Pseudomonas putida	Mayak et al. (1999)
	Rhizobium phaseoli	Atzorn et al. (1988)
Cytokinin	*Paenibacillus polymyxa*	Timmusk et al. (1999)
	Pseudomonas fluorescens	García de Salamone et al. (2001)
	Azotobacter vinelandii Azotobacter beijerinckii	Azcón and Barea (1975)
	Bacillus subtilis	Arkhipova et al. (2005)
	Azotobacter chroococcum	Nieto and Frankenberger (1989)
	Azotobacter vinelandii	Taller and Wong (1989)
	Paenibacillus polymyxa	Timmusk et al. (1999)
Gibberellins	*Azotobacter vinelandii* and *Azotobacter beijerinckii*	Azcón and Barea (1975)
	Rhizobium phaseoli	Atzorn et al. (1988)
	Acinetobacter calcoaceticus	Kang et al. (2009)

to maintain the pool of totipotent stem cells in their root and shoot meristems (Howell et al. 2003; Leibfried et al. 2005), while the gibberellins (GAs) stimulate growth and control many plant developmental processes including seed germination, stem growth, sex expression, and fruit formation (Bomke and Tudzynski 2009). Cytokinin production was reported in the cell-free medium of few strains of *Rhizobium* spp., *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Azotobacter* spp., *Bacillus subtilis*, *Rhodospirillum rubrum*, and *Paenibacillus polymyxa* (Glick 2012). Gibberellin production was also reported in the case of *Rhizobium phaseoli*, *Bacillus cereus*, and *Acinetobacter calcoaceticus* (Atzorn et al. 1988; Joo et al. 2005; Kang et al. 2009) (Table 8.1).

8.3 Role of Bio-priming in Nutritional Values of Plant

The present world population of about 7.2 billion is expected to be more than 9.6 billion by the end of 2050 (UNDESA 2013). Continuous increasing demand of food supply leads to the higher use of chemical fertilizers and pesticides which is harmful for the human health and environment also. Therefore researchers are concentrating more on the use of bio-fertilizers and biopesticides as an alternative to chemicals for sustainable agriculture. In this regard several attempts have been made toward nutrient-rich high-quality food production in sustainable agriculture

(Raja 2013; Patel et al. 2015). Seed bio-priming has the potentiality to enhance plant growth, yield, and nutritional values of the end product. It also has the ability to enhance uniform germination of seeds which leads to rapid, uniform, and high establishment of crops, hence improving harvest quality and yield (Mahmood et al. 2016). Several mechanisms are reported to define the role of bio-priming to enhance nutritional value of the plant products, viz., phosphate solubilization, increased N_2 fixation, increased production of plant growth-promoting compounds like phytohormones, production of antibiotics, and decomposition of organic matter (Sinha et al. 2014). Rhizosphere microbes play a very crucial role in enhanced uptake of three essential nutrients N, P, and K (Sarma et al. 2015). It is well elaborated that N, P, and K are the major constituents of several enzymes, hormones, amino acids, and genetic material in plants having role in various physiological processes in the plant (Maathuis 2009; Krouk et al. 2010; Chevalier and Rossignol 2011). Yadav et al. (2017) reported increased nutrient uptake of N, P, K, Na, Ca, and organic matter in the seed, foliage, and pericarp of the chickpea plants bio-primed with *Pseudomonas fluorescens* OKC and *Trichoderma asperellum* T42. Additionally, nutritional qualities of the seed, foliage, and pericarp of the chickpea plants, viz., total phenolic and protein content, carbohydrate content, total flavonoid content, and reducing power, were also found to be increased during seed bio-priming. There was also increment in number of branches, heads per plant, diameter of head, grain number per head, grains per plant, 1000 grain weight, oil content, and grain yield in safflower bio-primed with *Pseudomonas* spp. (Sharif 2012). Recently, Singh et al. (2018) demonstrated that treatment with *Trichoderma asperellum* T42 enhanced nitrogen utilization in tobacco. Treatment with microbial consortium of *Pseudomonas fluorescens* OKC, *Trichoderma asperellum* T42, and *Rhizobium* sp. RH4 was found to have a significant role in enhancement of seed germination and seedling growth in both chickpea and rajma (Yadav et al. 2013). It is also well reported that certain microbes also have the ability to produce vitamins and other nutrients. *Azotobacter vinelandii* strain ATCC 12837 and *A. chroococcum* strain H23 produce the vitamin B group members, viz., niacin, pantothenic acid, thiamine, riboflavin, and biotin, after 72 h of growth in chemically defined media (Revillas et al. 2000). Therefore, it can be claimed that suitable seed bio-priming can improve nutritional value of the plant and plant harvest.

8.4 4Role of Seed Bio-priming in Biotic and Abiotic Stress Management

In natural environment plants continuously face biotic and abiotic challenges, which are also increasing day by day as an outcome of the changing climate. These environmental stresses are becoming continuous threat to plant production as well as productivity (Sarma et al. 2012). The adverse effect of chemical pesticides is well known, and it is the main reason to emphasize the microbe-mediated alleviation of these stresses. Beneficial microbial consortium efficiently protects the plant and boosts the tolerance level of plants under stresses (Ray et al. 2017). Several reports are available in relation to PGPR-mediated stress tolerance and increased yield,

viz., in pea (Jain et al. 2013; Patel et al. 2016), lettuce (Kohler et al. 2009), chickpea (Sarkar et al. 2014), wheat (Jaderlund et al. 2008; Chakraborty et al. 2013; Nadeem et al. 2013; Kumar et al. 2014), maize (Rojas-Tapias et al. 2012), rice (Bal et al. 2013; Jha et al. 2013; Lavakush et al. 2014), broad bean (Younesi and Moradi 2014), soybean (Masciarelli et al. 2014), and groundnut (Paulucci et al. 2015). An increase in phenolic contents was found during pathogen infection site, i.e., in the collar region and leaves of chickpea during challenged inoculation with *Sclerotium rolfsii* (Singh et al. 2014). Similarly, a significant increment of total phenolic, flavonoid, ascorbic acid, and protein contents, free radical-scavenging activity, hydroxyl radical-scavenging activity, iron chelation, and reducing power were reported in the seeds and pericarp of pods of pea treated with the microbial consortium comprising *Bacillus subtilis* BHHU100, *Trichoderma harzianum* TNHU27, and *Pseudomonas aeruginosa* PJHU15 (Jain et al. 2014). It is also well recognized that the mixtures of various strains of *Trichoderma* perform better in plant disease management than an individual strain (Kumar et al. 2016). Singh et al. (2013a, b) also showed that high and uniform deposition of lignin in cambial cells of chickpea during challenge with *S. rolfsii* when bio-primed with the triple microbial consortium (*P. aeruginosa* PHU094, *T. harzianum* THU0816, *Mesorhizobium* species RL091) compared to the individual microbial applications.

Systemic resistance is the type of tolerance of plant against a wide range of plant pathogens induced by the interaction with a mild strain of the pathogen. Seed bio-priming provides significant resistance through induced systemic resistance. A wheat endophytic bacterium (*Pseudomonas aeruginosa* PW09) triggers the induced systemic resistance against *Sclerotium rolfsii* infection and increased biomass in cucumber under NaCl stress by enhancing the phenylpropanoid metabolism, antioxidant activities, and proline accumulation (Pandey et al. 2012). Patel et al. (2016) reported the *Pseudomonas fluorescens* OKC and *Trichoderma asperellum* T42 mediated induced expression of *LOX1* and *COI1* that lead to activation of JA-mediated resistance in pea against *Erysiphe pisi*. Several mechanisms of bio-priming mediated stress management classified and they are as follows:

- Mycoparasitism
- Antibiosis
- Induced phenolic production
- Activation of antioxidant production
- Vigorous growth of the plant
- Increased uptake and nutrient status
- Induced expression of plant defense-related genes
- Systemic defense activation

8.5 Conclusion and Future Prospects

Microbial seed priming or seed bio-priming is one of the most economical methods which require small volume of inoculums. Microbial seed priming enhances plant growth promotion by producing different phytohormones as well as enhances

nutritional value of the plant products. Seed bio-priming also induces defense responses in plants against biotic and abiotic stresses. But it is important to note that different crop seeds show variation in shape, size, and nature of seed coat. Due to this, it is important to standardize the microbial concentration and time of priming for each crop. Few available records explain that some crops enhance plant growth even at very low microbial concentration. This will not minimize the application of higher microbial concentration but also reduce the cost of seed priming. Several aspects such as proper formulation development and mechanistic insight of the defense responses as well as plant growth promotion require elaboration in near future. Seed bio-priming will be the best tool to overcome the environmental stresses in a sustainable way under the continuously increasing demand of chemical-free produces in the present scenario.

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Potential of Biopriming in Enhancing Crop Productivity and Stress Tolerance

9

Ahmad Mahmood and Ryota Kataoka

Abstract

Appropriate application of beneficial microbes to the seed or soil is essential for their efficacy and efficiency. Several methods like soil application, seed inoculation, root dipping, foliar application, and seed coating or covering have been employed, yet with the increase in concerns regarding survival of microbes on seed surface, and increasing focus on endophytes has led to search for potential methods ensuring the survival and colonization of the seed by the required microorganisms. To overcome the problems of lack of uniformity in seed emergence, poor seedling vigor, and establishment, researchers have come up with the technique of seed priming, which employing activation of physiological processes prior to sowing has certain advantages. Incorporation of hydration or immersion of seeds in microbial suspension, and/or seed covering in case of fungi, followed by incubation for a predetermined duration, and subsequent drying termed as biopriming have shown better survival, and colonization of desired microbes on/in the seeds providing plant growth promoting, and stress tolerance activities when used in many crops. A diversity of biopriming methods have been used with varying potential of plant growth-promoting activities as well as promising results in biocontrol of pathogens.

Keywords

Seed biopriming · Plant growth-promoting microorganisms · Crop growth · Stress tolerance

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Abbreviation

CFUs Colony forming units

9.1 Introduction

Increasing food requirement for continuously on the rise population has forced the scientific and farming community to look for enhancing crop yields within the available resources (Seufert et al. 2012) besides avoiding damage to the environment (Goswami et al. 2016). The productivity of crops is hindered by several factors, where poor stand establishment (Grassbaugh and Bennett 1998) and incidence of seed-borne or early pathogens on seedling stage lead to reduced crop yields (Orzolek 1991). Enhancing the production on the cost of environment is also discouraged, so there is need to search for sustainable production technology to meet the requirements. Pre-sowing techniques come as an opportunity to enhance the stand establishment inclusive of enabling the plant to cope the stress without compromising on yield, coupled with no or minute deleterious effect on the surroundings.

Activation of physiological processes without the emergence of the radicle of seed through seed priming has gained importance in crop production. Initially, the technique aimed to bring up uniform seed germination (Parera and Cantliffe 1994) and better stand establishment, but with the time and research, it has been used for enhancing the crop growth jointly with stress resistance (Hossain et al. 2015), where its economical and effective application has after 'been debated' Ashraf and Foolad (2005). The priming treatment has been used extensively in crops like barley, lentil, rice, and sunflower, for speeding up their germination and emergence (Saglam et al. 2010) and growth, viz., stand establishment, also under stress conditions like drought (Ahmed et al. 2016; Farooq et al. 2013; Kaya et al. 2006; Rouhollah 2013; Sun et al. 2010; Tabatabaei 2013; Yan 2015), salinity (Abraha and Yohannes 2013; Ellouzi et al. 2017; Jisha and Puthur 2016), heavy metal stress (Galhaut et al. 2014; Moulick et al. 2016), low temperature or chilling (Ahmad et al. 2015; Yadav et al. 2011), and high temperature (Parera et al. 1993). Seed priming includes different techniques, namely, hydropriming, osmopriming, thermopriming, matrix priming, hormonal priming, osmohardening, halopriming, and biopriming (Farooq et al. 2017; Jisha et al. 2013).

Seed priming enhances the plant growth and stress tolerance through different mechanisms (Shehab et al. 2010). The mechanisms in enhancing plant growth (uniform and quick germination, better plant establishment) and stress tolerance (activation of enzymes (Farhad et al. 2011; Posmyk et al. 2009), enhancing the water uptake (Farooq et al. 2009), and accumulation of sugar and proline within the seedlings (Yan 2015)) are notable. The enhanced and uniform germination is a response to reduced imbibition duration (Brocklehurst and Dearman 2008), activation of the enzymes (Lee and Kim 2000), and enhanced metabolism (Farooq et al. 2006). Similarly, for the stress tolerance, activation of the cellular defense mechanisms and enzymes (Hussain et al. 2016) prior to the stress in the field leads to better

stress tolerance in the plants, be it biotic or abiotic. Activation of antioxidant mechanism in tobacco through seed priming has been reported (Xu et al. 2011).

The environmental and sustainability factors are major motives behind the preference of biological or organic fertilizers over the chemical ones. The biological fertilizers also termed as biofertilizers include certain microorganisms, plant growth-promoting microorganisms (PGPM), including plant growth-promoting bacteria (PGPB), plant growth-promoting fungi (PGPF), and plant growth-promoting cyanobacteria (PGPC). Potential of beneficial microorganisms in enhancing crop productivity and combating stress conditions has been widely discussed and reviewed (Ahemad and Kibret 2014; Glick 2012; Goswami et al. 2016; Hayat et al. 2010; Santoyo et al. 2016; Souza et al. 2015). PGPB include the bacteria showing positive effects on the plants, either free living in the rhizosphere or endophytic, living within the plants without showing symptoms of any damage, and enhance the plant growth and stress tolerance through different mechanisms, viz., symbiotic and nonsymbiotic nitrogen fixation; facilitation of nutrient uptake including phosphorus, potassium, and iron; release of certain metabolites involved in plant growth promotion and stress tolerance; and remediation of organic and inorganic pollutants (Ahemad and Kibret 2014; Meena et al. 2015; Santoyo et al. 2016). Similarly, PGPF have showed different mechanisms of PGP, including supply of nutrients to the plants, mobilizing micronutrients, increasing the surface area of the roots, and release of certain metabolites (Kumar 2016), and also reduce the infestation of plant diseases (Hossain et al. 2017), where arbuscular mycorrhizal fungi (AMF) and *Trichoderma* hold important position. PGPC are responsible for enhancing the crop growth through nitrogen fixation and release of metabolites, improving soil fertility by soil aggregation, and increasing water-holding capacity (Shariatmadari et al. 2013). Efficient application of these PGPM is necessary for taking better advantage of all the PGP mechanisms showed by them (Compant et al. 2005; Fan et al. 2012; Ongena and Jacques 2008; Weller 2007).

Application of biofertilizers mainly bacteria has long been carried out through seed inoculation by the farming community, whereas instances of root dipping, soil incorporation, and foliar spray can also be observed, usually at research level. All the application methods have different merits and demerits (Mahmood et al. 2016) where there is need for a more effective application method, viz., survival of the bacteria collectively with efficiency of the latter. Among all the methods used, the bacteria are applied to the seeds either directly or in a formulation and are usually used immediately after application; however their survival remains inefficient (Wright et al. 2003a) so application of beneficial bacteria through priming is an attractive alternative. It has also been observed that a number of microorganisms increased during the process of priming, and the survival rate was not affected even by the subsequent drying (Wright et al. 2003b).

Biopriming or biological seed priming involves biological material for seed priming (Farooq et al. 2017). It has been explained as application of bacteria to the seeds during the hydration process as carried out in other seed priming techniques (Prasad et al. 2016), where application of fungus through seed biopriming has also been carried out. Among other biological materials used for seed priming, aqueous extracts

of the plants have been used (Aiyaz et al. 2015; Ghezal et al. 2016). This technique has been defined and explained differently by different researchers (Ashraf and Foolad 2005; McDonald 1999) and simply involves application of beneficial microbes to the seed in integration with the seed hydration (Rawat et al. 2011; Singh et al. 2016b), where application to the seedlings or plantlets has also been reported (Harish et al. 2008), also termed as biohardening (Panigrahi et al. 2016).

Application of endophytic bacteria or fungi through biopriming has potential benefits, as it allows sufficient time and environment for successful colonization of the seeds. The incubation duration allows the beneficial microbes to colonize the seed surface, also enter the seeds where needed. Biopriming and other seed-coating techniques have long been advocated for uniform application of biocontrol agents on the seeds (Bennett et al. 1992; Harman and Taylor 1988; Khan 1992; Kubik 1995; Lewis et al. 1987; Pill 1995; Warren and Bennett 1997), but the drying phase lacked information (Bennett 1998), which has been differently introduced by other researchers, usually drying in the shade for 24 h or overnight (Abuamsha et al. 2010; Carrozzi et al. 2012; Nandakumar et al. 2001). A 100% bacterial population increase was observed on the seeds of chickpea after 20-h incubation on sterilized vermiculite in talc-based formulation (Vidhyasekaran and Muthamilan 1995) which indicates that seed biopriming provides a microclimate favorable for the proliferation of microbes on the seeds.

Keeping in view the potential of biopriming in crop production, this chapter focuses on development of the technique, different methods used, and potential of this technique in enhancing plant growth, and productivity, and its role in ameliorating biotic and abiotic stresses.

9.2 Methods Used in Biopriming

Beneficial microorganisms can be applied to the soil, seed, and seedlings in different ways, mostly associated with their required function. In case of biological control, bacteria are generally coated on the seeds or the seedlings which create a competition environment and stop the pathogenic microbes' adhesiveness with the seeds or the seedlings. In general, when the aim of bacterial application is just to introduce the beneficial bacteria in the soil environment, then conventional inoculation method is accompanied with other methods discussed by Malik and Williams (2008). Uniformity in germination and better stand establishment options when considered, biopriming is favored method. Biopriming has been practiced and explained by different researchers (Bennett et al. 2009; Callan et al. 1991; Chakraborty et al. 2011; Gururani et al. 2012; Mirshekari et al. 2012; Moeinzadeh et al. 2010; Sharifi 2011; Sharifi and Khavazi 2011; Sharifi 2012) in several ways but needs to be explored and discussed further.

The term biopriming was first introduced by Callan et al. (1990), where they coated the sweet corn seeds with bacteria and immersed the seeds in warm water for imbibition of water up to 35–40%. Almost all of the later methods employed the soaking or immersion of the seeds in microbial suspension or similar treatments, except for the

biopriming of seedlings or plantlets (Harish et al. 2008; Panigrahi et al. 2016). Different incubation or hydration procedures have been reported, where the use of powdered lignite or coal has been reported by Harman and Taylor (1988) and plastic bag by Callan et al. (1990), both including moist conditions. The priming treatment should be carried out after surface disinfecting the seeds (Singh et al. 2016b), as there is a chance of pathogens presence on the seeds, which can reduce the proliferation of desired microbe on the seed by inducing competition (Wright et al. 2003b).

Among the methods reported, trash was removed from the wheat seed and was surface sterilized for 1 min in 70% ethanol and then treated in 5% sodium hypochlorite solution for 40 min, followed by rinsing six times with autoclaved distilled water. Seeds were further dried at room temperature and were subjected to priming in bacterial suspension (Saber et al. 2012). Abuamsha et al. (2010) primed the rapeseed seeds in a bacterial suspension having $\log_{10}$ 11 CFUs (colony forming units) mL^{-1}, which were prepared from 24 h TSB cultures by centrifugation at 25,000 × g for 10 min and subsequent dilution with the culture supernatant. Bacterial suspension was applied at the ratio 1 mL to 1 g seed, and the suspension kept on shaking at 150 rpm at 22 °C for 4 h followed by drying the seeds at 28 °C overnight. In another method, surface-sterilized seeds with 1% NaOCl for 1 min followed by rinsing with sterilized water were immersed in *Azospirillum brasilense* Sp245 inoculum containing 10^7 bacteria per seed suspended in 66 m mol L^{-1} phosphate buffer, pH 7 for 2 h at 20 °C. The seeds were then dried at 20 °C under airflow (Carrozzi et al. 2012). Similarly, surface disinfection of the seeds was carried out by 10% H_2O_2 according to method given by Miche and Balandreau (2001), and seeds were further soaked in bacterial suspensions containing 10^7 bacteria mL^{-1} for 2 h at 28 °C (Kasim et al. 2013). In another method explained by Gholami et al. (2009), they surface sterilized the maize seeds with 0.02% sodium hypochlorite for 2 min, rinsed thoroughly in sterile distilled water followed by coating of seeds with 20% Arabic gum, and then suspended the seeds in bacterial perlite mixture until the seeds were uniformly coated (Firuzsalari et al. 2012; Sharifi and Khavazi 2011; Sharifi et al. 2011).

For instance, Reddy (2013) suggested the soaking of the seeds in the water for 12 h followed by mixing of the bacteria at the rate of 10 g kg^{-1} seed. Then a heap of the treated seeds should be made which should be covered with moist jute bag to ensure enough humidity. Seeds should be further incubated in highly humid conditions for 48 h at temperature range of 25–32 °C. Bio-agent thus added gets very good conditions for the growth and forms a protective layer around the seed which restricts the attachment of pathogenic bacteria to the seeds. This method is mainly characterized to be used in biocontrol. In another report, trash was removed from the wheat seed and the former was surface sterilized for 1 min in 70% ethanol and then treated with 5% sodium hypochlorite solution for 40 min, followed by rinsing six times with autoclaved distilled water. Seeds were further dried at room temperature and were subjected to priming in bacterial suspension (Saber et al. 2012).

The other two methods widely used in seed biopriming include talc-based formulation and drum priming. In the talc-based priming, the bacterial suspension is mixed with talc powder, augmented also with calcium carbonate and carboxymethyl cellulose followed by subsequent drying of the seeds in shade for 24 h (Nandakumar

et al. 2001). In drum priming, used commercially for pesticide application to the seeds and initially introduced by (Rowse 1996), the seeds are soaked in the aqueous bacterial suspension in the continuously shaking drum for predetermined time and suspension volume, followed by drying (Wright et al. 2003a).

9.3 Role of Biopriming in Increasing Crop Productivity

Biopriming plays its role in improving seed viability, germination, plant vigor, growth, and yield (Prasad et al. 2016) and can significantly enhance the seed germination and uniformity in emergence of seedlings (Bhatt et al. 2015) along with stand establishment (Nakkeeran et al. 2005). Like the other seed priming treatments, biopriming helps in starting the physiological processes pre-sowing and helps in multiplication of PGPR in the area surrounding the seed (Taylor and Harman 1990). Several researchers have employed the biopriming in enhancing the crop growth and productivity. Table 9.1 presents the studies documenting positive effects of biopriming with different bacterial and fungal strains on various crop plants. Different applications on barley, carrot, maize, safflower, and sunflower have been reviewed previously by Mahmood et al. (2016).

Application of *Pseudomonas aureofaciens* through drum priming system enhanced the stand establishment in tomato (Warren and Bennett 1997). Application of various other PGPM in different crops through biopriming as compiled in Table 9.1 presents that wide application of *Trichoderma* species to plants for plant growth promotion and biocontrol has long been practiced (Altomare et al. 1999). Application of *Trichoderma* has gained more importance in plant stress tolerance, especially biotic stress; however its application for PGP has also been documented. Applied to pea seeds as biopriming agent, *Trichoderma* significantly enhanced the plant growth parameters, with biopriming concluded as an efficient method (Singh et al. 2016a). Among other biopriming applications of *Trichoderma*, it increased the growth of wheat, furthermore nitrogen uptake and recovery, and agronomic and physiological use efficiency and also performed well even under 75% of the recommended dose of the fertilizer (Meena et al. 2016). Similarly, in an experiment featuring six crops, namely, brinjal, chili, guar, okra, ridge gourd, and tomato, Singh et al. (2016b) observed that the seed germination depended on specific dose of *Trichoderma* spores, where the latter enhanced plant growth and induced systemic resistance-related enzyme activity. Other than that, response of snapdragon to the biopriming with *Trichoderma* and *Bacillus subtilis* was documented, where the authors observed increased germination and vegetative and reproductive growth (Bhargava et al. 2015). In another experiment, *Trichoderma* and *Pseudomonas* application through biopriming with varying duration was investigated, and *Pseudomonas* enhanced the seedling growth and vigor better than *Trichoderma*, but both proved to be way better when compared with non-primed seeds (Ananthi et al. 2014). Application of *Trichoderma* through seed biopriming also enhanced the enzyme activity through release of certain metabolites in maize plant (López-Coria et al. 2016). Finally, the application of two PGPR strains through priming enhanced

Table 9.1 Role of seed biopriming with different plant growth-promoting microorganisms in promoting plant growth

Strain	Crop	Crop response	Experiment	Priming duration	References
Trichoderma asperellum	Maize	Increased root length, shoot length, plasma membrane, and H^+-ATPase activity	Laboratory	1.5 h	López-Coria et al. (2016)
Trichoderma harzianum	Wheat	Increased plant height, chlorophyll content, root length, and effective tillers	Pot	30 min	Meena et al. (2016)
Trichoderma asperellum	Pea	Increased shoot length, root length, number of leaves, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight	Pot	–	Singh et al. (2016a)
Trichoderma asperellum	Brinjal, chili, guar, okra, ridge gourd, tomato	Increased seed germination, radicle length, number of leaves, shoot length, root length, chlorophyll, phenylpropanoid, and lignifications activity	Laboratory and pot	24 h	Singh et al. (2016b)
Trichoderma harzianum Bacillus subtilis	Snapdragon	Increased germination, number of leaves, shoot length, root length, plant height, spreading of the plant, number of spikes, total number of florets per spike, and flowering duration	Laboratory	24 h	Bhargava et al. (2015)
Trichoderma viride or Pseudomonas fluorescens	Chili	Increased germination, root length, shoot length, biomass, and seedling vigor index	Laboratory	3 h, and 12 h	Ananthi et al. (2014)
Azotobacter chroococcum Azospirillum lipoferum	Barley	Increased 1000 grain weight, grain yield, biological yield, and harvest index	Field	–	Mirshekari et al. (2012)

– Indicates no data given, h stands for hours, and min stands for minutes

the growth and yield of barley at different fertilizer levels (Mirshekari et al. 2012). Promotion of plant growth and productivity by the PGPR and PGPF applied through priming has been linked to PGP activities, and mechanisms of growth promotion by beneficial bacteria and fungi have been extensively reviewed (Goswami et al. 2016). Major microbial contributions toward enhanced plant productivity include nitrogen fixation; enhanced solubility, and availability of phosphorus, potassium, and iron; production of hormones, vitamins, enzymes, and organic acids from bacteria; facilitation of nutrient uptake through hyphal networks; and release of several metabolites from fungi, inclusive of quicker seed emergence, more vigorous seedlings, and better stand establishment due to seed priming treatments.

9.4 Role of Biopriming in Stress Tolerance

The crop plants are prone to seed- and soilborne diseases, resulting in heavy yield losses, and are usually controlled chemically; however, biopriming, being an environment-friendly, economical, and effective technique (Ashraf and Foolad 2005), is being focused for control of biotic and abiotic stresses. Bennett (1998) advocated the use of microorganisms as biopriming agents for enhanced seedling growth under stress conditions. An increase of tenfold on the seeds was observed in the population of applied antagonist by Callan et al. (1990), which ultimately helps to control the plant pathogens in the rhizosphere. Biopriming with PGPB can provide systemic resistance against multitude of pathogens (Compant et al. 2005), in combination with uniform germination in stress conditions (Singh et al. 2003), which suggests its use against different type of stresses.

9.4.1 Role of Biopriming Against Biotic Stress

Seed- and soilborne diseases pose threat to crop productivity and are often dealt with chemical pesticides, which are not sufficient against pathogenic attack on late stages of the plant from aerial parts. Under these circumstances, biopriming serves as an attractive approach, in which microbes keep on multiplying and form a biofilm around the root surface supplementary to putting competition by occupying the space, which helps reduced or no infestation by the pathogens, even in the later stages of the plant (Prasad et al. 2016). The biopriming with plant beneficial bacteria also helps in initializing the systemic resistance in the plants.

From the initial reports, it was observed that the seeds of carrot already infested with *Alternaria radicina* and *Alternaria dauci* when bioprimed with antagonistic fungi *Clonostachys rosea* reduced the incidence of these pathogens (Jensen et al. 2001). Similar instances including different bacterial and fungal biopriming agents against several pathogens including *Colletotrichum* (Begum et al. 2010), *Fusarium* (Mnasri et al. 2017; Nayaka et al. 2010; Srivastava et al. 2010), *Rhizoctonia*, *Sclerotium* (El-Mougy and Abdel-Kader 2008), and *Verticillium* (Rybakova et al. 2016) have been compiled in Table 9.2.

Table 9.2 Role of seed biopriming with different plant growth-promoting microorganisms in combating biotic stress

Strain	Crop	Pathogen/disease controlled	Crop response	Experiment	Priming duration	References
Rhizospheric bacteria, Endophytic bacteria	Wheat	*Fusarium culmorum*	Increased germination and seedling vigor, decreased disease incidence	Pot	24 h	Mnasri et al. (2017)
Serratia spp. *Paenibacillus* spp.	Oilseed rape and cauliflower	*Verticillium dahlia*, *Verticillium longisporum*	Increased root weight, weight of the green parts, and germination	Laboratory and pot	4 h	Rybakova et al. (2016)
Trichoderma harzianum, *Trichoderma virens*, *Pseudomonas aeruginosa*	Soybean	*Colletotrichum truncatum*	Increased seed germination and seedling establishment	Field	12 h	Begum et al. (2010)
Trichoderma harzianum	Maize	*Fusarium verticillioides*	Increased seed germination, vigor index, field emergence, yield, and thousand seed weight	Pot	24 h	Nayaka et al. (2010)
Pseudomonas fluorescens, *Trichoderma harzianum*	Tomato	*Fusarium oxysporum*	Increased seed germination, decreased germination time, and disease incidence	Pot and field	24 h	Srivastava et al. (2010)
Trichoderma viride, Trichoderma harzianum, Bacillus subtilis, Pseudomonas fluorescens, Trichoderma hamatum, Bacillus cereus	Faba bean	*Rhizoctonia solani, Fusarium solani*, *Sclerotium rolfsii*	Seed storage experiment[a]	Greenhouse and field	16 h	El-Mougy and Abdel-Kader (2008)

[a]The experiment investigated the efficacy of priming agents against disease causing pathogens in different storage durations
– Indicates no data given, h stands for hours, and min stands for minutes

Similar to the crop productivity, biopriming with *Trichoderma* has been used extensively in controlling plant pathogens. The seeds of soybean were bioprimed with *Trichoderma harzianum*, *Trichoderma virens*, and *Pseudomonas aeruginosa* separately and in consortium against damping-off causing *Colletotrichum truncatum*, and it was observed that population of bacteria on the seeds increased more than four times after 12 h, where germinating hyphae were observed in case of fungi, and reduced the disease incidence up to 97.2% together giving healthy seedlings (Begum et al. 2010). In case of biopriming through immersion of the maize seeds in conidial suspension of *Trichoderma harzianum* against *Fusarium*, it was observed that *Trichoderma* pure culture was more effective in controlling disease incidence, whereas talc formulation stood second when compared with no priming and fungicide applied treatments (Nayaka et al. 2010). Similarly, another experiment employing *Trichoderma*, *Pseudomonas*, and *Glomus* spp., tomato wilt was significantly decreased up to 74%, where seed germination time decreased and seed germination was enhanced (Srivastava et al. 2010). In a recent report, biopriming of wheat seeds with free-living and endophytic bacteria resulted in antagonism through contact and production of volatile compounds, thus reducing the disease incidence and also enhancing the stand establishment (Mnasri et al. 2017). Application of *Serratia* and *Paenibacillus* spp. through biopriming helped control the pathogen *Verticillium* in rapeseed and cauliflower where the authors recommended use of non-sterile soil for related experiments (Rybakova et al. 2016). In a different experiment, investigating the efficacy of biopriming agents against root rot pathogens of faba bean, the seeds were stored for long durations, and it was concluded that disease incidence was completely controlled up to 2 and 3 months of storage; however less protection was observed when the seeds were stored for 4 and 6 months (El-Mougy and Abdel-Kader 2008).

The biopriming agents successfully colonize the seed surface and inner parts in case of endophytes, which compete with pathogens for space and nutrients, thus leading to decreased incidence of the disease-causing organisms. For instance, the seed-inhabiting microbes also release certain antibiotics, organic acids, and metabolites to limit the approach of foreign organisms. Additionally, the preference showed by the plant through certain exudates also helps the applied microorganisms retain the spermosphere combined with xylem and phloem of the plant roots and stem. Briefly, the microbial antagonistic activities include ACC deaminase assisted regulation of plant ethylene levels in response to pathogenic infections (Van Loon 2007), release of siderophores and antibiotic metabolites (Beneduzi et al. 2012), production of enzymes responsible for pathogenic fungus cell lysis (Maksimov et al. 2011; Neeraja et al. 2010), and antagonism in case of space and nutrients (Kamilova et al. 2005).

9.4.2 Role of Biopriming Against Abiotic Stress

Biopriming significantly enhances the stress tolerance in the plants through various mechanisms as discussed above. Biopriming has gained more importance as a biocontrol agent due to its effectivity against pathogenic microbes; however use of biopriming agents against abiotic stress conditions can also be found. The use of biopriming against drought, osmotic, and salinity stress, as compiled in Table 9.3,

Table 9.3 Role of seed biopriming with different plant growth-promoting microorganisms in combating abiotic stress

Strain	Crop	Stress ameliorated	Crop response	Experiment	Priming duration	References
Trichoderma lixii	Maize	Salinity	Increased length, fresh and dry weights of root/shoots, relative water content, soluble protein, proline, chlorophyll, and carotenoid content	Pot	1 h	Pehlivan et al. (2017)
			Decreased lipid peroxidation, hydrogen peroxide, and lipid peroxidation			
Thalassobacillus denorans Oceanobacillus kapialis	Rice	Salinity	Increased germination, root and shoot lengths, fresh and dry weight of seedlings, chlorophyll and carotenoid contents, total nitrogen and protein contents, Ca^{+2} and K^{+} ion concentration	Pot	30 min	Shah et al. (2017)
			Decreased Na^{+} ion concentration			
Enterobacter spp.	Tomato	Osmotic/drought	Increased germination percentage and germination rate	Laboratory	24 h	Bhatt et al. (2015)
Citricoccus zhacaiensis	Onion	Osmotic	Increased germination, seedling vigor, and germination rate	Laboratory	24 h	Selvakumar et al. (2015)
Trichoderma harzianum	Wheat	Drought	Decreased proline, malondialdehyde, and hydrogen peroxide concentration, increased total phenolics content, and L-phenylalanine ammonia-lyase activity	Pot	24 h	Shukla et al. (2015)
Pseudomonas aeruginosa	Sunflower	Salinity *Also biocontrol against *Macrophomina phaseolina*	Increased germination, root length, shoot length, fresh weight, dry weight	Laboratory	10 min	Tewari and Arora (2014)
Trichoderma harzianum	Wheat	Salinity	Increased germination, root and shoot lengths, chlorophyll and membrane stability index, proline content, and phenolics concentration, Decreased salinity and malondialdehyde	Pot	–	Rawat et al. (2011)

– indicates no data given, h stands for hours, and min stands for minutes

has been observed in onion (Selvakumar et al. 2015), sunflower (Tewari and Arora 2014), tomato (Bhatt et al. 2015), and wheat (Rawat et al. 2011; Shukla et al. 2015).

Among abiotic stress amelioration by biopriming, *Trichoderma* has been used in controlling salinity and drought stresses, where *Trichoderma lixii* when used in maize (Pehlivan et al. 2017) and *Trichoderma harzianum* in wheat (Rawat et al. 2011) showed better physiological and morphological parameters when compared with untreated control. Use of *Trichoderma harzianum* as biopriming agent against drought stress was studied in wheat crop, where it was reported that no application of water from 4 to 13 days resulted in increased concentration of stress-related enzymes and metabolites such as phenolics and decreased concentration of hydrogen peroxide, malondialdehyde, and proline (Shukla et al. 2015). The bacterial applications have been employed in combating the salinity, osmotic, and drought stress. *Thalassobacillus denorans* and *Oceanobacillus kapialis* isolates from salt mine showing halophilic behavior enhanced the growth of fine rice variety under varying salinity concentrations and showed improvement in morphological and physiological parameters after 15 and 28 days, respectively, when applied through biopriming (Shah et al. 2017). In a laboratory experiment, tomato seeds were treated with different *Enterobacter* strains and under five different levels of osmotic stress, viz., 0, −0.2, −0.4, −0.6, −0.8, and −1.0 MPa, and it was observed that seedling vigor was enhanced in combination with germination (Bhatt et al. 2015). In another similar instance, with an exception of stress levels, i.e., 0, −0.2, −0.4, −0.6, and −0.8 MPa, *Citricoccus zhacaiensis* showing production of hormones, ACC deaminase, and ammonia and solubilization of zinc and phosphate under stress conditions, when applied in onion, enhanced the seedling growth under stress conditions (Selvakumar et al. 2015). Additionally, application of *Pseudomonas aeruginosa* having the capability of producing exopolysaccharides at a concentration as high as 2000 mM NaCl, through seed biopriming in sunflower, significantly enhanced plant growth, along with control of pathogen *Macrophomina phaseolina* under stress conditions (Tewari and Arora 2014).

Abiotic stress tolerance can be due to several mechanisms where microorganisms contribute toward the drought tolerance by production of abscisic acid and gibberellic acid (Cohen et al. 2009). Other physiological mechanisms helping the plants tolerating drought stress include modifications in root architecture, photosynthetic and photo-protective pigments, increase in proline content, and decreased stomatal conductance (Beckett et al. 2012; Piccoli and Bottini 2013). Similarly, the 1-aminocyclopropane-1-carboxylate (ACC) deaminase production by the PGPB leads to regulation of ethylene levels within the plants, helping them cope better with salinity stress. Additionally, the stimulation of the tree for exudation by the PGPM to release organic acids for chelating sodium ions in the soil (Li et al. 2007), and siderophore production can also ameliorate the effects of certain stress conditions. The PGPR also change the selectivity of certain ions like sodium, potassium, and calcium in the plants to tolerate the salinity (Hamdia et al. 2004).

9.5 Limitations and Future Prospects

Storage and short shelf life of the bioprimed seeds are a limitation of this technology. Instances of reduced population of bacteria or fungi on the seed indicate that there is need for appropriate storage technology (Callan et al. 1997; Warren and Bennett 1999), which may include environment regulation along with use of additives for long-term survival of microbes. Investigation of seed storage duration on the population of biopriming agents on the seeds concluded lesser protection after seed storage for 4–6 months (El-Mougy and Abdel-Kader 2008), so enhancing the shelf life of bioprimed seed lots needs to be explored. Secondly, the contamination from the endophytic pathogens remains a problem, although the population of beneficial endophytes applied can help limit the formers' activity, yet the seed disinfection prior to biopriming should include this aspect and should be further investigated. Similarly, the introduction of on-farm seed biopriming would help the farmers apply desired microbes when necessary, but for this, priming technique should be as easy as hydropriming which will encourage farmers to use and also will reduce the chances of contamination, desiccation, and seed damage.

9.6 Conclusion

Application of beneficial microbes with the aim of enhancing crop productivity has long been practiced, and with the time and research, new methods of application have been introduced, including biopriming. Biopriming, an amalgamation of seed priming with application of plant beneficial fungi and bacteria, can significantly improve seed germination and emergence, seedling establishment, crop growth, and yield parameters under normal and stress conditions. Its use has been investigated in diverse field crops and has shown potential benefits, with diversity of methods reported.

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Stimulating Plant Tolerance Against Abiotic Stress Through Seed Priming

10

Mona Gergis Dawood

Abstract

The seed priming process is a physiological method that involves seed hydration (usually within 10–20% of full imbibition) and effective enough for enhancement of seed germination, early seedling growth, and yield under stressed and non-stressed conditions but insufficient to allow radicle protrusion. Primed seeds germinate faster and more uniformly than the non-primed ones. Seed priming is influenced by many factors such as aeration, light, temperature, time, and seed quality and induced a set of biochemical changes in the seed which are required for initiating the germination process. These changes include activation of enzymes, breaking dormancy, metabolism of germination inhibitors, and imbibition. The positive effects of priming on the germination performance of many species are attributed to the induction of biochemical mechanisms of cell repair: the resumption of metabolic activity can restore cellular integrity, through the synthesis of nucleic acids (DNA and RNA) and proteins and the improvement of the antioxidant defense system. Several methods of seed priming were successfully used in agriculture for seed conditioning to accelerate the germination rate and improve the seedling uniformity such as seed priming with water (hydropriming), plant growth regulators, beta-aminobutyric acid, 5-aminolevulinic acid, osmoprotectant, melatonin, chitosan, plant extract, polyethylene glycol, and inorganic salts. It is worthy to mention that all these methods showed pronounced effect on germination, seedling growth, and yield of different crops under normal or stress conditions.

Keywords

Seed soaking · Plant resistance · Abiotic stress · Germination quality

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10.1 Introduction

In nature, plants often face the challenge of severe environmental conditions, which include various biotic and abiotic stresses that exert adverse effects on plant growth and development causing considerable losses in the crop productivity. Plants are subjected to a variety of abiotic stress such as salinity, drought, high temperature, low temperature, etc. that reduced germination rate and seedling growth with significant variations from crop to crop (Hamidi and Safarnejad 2010). Abiotic stress causes many physiological and biochemical changes in the seedlings, which include the generation of reactive oxygen species (ROS), leading to membrane damage and cell leakage and destruction of photosynthetic components (Jisha and Puthur 2014). ROS are known to exacerbate imbalance between light absorption and its utilization by inhibiting Calvin-Benson cycle activity (Logan et al. 2006). ROS also reduce content and activity of ribulose bisphosphate carboxylase oxygenase (RUBISCO) enzyme which leads to higher electron flux to O_2 coupled with reduced CO_2 accumulation (Zhou et al. 2006; Ahmad et al. 2015), and they can be extremely reactive with several cellular constituents such as proteins, lipids, and nucleic acids (Hasanuzzaman et al. 2013), which in turn results in negative effects on metabolism and cellular structures (França et al. 2007; Mouradi et al. 2016). Free radical oxidations and protein enzymatic dehydrogenation and aldehyde oxidation might contribute to seed quality reduction (Ghassemi-Golezani et al. 2010).

In many plants, germination, seedling growth, and subsequent crop yield can be inhibited by salinity stress (Soccio et al. 2010; Carvalho et al. 2011). *Salinity* has an adverse effect on seed germination and seedling growth of several crops either by creating an osmotic potential in the rhizosphere of the plant that inhibits the absorption of water or creates toxic effect to the roots and whole crop because of Na^+ and Cl^- (Khajeh-Hosseini et al. 2003; Munns and Tester 2008). Moreover, salinity has a negative effect on plasma membrane by affecting on its permeability which in turn modulates the pattern of ion leakage (Sairam et al. 2002). Salt tolerance of plants can be increased by treatment of seeds with NaCl solution prior to sowing (Sivritepe et al. 2003). Farhoudi et al. (2007) reported that priming of canola seed decreased the absorption of harmful ion and cell damage of canola seedling under salinity stress.

Drought is one of the most important environmental factors limiting plant growth and productivity. Soltani et al. (2008) mentioned that as the drought severity increased, the germination rate linearly decreased in unprimed cotton seeds, but primed seeds had lower reduction slope. Moreover, Khatami et al. (2015) stated that some priming treatments were sufficient to invigorate the corn seed germination under drought stress, since hormone priming under moderate drought and osmopriming under severe drought were the best methods for seed improvement.

Low-temperature conditions decreased plant growth rate because of inhibition of photosynthesis and increasing photooxidative injury of the photosystems. Photooxidative damage caused lipid peroxidation and degradation of chlorophyll and carotene. Plants exert many physiological and biochemical changes under low-temperature conditions that make them survive under these

conditions (Xin and Browse 2000). In general, physiological and biochemical dysfunctions induced by low temperature in plants are not equal and most of them can be convert if plant return to optimum condition before appearance of damage, since disturbances of physiological and biochemical functions are reversible.

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. In general, a transient elevation in temperature, usually 10–15 °C above ambient, is considered heat shock or heat stress. However, heat stress is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature. The extent to which it occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or the night (Wahid et al. 2007a).

The time from sowing to plant establishment is a crucial period in crop growth (Bray 1995), with a direct impact on final yield and quality (Gupta et al. 2008) especially under abiotic stress. So, several ways for enhancing the plant tolerance toward abiotic stress have been experimented like breeding of plants and developing transgenics (Jisha et al. 2013). Moreover, some studies on different plants showed that seed priming treatments have effectively increased germination and seedling growth either at normal conditions or stressed conditions (Bradford 1986). Since, efficient seed germination, rapid and uniform seedlings emergence lead to successful culture establishment (Chen and Arora 2011).

10.2 Seed Priming

Seed priming is a simple, safe, economic, and effective approach for enhancement of seed germination, early seedling growth, and yield under stressed and non-stressed conditions (Sedghi et al. 2010). Seed priming is a form of seed preparation in which seeds are presoaked before planting with a certain solution that allows partial hydration but not germination and redried to original moisture content (Ahmad et al. 2012). The seed priming process is a physiological method that involves seed hydration (usually within 10–20% of full imbibition) (Pill 1995), sufficient to permit pre-germinative metabolic events to proceed, but insufficient to allow radicle protrusion (Bradford 1986). Primed seeds germinate faster and more uniformly than the non-primed ones.

During priming, the germination process is not completed, but metabolic activities for radical protrusion may be initiated (Heydecker et al. 1973). At the cellular level, few processes have been described to act during priming, some of these being activation of cell cycle (De Castro et al. 2000) and mobilization of storage proteins (Gallardo et al. 2001). The priming process induces the rate of seed germination and is associated with the initiation of germination-related processes (Soeda et al. 2005) and repair processes (Sivritepe and Dourado 1995) and increases various free radical-scavenging enzymes, such as superoxide dismutase, catalase, and peroxidase (Gallardo et al. 2001).

Several seed priming methods were successfully used in agriculture for seed conditioning to accelerate the germination rate and improve the seedling uniformity (Nouman et al. 2012; Aghbolaghi and Sedghi 2014; Bagheri 2014; Lara et al. 2014). Moreover, seed priming helps many crops to neutralize the adverse effects of abiotic stress (Ashraf and Foolad 2005; Zhang et al. 2012; Hameed et al. 2013; Jisha et al. 2013; Jisha and Puthur 2016).

10.2.1 There are Three Principal Methods of Seed Priming

1. *Hydropriming (water-only treatments)*: soaking of seeds with water overnight and then drying before sowing markedly improve plant stand, establishment, vigor, and the final yield (Harris et al. 1999). Seeds are submerged in water with or without aeration. Therefore, water is freely available to seeds, its uptake only being governed by the affinity of the seed tissue for water (Taylor et al. 1998). As a main drawback, seed germination can proceed until radicle protrusion. Thus, the process needs to be stopped at a precise moment, before phase III begins. Another disadvantage is that seeds are not equally hydrated, which results in a nonuniform activation of the physiological processes necessary to synchronize and improve germination (McDonald 2000).
2. *Solid matrix priming or matri-priming*: hydrated solid matrices soaked with osmotic solutions (McDonald 2000), such as hydrated sand (Hu et al. 2005), peat, and vermiculite (Taylor et al. 1998), or cotton soaked with osmotic solutions; involves the use of a wet organic or inorganic material (Parera and Cantliffe 1994), which simulates the natural imbibition processes taking place in the soil (McDonald 2000). The substrate must possess the given characteristics: low matric potential, high seed safety, high specific surface (i.e., high surface to volume ratio), negligible water solubility, high adhesiveness to seed surface, and high capacity to retain water (Khan 1991).
3. *Osmotic priming (osmopriming)*: osmopriming is the process that involves the use of osmotic solutions with a low water potential to control seed water uptake. The most common substances used for osmopriming are polyethylene glycol (PEG), inorganic salts, mannitol, glycerol, and plant hormones (Taylor et al. 1998; Foti et al. 2002; Tiryaki and Buyukcingil 2009; Afzal et al. 2013; Farooq et al. 2015).

The three methods may be grouped into two categories: non-controlled water uptake (hydropriming) and controlled water uptake (osmopriming and solid matrix priming) (Taylor et al. 1998).

Bio-priming: Additional method includes seed coating with bacteria (bio-priming or bio-osmopriming), e.g., *Trichoderma* spp. (Pill et al. 2009; Begum et al. 2010) and *Pseudomonas aureofaciens* (Warren and Bennett 1999). Bio-priming has been able to control damping-off of seedlings in sweet corn (*Zea mays* L.) (Callan et al. 1990),

cucumber (*Cucumis melo* L.) (Pill et al. 2009), pea (*Pisum sativum* L.), and soybean (*Glycine max* (L.) Merr.) (Taylor et al. 1994).

10.2.2 Factors Affecting Seed Priming Process

Seed priming is influenced by many factors such as aeration, light, temperature, time, and seed quality.

1. *Aeration* is considered an important step to assist seed respiration (Bujalski and Nienow 1991) and seed viability and contributes to synchronize the emergence (Heydecker et al. 1975) and ensures a safer seed habitat. However, the effect of aeration varies according to species: in onion, aeration of the PEG solution increased the germination percentage, compared to non-aerated treatment (Heydecker and Coolbear 1977; Bujalski et al. 1989). By contrast, no difference was observed in the germination of lettuce between aerated and non-aerated K_3PO_4 priming (Cantliffe 1981).
2. *Light* effect is widely varied according to species. Khan et al. (1978) mentioned that illumination during priming of celery seeds may reduce dormancy. On the other hand, the best results with lettuce were obtained with priming in the dark (Cantliffe et al. 1981).
3. *Temperature* is another important variable, as it affects the speed of chemical reactions. Treatment temperature varied between 15 (several cases) and 30 °C (rice in hydropriming, Basra et al. 2005). Temperatures of about 15 °C during priming were shown to improve the overall seed performance in most species (Bradford 1986), whereas lower temperatures slowed the germination processes, requiring longer times to achieve the same results (McDonald 2000). The range of temperatures normally used in priming varies between 15 and 20 °C.
4. *Treatment duration* is widely varied according to species and experiment, from a minimum of 8 h (sunflower primed in a salt solution, Wahid et al. 2008) to a maximum of 14 days (four ornamental species, Finch-Savage et al. 1991). Treatment duration mainly depends on the type of osmotic solution, osmotic potential, temperature, and crop species. It also depends on the specific time and likelihood of radicle protrusion: long priming can more easily lead to this occurrence, creating irreversible damage during drying-back (Parera and Cantliffe 1994).
5. *Seed quality* is a key aspect influencing the effects of priming. A vigorous seed, free from pathogens is an essential requisite for a good priming result (Cantliffe et al. 1987), in contrast to the belief that this technique may improve the performance of seeds of intrinsic modest quality. Other seed characteristics may influence priming process. For instance, osmopriming with PEG solution is not suitable for seed treatment of sorghum with high content of tannin, because tannins can be removed with the solution treatment and determine a reduction of germination (Patanè et al. 2008). In fact, tannins reduce seed susceptibility to insects, birds, and mold diseases and protect from weathering (Beta et al. 1999).

In this specific case, it is advisable to adopt treatment solutions different from PEG or others technique, such as bio-priming (Patanè et al. 2008). Moreover, Bradford (1986) mentioned that composition of solution and osmotic potential had pronounced effect on seed priming.

10.3 Triphasic Model of Seed Imbibition

In orthodox seeds the dry seed, ready for germination, exhibits a triphasic pattern of water uptake (Bewley and Black 1978). *Phase I* is the rapid water uptake that is largely a consequence of the matric forces exerted by the seed. During this phase, DNA and mitochondria are repaired and proteins are synthesized using existing messenger ribonucleic acid (mRNA) (McDonald 2000). *Phase II* is a lag phase, in which seed water potential is in balance with that of the environment. In this phase the major metabolic changes preparing the embryo for germination occur, including the synthesis of mitochondria and proteins by new mRNA. Thus, phase II is also called activation phase. *Phase III* is a second rapid uptake of water occurs and the radicle emerges so called visible germination (Bewley and Black 1978; Bradford 1995). Phases I and II represent the most delicate phases for the process of germination and are crucial for a successful seed priming (Bewley 1997). The triphasic model has deep implications for seed viability. The seed tolerates a return to the initial moisture necessary for storage, a process known as drying-back or redrying, when it is in phase I or II, whereas phase III is too advanced to allow a drying-back without seed damage (Taylor et al. 1998). According to the triphasic model, the start of germination is associated with a rapid synthesis of RNA and proteins, to carry out the repairing processes before the beginning of DNA replication (Osborne 1983). Seed priming typically involves an extension of phase II, which in turn permits the completion of more repair processes (Bray 1995), and allows the drying-back, which is necessary when the final sowing is postponed (industrial seed production). The postponement of phase III involved in priming plus redrying results in a better seed performance under favorable conditions.

10.4 Biochemical Changes Induced by Priming

Seed priming induced a set of biochemical changes in the seed which are required for initiating the germination process. These changes include activation of enzymes, breaking dormancy, metabolism of germination inhibitors, and imbibition to start the germination process (Ajouri et al. 2004; Farooq et al. 2010). Some or all of these changes that precede germination are triggered by seed priming and persist following the redrying of seeds (Asgedom and Becker 2001). Thus, upon sowing, primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage (Rowse 1995). Moreover, seed priming may repair some damage in the membrane caused by deterioration and result in better germination pattern and higher vigor level compared with non-primed seeds (Ruan and Xue 2002).

Priming showed stimulatory effects in the early stages of germination by mediation of cell division in germinating seeds (Hassanpouraghdam et al. 2009). The increase in speed of germination and germination percentage may be due to the modification of physiological and biochemical nature of seed embryo and its associated structures, i.e., pre-enlargement of the embryo (Austin et al. 1969), and biochemical changes like enzyme activation; gibberellin-like substances may be released during phase II of germination which triggers the synthesis of hydrolytic enzymes that causes the early availability of high-energy compounds (Basra et al. 2005) and vital biomolecules to the germinating seedling (Renugadevi and Vijayageetha 2006).

The positive effects of priming on the germination performance of many species are attributed to the induction of biochemical mechanisms of cell repair: the resumption of metabolic activity can restore cellular integrity, through the synthesis of nucleic acids (DNA and RNA) and proteins and the improvement of the antioxidant defense system (Bewley and Black 1994; Di Girolamo and Barbanti 2012).

10.4.1 Effects on DNA

A strong increase in DNA synthesis occurs at the end of germination (phase III) in both primed and unprimed wheat grains, as shown by Dell'Aquila and Taranto (1986). Thereafter, an increase in DNA was observed 14 days after the seed priming, when the seed had entered the irreversible germination phase (Bray 1995). The positive effects of priming on DNA are offered by a study on *Brassica oleracea* L., where an aerated hydration determined an advance in DNA synthesis (Thornton et al. 1993). In spite of its crucial role, the amount of DNA which is needed in the repair processes is only 20–30% of the total DNA synthesized during priming. The rest is mainly represented by mitochondrial DNA; in fact, the number of mitochondria was shown to rapidly increase during priming in leek seeds (Ashraf and Bray 1993). The enhancement of DNA replication during priming depends on species, cultivar, seed lot quality (Lantieri et al. 1994), and treatment conditions (Ozbingol et al. 1999).

10.4.2 Effects on RNA

Priming allows the recovery of rRNA integrity (Coolbear et al. 1990), in turn ensuring a correct coding of amino acids for the synthesis of proteins during seed germination. Bray et al. (1989) proved that this accumulation involved rRNA (ribosomal RNA, 85% of total RNA) in a turnover between degradation of damaged rRNA and synthesis of new rRNA, while the level of mRNA (messenger RNA, 0.5% of total RNA) remained constant. rRNA is as much necessary to repair cell damages as DNA.

10.4.3 Effects on Protein Synthesis

Protein synthesis is an essential requisite for germination and starts after few minutes from hydration (Cheung et al. 1979). Bray (1995) showed that the amount of synthesized protein observed 2 days after germination in primed leek seed was the same as that observed 4 days after germination in unprimed seed. Chen et al. (2012) observed an increase of dehydrin in spinach (*Spinacia oleracea* L.) during osmopriming. This increase was also observed during germination of primed seeds in chilling stress and desiccation stress, suggesting that osmopriming may play a positive role in the tolerance to these stresses.

10.4.4 Effects on Enzymes

Osmopriming induce the synthesis and activation of enzymes catalyzing the breakdown and mobilization of reserve substances (Varier et al. 2010). Sung and Chang (1993) mentioned that enzymes responsible for the mobilization of reserve carbohydrates (α- and β-amylases) and lipids (isocitrate lyase) are activated due to priming process. This effect is associated with the water deficit induced by osmopriming, which is supposed to determine a mobilization of reserve proteins (Varier et al. 2010).

In fact, priming appears to strengthen defense system (antioxidant enzymes): seed priming was associated with an increase in CAT expression in *Arabidopsis* (Gallardo et al. 2001) and sunflower (*Helianthus annuus* L.) (Kibinza et al. 2011) and maize seeds (Chiu et al. 2002). In sunflower seeds, Bailly et al. (1998, 2000) showed that osmopriming with PEG led to an increase of SOD and CAT, in response to the rise of metabolic activity during priming, which is responsible for a secondary production of AOS from mitochondrial respiration and/or lipid peroxidation. It appears, therefore, that the defense system of the antioxidant enzymes is enhanced in response to a higher amount of potential threats.

Several methods of seed priming were successfully used in agriculture for seed conditioning to accelerate the germination rate and improve the seedling uniformity as seed priming with water, plant growth regulators, beta-aminobutyric acid, 5-aminolevulinic acid, osmoprotectant, melatonin, chitosan, plant extract, polyethylene glycol, and inorganic salts. It is worthy to mention that all these methods showed pronounced effect on germination, seedling growth, and yield of different crops under normal conditions or stressful conditions.

10.5 Seed Priming with Water (Hydropriming)

Soaking seeds with water overnight and then drying before sowing markedly improved seedling emergence, plant growth, establishment, vigor, and the final yield as reported by Harris et al. (1999) and Ahmad and Shad (2010). On the other hand, Harris et al. (2002) and Basu et al. (2005) reported that effects of

hydropriming persisted only till early vegetative growth of maize. The primed seed germinated soon after planting compared with untreated dry seed, since the early emergence and its effect on early maturity of primed seed may be due to the completion of pre-germinative metabolic activities that making the seed ready for radical protrusion (Rajpar and Wright 2000). Moreover, Rajpar et al. (2006) reported that primed seeds overnight took significantly fewer days to emerge and reach maturity when compared to the untreated dry seed, whereas 36 h primed seeds showed poor germination and 48 h primed seeds inhibited germination. This inhibition may be attributed to the long period of priming that led to excess water in the seeds and greater reduction in the O_2 availability to the embryo. This was reported by Perry and Harrison (1974) who stated that excess of water during priming had inhibitory effect on seed germination of *Beta vulgaris*. The reduction in O_2 availability could lead to an inhibition of ethylene synthesis (Beyerjr et al. 1985) and consequently seed germination. Dawood (2005) demonstrated that the changes in the chemical composition and anti-nutritional factors under the effect of soaking of canola seeds with water for 24 h depend on the variety and caused a marked decrease in tannins and glucosinolates, total polyphenols, and phytic acid of canola meal. In this regard, Kibinza et al. (2011) showed that priming of sunflower seeds improves germination percentages due to the significant drop in H_2O_2 accumulation and restoration of catalase activity that protects stressed seeds against damages caused by ROS activities. Moreover, Kester et al. (1997) reported that seed priming is able to increase protein content in plant tissue via conferring protection to the cellular proteins damaged, improving the performance of the protein synthesis system, and also increase in protein L-isoaspartyl methyltransferase enzyme that repairs plant tissue protein.

10.6 Seed Priming with Plant Growth Regulators

Priming seeds with plant growth regulators has been reported to be beneficial to growth stages and yield of some crops and known as a strategy to alleviate the harmful effect of environmental stresses (Bahrani and Pourreza 2012; Jisha et al. 2013). For instance, soaked rye seeds with gibberellic acid increased germination percentage under water stress conditions (Ansari et al. 2013). The germination of pretreated pepper seeds with salicylic acid improved significantly under high salinity levels (Khan et al. 2009). In addition, Nascimento (2004) documented that seed priming with ethylene minimizes the effect of high temperatures on lettuce seed germination. Seed priming with brassinosteroids (BR) increased peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activities in *Medicago sativa* seedlings under salinity stress (Zhang et al. 2007). Priming *Vicia faba* seed with salicylic acid improved salinity tolerance due to enhanced activities of CAT, ascorbate peroxidase (APX), POD, and glutathione reductase (GR) (Azooz 2009). Priming seeds of *Agropyron elongatum* with gibberellin (GA) and abscisic acid (ABA) induced CAT and SOD activities under drought conditions when compared to unprimed seeds (Eisvand et al. 2010). Maize seeds primed with hormones exhibited acquired abiotic stress tolerance through a more responsive antioxidative system (Afzal et al. 2008;

Farooq et al. 2008). Moreover, phytohormonal priming can enhance seed germination through enhancing some enzymes such as amylase activities and protease that hydrolyzed starch and protein molecules into simple forms available for the embryo to germinate (Miransari and Smith 2014). Hormonal priming was found also to reduce the reactive oxygen species (ROS) produced under $PbCl_2$ stress, and it can alleviate the stress by controlling the oxidative damage on seed germination and embryo growth under stress conditions (Sajedi et al. 2011). Although hydropriming and hormonal priming on soybean can improve seedling establishment and field performance, hormonal priming using auxin, cytokinin, and GA_3 were the most appropriate priming treatments for soybean seeds grown under Pb stress conditions (Abu-Muriefah 2017). ROS produced under stress conditions often interact with the phytohormone priming, and this interaction between ROS and phytohormone could be antagonistic or synergistic (Golldack et al. 2013).

10.6.1 Auxin

Generally, auxins are the best hormones to use because they are nontoxic to plants over a wide range of concentration and effective in promoting root system of large number of plant species. Auxins might regulate cell elongation, cell division, tissue swelling, formation of adventitious roots, callus initiation, and induction of embryogenesis at very low concentrations (Vanderhoef and Dute 1981). The principal auxin in plants is indole-3-acetic acid (IAA) that produced mainly in the shoot apex bud and young leaves of plants. Other meristematic tissues, flowers, fruits, and young seeds have also been shown to be sites of this hormone production. IAA has wide range of effects on many processes such as cell division, vascular tissue differentiation, root initiation, flowering, fruit setting, ripening, senescence, and gravitropism (MacDonald 1997). Furthermore, IAA stimulated cell elongation and apical dominance (Wang et al. 2001), increased photosynthetic activities (Naeem et al. 2004), and activated the translocation of carbohydrates during their synthesis (Awan et al. 1999).

It is well known that IBA is an auxinic hormone and plays a regulatory role in many vital processes within plants such as growth, development, and vascular and pollen formation. Its function on growth, including embryo development, is controlled by its transport which is regulated by transcriptional factors (Hayashi 2012). Another important function of the IBA is its role in cell elongation in the growing embryo (Hauvermale et al. 2012). Auxin by itself is not a necessary hormone for seed germination (Hentrich et al. 2013); however, according to the analyses regarding the expression of auxin-related genes, auxin is present in the seed radicle tip during and after seed germination.

10.6.2 Cytokinins

It was found that cytokinins are able to stimulate seed germination by alleviating the stresses such as drought, salinity, and heavy metal as well as oxidative stress (Peleg and Blumwald 2011; Miransari and Smith 2014). Cytokinins have the ability to regulate a range of cell activities including cell division and seed germination. Heyl et al. (2012) mentioned that cytokinins were active at all stages of germination and enhance the activity of meristematic cells in epicotyls and hypocotyls. These hormones have potent effects on plant physiology and are intimately involved in the regulation of cell division, apical dominance, chloroplast development, anthocyanin production, and maintenance of the source-sink relationship (Hutchkinson and Kileber 2002). In addition, cytokinins are regarded as the most important senescence-retarding hormones, and their application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins as well as reverse leaf and fruit abscission (Pospíšilová et al. 2000). Kinetin is a synthetic cytokinin known to significantly improve plant growth and development even grown under environmental stress. It stimulates leaf expansion and development of reproductive organs and delays senescence (Shah 2007).

10.6.3 Gibberellins

Gibberellins are diterpenoid, regulating seed germination and plant growth through its antagonistic effects with ABA. Abu-Muriefah (2017) stated that GA_3 priming was found to enhance seed germination, maybe through its effect on stored food within seeds, and makes it available for embryos during germination processes. The endosperm within seeds becomes available to the embryo via the activities of some hydrolase enzymes. It is well known that GA_3 stimulates the synthesis and production of the hydrolases, especially α-amylase, resulting in the germination of seeds. In this regard, Yamaguchi (2008) found that gibberellins were able to induce a range of enzymes necessary for seed germination including amylase, protease, and glucanase. Moreover, seed germination is often controlled through suppression effects of excess ABA on the expansion of embryo organs caused by inhibition of GA_3 effects on the growth of radicle and hypocotyl (Voegele et al. 2011). In addition, gibberellins are essential in seed germination for the production of mannanase that is necessary for seed germination (Wang et al. 2005).

10.6.4 Abscisic Acid (ABA)

ABA inhibited the activity of many enzymes involved in germination of pigeon pea, barley grains, and soybean as reported by Sneideris et al. (2015), Staroske et al. (2016), and Abu-Muriefah (2017), respectively. Although ABA had negative effects on seed germination process, it positively affects seed dormancy and plant activities under biotic and abiotic stresses (Popko et al. 2010). Chiu et al. (2016) stated that

high ABA concentrations can inhibit seed germination in many species probably due to the inhibitory effect of ABA on the activity and/or the synthesis of some enzymes involved in the degradation of endosperm cells such as α-amylase within seeds, which considered an important process for seed germination. Moreover, the inhibition of seed germination at high levels of ABA was through inhibition of the radicle expansion and suppression of some transcriptional factors, which can negatively affect the process of seed germination (Graeber et al. 2010).

10.6.5 Ethylene

Ethylene regulates plant responses under different stress conditions and controls many processes in plants, including seed germination (Keunen et al. 2016) and embryo radicle growth (Baskin et al. 2003). It was found that ethylene concentration increased during seed germination process of many species including wheat, corn, soybean, and rice (Zapata et al. 2004). 1-Aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene, can enhance seed radicle emergence through the production of ethylene which is produced in the radicle (De Poel and der Straeten 2014). In this regard, seed priming with ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid) increased the rate of germination in lettuce seeds (Nascimento 2004), but it didn't affect significantly the rate of germination in ryegrass (Tiryaki et al. 2004). However, under abiotic stress conditions and GA_3-deficient mutants, ethylene can play the same role of GA_3; therefore ethylene priming makes seeds able to germinate completely at such conditions (Matilla and Matilla-Vazquez 2008).

10.6.6 Salicylic Acid

Salicylic acid (SA) acts as an endogenic phytohormone from phenolic compounds (among the group of ortho-hydroxyl benzoic acid), having the ability of antioxidant defense system, and regulates various physiological and biochemical processes in plant such as stomata conductivity (Hayat et al. 2010), activity of photosynthesis pigments (Hayat et al. 2005), maintenance of tissue water contents and reduced membrane permeability (Farooq et al. 2008), adjustment of the activity of antioxidant enzymes (Carvalho et al. 2011), and tolerance to environmental stresses (Kabiri et al. 2012). In addition, Sakhabutdinova et al. (2003) reported that salicylic acid treatments maintain IAA and cytokinin levels in the plant tissues, which enhanced the cell division.

Regarding priming process, it was reported that salicylic acid pretreatment produced a higher total biomass and seed vigor index (Kabiri et al. 2012; Sharifzadeh et al. 2013) and increased seedling field emergence because the salicylic acid treatment prevented the decrease in indoleacetic acid and cytokinin content completely which reduces inhibition of plant growth (Afzal et al. 2006; Ansari and Sharifzadeh 2012).

Seed priming with salicylic acid induced salinity tolerance (Kumar et al. 2010; Ahmad et al. 2012, 2015) via increasing SOD activity which quenches oxygen radicals (Gautam and Singh 2009; Orabi et al. 2015) and reducing membrane permeability and leakage of ions like NO^{3-} in wheat and canola (Wahid et al. 2007b; Sakr and Arafa 2009) and enhancing chlorophyll contents and sugars which is necessary for osmotic regulations as well as retards ethylene biosynthesis (Hamid et al. 2008). Moreover, Dellali et al. (2012) recognized that salicylic acid priming at the seedling growth stage alleviated salt-induced oxidative stress by reducing malondialdehyde (MDA) and H_2O_2 content.

Furthermore, seed priming with salicylic acid improved chilling tolerance and increased germination (Sedghi et al. 2010), via activation of antioxidants, maintenance of tissue water contents, and reduced membrane permeability (Farooq et al. 2008), as well as protected structure of plant cell (Horvath et al. 2007). Farooq et al. (2008) showed that maize seed priming by salicylic acid solution improved germination characteristics and seedling root and shoot growth under both normal and low temperature conditions through activation of antioxidant enzymes system, including catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxides (APX). Meanwhile, Pouramir-Dashtmian et al. (2014) revealed that seed priming with salicylic acid solution improved rice seedling growth under low temperature condition via increasing proline, soluble sugar, and protein content.

Regarding optimum concentration, it was found that seed priming with either 20 mg L^{-1} acetylsalicylic acid or salicylic acid seems to be the suitable concentration that showed maximum seed invigoration and better performance in maize through inducing SOD activity and improving nutrient contents in root and shoot as reported by Ahmad et al. (2015). In addition, Hayat et al. (2005) reported that soaking of wheat grains with low concentrations of salicylic acid significantly promoted growth of wheat seedlings. Pretreatment of barley seeds with 1150–1252 μmolar salicylic acid significantly increased the number of seed in spike and thousand seed weight and thus enhanced seedling emergence in field condition (Khaliliaqdam and Mir-Mahmoodi 2013.

10.7 Seed Priming with Beta-Aminobutyric Acid (BABA)

Recently, nonprotein amino acids like β-aminobutyric acid (BABA) were employed in seed priming of various crops against biotic and abiotic stress (Worrall et al. 2012). BABA is known as a potent inducer of resistance in plants against nematodes (Oka et al. 1999), microbial pathogens (Cohen 2002), insects (Hodge et al. 2005), and abiotic stress (Jakab et al. 2005; Zimmerli et al. 2008). According to Zhong et al. (2014), BABA can bring plants into a sensitization state in which defenses are not expressed, but are able to react more rapidly and/or more strongly to various stress. BABA-induced priming functions by the interaction with several hormones like salicylic acid (SA), abscisic acid (ABA), and ethylene (Jakab et al. 2005) or by causing a cascade of signaling processes mediated through H_2O_2 (Cohen et al. 2010). Jisha and Puthur (2016) mentioned that priming rice grain with BABA reduced MDA content of seedlings through reducing the lipid peroxidation of

biomembranes and increasing antioxidant enzyme activities and thus improved the PEG-6000 and NaCl stress tolerance of rice seedlings.

10.8 Seed Priming with 5-Aminolevulinic Acid (ALA)

5-Aminolevulinic acid (ALA) is an aliphatic precursor in the biosynthesis of all porphyrin compounds such as chlorophyll and heme (Wang et al. 2004). ALA is a biodegradable herbicide and insecticide, but it has promotional effects on the growth and photosynthesis of crops and vegetables and is harmless to humans, animals, and crops (Hotta et al. 1997a, b). Rae-hyun and Song (2007) demonstrated that the application of *Rhodopseudomonas* sp. which produced indole-3-acetic acid and 5-aminolevulinic acid could increase the germination percentage of tomato seed by 30.2%. ALA is able not only to act as an antioxidant enzyme promoter but also to stimulate nitrate reductase activity (Beyzaei et al. 2014) and the respiration rate and ATP synthesis (Fu et al. 2014). ALA not only promotes the growth and yield of crop plants but also acts as an alleviator of oxidative damage and enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) to encounter radicals under stress conditions, i.e., drought stress and salinity stress (Li et al. 2011; Liu et al. 2011; Zhang et al. 2013a). Korkmaz et al. (2010) reported that seed soaking with 25 ppm ALA enhanced the germination rate and seedling uniformity, thereby providing chilling stress tolerance of pepper seedling. Kanto et al. (2015) suggested that rice seed priming with ALA at 0.5 μg mL^{-1} could ameliorate the germination capacity even after exposure to the accelerated ageing treatment by enhancing the activity of antioxidant enzymes to scavenge ROS during rice seedling growth under accelerated ageing.

10.9 Seed Priming with Osmoprotectants

Many plants tolerate stress by production of different types of organic solutes called osmoprotectants (compatible solutes or osmolytes) which lower the osmotic potential and attract water molecules into the cell and ultimately maintain the cell turgor. These compatible solutes including soluble sugars, sugar alcohols, proline, glycinebetaine, etc. are low molecular weight, highly soluble in water, and nontoxic to plant even at higher cytosolic concentration (Hoque et al. 2007).

Generally, these compatible solutes protect plants from stress injury through different means, including protection of cytoplasm and chloroplasts from Na^+ damage and scavenging of reactive oxygen species (Smirnoff and Cumbes 1989), stabilization of proteins and protecting membrane structure (Bohnert and Jensen 1996), maintaining the osmotic balance, and general maintenance of physiological stability of plants under stressful conditions (Farooq et al. 2009).

10.9.1 Glycinebetaine (GB)

Glycinebetaine (GB) is a quaternary ammonium compound, an amino acid derivative, and regarded as one of the most effective compatible solutes that protect plants from injury of abiotic stresses. GB application improves growth, survival, and tolerance of a wide variety of plants under various stress conditions (Ashraf and Foolad 2007) by regulating a number of physiological and biochemical processes (Qureshi et al. 2013), maintaining turgor pressure (Agboma et al. 1997), enhancing net CO_2 assimilation rate (Lopez et al. 2002), protecting the functional proteins and enzymes (e.g., Rubisco) and lipids of the photosynthetic apparatus, and maintaining electron flow through thylakoid membranes (Allakhverdiev et al. 2003) and regulation of photosynthetic machinery and ion homeostasis (Raza et al. 2014). Further, GB induces defense response in crops against reactive oxygen species (ROS) produced due to biotic and abiotic stresses and plays a vital role in the process of osmotic adjustment in many crops under environmental stresses (Gadallah 1999). Moreover, it may act as an antitranspirant which allowed the plant to access more water for a long period and facilitates photosynthesis as reported by Agboma et al. (1997).

The main role of GB in plants exposed to saline soil is considered to be maintaining osmotic regulation in cells (Gadallah 1999). It was suggested that GB supports various transporters for normal functioning under salt stress. From this, it can be proposed that GB has a protective effect in discriminating Na^+ against K^+ under saline states (Mansour 1998). The second possible role of GB is that it might enhance vacuolar efficiency in the roots of salt-stressed plants for accumulating more Na^+ as reported by Rahman et al. (2002). Gadallah (1999) reported that GB improves germination and seedling growth of many crops under stressful condition. Plants treated with GB also maintain higher antioxidative enzyme activities that minimize oxidative stress (Ma et al. 2006). The application of GB has an important role in water availability for the imbibition of germinating seeds during limited water. Under salinity conditions, GB increased seed germination by enhancing the osmotic pressure (Sakamoto and Murata 2000) and alleviated lipid peroxidation and facilitated the protection of membrane functions (Hu et al. 2012), protected the photosynthetic machinery by stabilizing the activity of repair proteins (Murata et al. 2007), and provided a direct protective effect on antioxidant enzyme activities under salinity stress (Hoque et al. 2007).

Cuin and Shabala (2005) suggested that compatible solutes, and GB in particular, regulate the net fluxes of Na^+ and K^+ across the plasma membrane at the cellular level in response to NaCl stress. Priming safflower seeds with GB (60 mM) was the most effective treatment that enhanced the plant's tolerance to salt stress during the germination stage. Dawood and Sadak (2014) mentioned that soaking canola seeds with different concentrations with GB caused significant increases in IAA, proline, and total soluble sugars and significant decreases in MDA, H_2O_2, and antioxidant enzymes (POX, PPO, SOD, CAT, APX, and NR) in canola plants irrigated with different levels of water relative to corresponding controls. All GB treatments caused significant increases in seed yield, oil, carbohydrate, protein, total phenolic content, tannins, and antioxidant activity of the yielded seeds and nonsignificant increases in

flavonoids in the yielded canola seeds either in plants irrigated with 75% FC or 50% FC relative to corresponding controls. The increases in seed yield/plant due to 20 mM GB were 30.80% and 60.28% at 75% FC and 50% FC, respectively, relative to corresponding controls. The fatty acid profile of canola oils showed different responses to GB treatments either in unstressed plants or drought-stressed plants. Oleic and linoleic acids were increased accompanied by decreases in linolenic and erucic acids under the interaction effect of GB treatments and drought stress (75% FC and 50% FC), and these results led to decreases in total saturated fatty acid and increases in unsaturated fatty acid relative to corresponding controls. Generally, 20 mM GB was the most pronounced and effective treatment in alleviating the deleterious effect of moderate or severe drought stress on canola plants. Meanwhile, 60 mM GB had the most pronounced effect on tolerance to salinity stress in safflower seedling. The GB-increased tolerance to salt in safflower was mainly related to increased CAT and SOD activities and the prevention of cell membrane damage as a result of reduced lipid peroxidation and improved ion homeostasis under salinity stress condition (Alasvandyari et al. 2017).

10.9.2 Proline

It is evident from different reports that application of proline induces abiotic stress tolerance in plants (Ali et al. 2007; Taie et al. 2013; Dawood et al. 2014). Its further role in salinity appears to involve the induction of salt-responsive genes, with the resultant formation of new proteins which may improve the adaptation to salinity stress (Khedr et al. 2003). Moreover, proline may be having a role in stabilization of cellular proteins and membranes in presence of high concentrations of osmotic stress. Proline accumulation in plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism (Jahari et al. 2010). In addition, Vendruscolo et al. (2007) reported that proline is involved in tolerance mechanisms against oxidative stress, and this is the main strategy of plants to avoid detrimental effects of water stress. Proline plays an important role as a sink for energy to regulate redox potentials (Simiroff and Cumbes 1989), alleviates salt stress induced by enhancement in oxygenase and carboxylase activities of Rubisco (Sivakumar et al. 2000), and protects plants from free radical that induced damage by quenching of singlet oxygen (Matysik et al. 2002). Several functions are proposed for the accumulation of proline in tissues exposed to salinity stress: osmotic adjustment (Voetberg and Sharp 1991), C and N reserves for growth after stress relief (Hellmann et al. 2000), detoxification of excess ammonia (Skopelitis et al. 2006), stabilization of proteins and membranes (Mansour 1998), protection of macromolecules from denaturation (Hamilton and Heckathorn 2001), osmoprotection (Kishor et al. 1995), free radical scavenging (Chen and Dickman 2005), antioxidation (Hoque et al. 2007), and regulation of cytosolic acidity (Sivakumar et al. 2000).

Taie et al. (2013) mentioned that pre-sowing seed treatment with proline seems to enhance faba bean salt tolerance via improving growth parameters, photosynthetic pigments, soluble carbohydrate, and total carbohydrate; meanwhile phenolic,

proline, Na^+, and Cl^- contents were decreased relative to their corresponding salinity controls. Proline treatments induced over expression for new protein bands with high density.

10.10 Seed Priming with Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indolic compound (biogenic indoleamine) structurally related with other important substances, such as tryptophan, serotonin, indole-3-acetic acid (IAA), etc. Several authors hypothesized that melatonin may possess some auxin-like effects and may act as a regulatory molecule in plants (Van Tassel et al. 2001). Many evidences have shown melatonin could alleviate biotic and abiotic stresses such as temperature, salinity, light, drought, and pathogen (Li et al. 2012; Zhang et al. 2013a, b, 2014; Meng et al. 2014). Moreover, melatonin also could regulate the expression of a large number of genes involved in plant stress defense (Weeda et al. 2014; Zhang et al. 2014). Melatonin application could be a good bio-stimulator improving not only seed germination and seedling/plant growth but also crop production especially under stress conditions (Janas and Posmyk 2013). Melatonin is soluble in both water and lipid so it may act as a universal hydrophilic and hydrophobic antioxidant (Janas and Posmyk 2013). Tan et al. (2007) mentioned that elevated levels of melatonin probably protect plants against water and soil pollutants through acting as a direct free radical scavenger and as an indirect antioxidant. One melatonin molecule may scavenge up to ten free radicals (Tan et al. 2007), which contrasts with the classic antioxidants that typically detoxify one radical per molecule. Its antioxidant activity may manifest itself in several ways: (1) direct free radical scavenging, (2) elevating the antioxidant enzyme activity, (3) protecting antioxidant enzymes from oxidative damage, (4) increasing the efficiency of mitochondrial transport chain, and (5) reducing the generation of free radicals (Tan et al. 2010). Paredes et al. (2009) reported that melatonin functions in plants can be recognized into three categories: growth promoters as auxins, antioxidants for free radicals which serve as a first-line defense against oxidative stress, and other functions (signal molecules for circadian maintenance, regulation of flower development, or maintenance of developmental stages in fruit tissues). Hernandez-Ruiz et al. (2004) mentioned that a higher concentration of melatonin (200 μM) had no significant effect or even inhibitory effect on seed germination. However, lower concentrations of melatonin (50 or 100 μM) promoted seed germination as mentioned by Wei et al. (2015).

Pretreatment of melatonin attenuates cold-induced apoptosis in carrot suspension cells (Lei et al. 2004) and decreased lipid peroxidation caused by toxic copper ion in red cabbage seedlings (Posmyk et al. 2008). Melatonin prevented chlorophyll degradation during the senescence of barley leaves (Arnao and Hernandez-Ruiz 2009) and protected membrane structures against peroxidation during chilling stress and recovery in cucumber seeds (Posmyk et al. 2009). Posmyk et al. (2008) reported that the pre-sowing seed treatment with melatonin-protected red cabbage seedlings against toxic Cu ion concentrations as well as melatonin application to cucumber

seeds had a beneficial effect on seed germination, the growth of seedlings, and crop production of plants especially those subjected to cold stress (Posmyk et al. 2009) and water stress (Zhang et al. 2013a, b). Jiang et al. (2016) mentioned that seed priming with 0.8 mM melatonin alleviates the salinity damage to maize by improving SOD, CAT, and PAL activities, relative water content, and proline and total phenolic contents and decreasing membrane relative electrolyte leakage and lipid peroxidation product. Szafrańska et al. (2012) and Jiang et al. (2016) mentioned that either seed priming or exogenous application with melatonin significantly increased PAL activities and total phenolic content. Janas and Posmyk (2013) showed that hydroprimed or osmoprimed seeds of corn (*Zea mays* L.), mung bean (*Vigna radiata* L.), and cucumber (*Cucumis sativus* L.) with melatonin had higher crop yield than the control ones under field conditions, since the production of corn, cucumber, and mung bean primed with melatonin was about 10–25% greater in comparison to those primed without melatonin and is dependent on plant species. Zhang et al. (2013b) reported that 100 μM melatonin alleviated polyethylene glycol-induced inhibition of cucumber seed germination, showing the greatest germination rate and photosynthetic rate, and at the same time significantly reduced chlorophyll degradation. Furthermore, the ultrastructure of chloroplasts in water-stressed cucumber leaves was improved after melatonin treatment, thus reversing the effect of water stress. Szafrańska et al. (2014) mentioned that hydropriming *Vigna radiata* seeds with melatonin (50 μM L^{-1}) increased level of melatonin in roots derived from hydroprimed seeds with melatonin by sevenfold higher than roots derived from non-primed seeds. Wei et al. (2015) concluded that coating soybean seeds with melatonin promoted soybean plant growth, increased yield, and improved salinity stress tolerance. Recently, Dawood and EL-Awadi (2015) concluded that melatonin treatments (100 and 500 mM) improved growth parameters, relative water content, photosynthetic pigments, and total carbohydrate, total phenolic, indoleacetic acid, K^+, and Ca^{+2} contents and reduced the levels of compatible solutes and Na^+ and Cl^- contents in leaf tissues of faba bean plants irrigated with diluted seawater (3.85 and 7.69 dS/m). Melatonin at 500 mM had a more pronounced effect in alleviating the adverse effects of the two salinity levels on the performance of faba bean plants than 100 mM melatonin. The beneficial effects of melatonin treatments in alleviating the harmful effect of salinity stress on the growth parameters were more pronounced in the plants grown under the higher salinity level (S2 = 7.69 dS/m) than those grown under lower salinity level (S1 = 3.85 dS/m) relative to corresponding controls. The increases in total photosynthetic pigments were 22.31%, 12.87%, and 15.85% in the plants treated with 500 mM melatonin and irrigated with tap water (S0) and diluted seawater at lower (S1) and higher (S2) concentrations, respectively, as compared with corresponding controls.

Jiang et al. (2016) suggest that seed priming with 0.8 mM melatonin significantly improved germination energy, germination percentage, seedling vigor index, shoot and root lengths, seedling fresh and dry weights, K+ content, relative water content, proline and total phenolic contents, superoxide dismutase, and catalase and phenylalanine ammonia lyase activities and significantly decreased mean emergence time, Na+ content, electrolyte leakage, and malondialdehyde content compared with

untreated seeds under salinity stress; thereby seed priming with melatonin alleviates the salinity damage to maize.

10.11 Seed Priming with Plant Extract

The allelopathic action of various natural compounds on the growth and development of many plants may be inhibitory or stimulatory depending on their concentration in the surrounding medium and on their physiological activity within plants (El-Daly and Soliman 1997). Allelochemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, and other compounds, sometimes have a greater allelopathic effect than individual compound alone. Einhellig (1995) mentioned that these metabolites may be selective in their action or plants may be selective in their responses. Moreover, allelochemicals which inhibit the growth of some species at certain concentrations may stimulate the growth of the same or different species at different concentrations (Narwal 1994). These allelochemicals promote or inhibit the crop growth based on species-specific concentrations (Ambika et al. 2003). Phenolic compounds can act on enzymes, phytohormone activity, and mineral content (Einhellig 2004). Saponins are readily soluble in water which may enhance the nutrient absorption (Satish et al. 2007). Alkaloids, phenolic compounds, and saponins protect the plants against pathogens and also produce antioxidant activity.

Leaves of different plants contain alkaloids and phenolic compounds which protect the plants against pathogens and also produce antioxidant activity (Satish et al. 2007). Rathinavel and Dharmalingam (1999) mentioned that the presence of bioactive substances in the leaf extracts enhanced lipid utilization and enzyme activity that increased dry weight and development of seedling to reach autotropic stage, enabling them to produce relatively more quantity of dry matter which is discerning the cause for the hike in vigor index by hardening treatment. It is generally assumed that physiologically active substances might have activated the embryo and other associated structures which resulted in the absorption of more water due to cell wall elasticity and development of stronger and efficient root system, and that would have ultimately resulted in higher vigor index (Rangaswamy et al. 1993). Many researchers also reported the benefits of seed hardening with different medicinal plants leaf extract to overcome the adverse condition (Renugadevi et al. 2008; Kamaraj and Padmavathi 2012).

It is worthy to mention the most important plants containing allelochemicals as follows: *Chlorophytum* leaves are high in saponin, carbohydrate, proteins, and various alkaloids (Chakraborthy et al. 2014). Leaves of *Vinca rosea* are also high in alkaloids and phenolic compounds (Tiong et al. 2013). Neem leaves contain flavonoids, steroids, carbohydrates, glycosides, antiquinone, terpenoides, and alkaloids (Raphael 2012). The seeds of fenugreek contain lysine and L-tryptophan-rich proteins, mucilaginous fiber, and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline (Bukhari et al. 2008). Furthermore, intercropping fenugreek with faba bean can reduce *Orobanche crenata* infection (Fernández-Aparicio et al. 2006).

Guava leaf contains volatile oil such as quercetin, avicularin, guaijaverin, etc. (Morant et al. 2008). Previous studies on the chemical composition of guava leaves have identified chemical products belonging to the groups with allelopathic properties (Monteiro and Vieira 2002) such as terpenoids, flavonoids, coumarins, and cyanogenic acids, among others (Gutiérrez et al. 2008). *Some studies have already identified guava allelopathic effects on other species, the effect of guava fruit extracts on cucumber germination* (*Cucumis sativus*) (Bovey and Diaz-Colon 1968) as well as the effect of guava root exudates on lettuce (*L. sativa*) germination and root growth and the root growth of bristly foxtail (*Setaria verticillata*) (Brown et al. 1983). Chapla and Campos (2010) reported that allelopathic effect of the guava leaf aqueous extract on the germination and growth of lettuce occurred only at 20% concentration. Regarding *Lantana camara*, *their* leaves, roots, and fruits contain allelochemicals mainly phenolics, flavonoids, tannins, and carbohydrates as mentioned by Gopie-shkhanna and Kannabiran (2007). Moreover, Basu and Hazra (2006) indicated that lantana plant has strong antioxidant activities. Yi et al. (2005) reported the presence of several phenolic compounds in lantana leaf extract identified by HPLC as salicylic, gentisic, β-resorcylic, vanillic, caffeic, ferulic, and p-hydroxybenzoic acids, coumarin, and 6-methylcoumarin. Ahmed et al. (2007) found that different concentrations of aqueous leaf extracts of lantana caused significant inhibitory effect on germination, root and shoot elongation, and development of lateral roots of receptor crops. The inhibitory effect was much pronounced in root and lateral root development rather than shoot and germination. Earlier, Dawood and Taie (2009) soaked lupine seeds with freshly prepared aqueous leaf extract (5, 10, 15% w/v) of eucalyptus and lantana separately for 24 h. The phenolic compounds and flavonoids (allelochemicals) are detected in higher amounts in aqueous leaf extract of eucalyptus than that of lantana. Aqueous leaf extract of either lantana or eucalyptus caused a gradual decrease in germination % of both sweet and bitter lupine varieties. Their effect was proportional to the extract concentration. Germination % increased by extending the germination period. Phenolic and alkaloid contents of 9-day-old seedlings increased by all treatments. All applied treatments had positive effect on photosynthetic pigments and yield as well as protein, carbohydrate, ash, and phenolic contents of the yielded seeds, whereas alkaloid % decreased. In respect to oil content, it was decreased by lantana treatments. Moreover, Dawood et al. (2012) mentioned that incorporated 10% fenugreek seeds or 20% guava leaves or 20% lantana leaves into the soil caused significant increases in the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid) and the total carbohydrate content of sunflower leaf tissues accompanied by a significant decrease in the total phenolic content. The head weight, seed weight/head, and 100-seed weight were increased in the following order: fenugreek treatment > lantana treatment > guava treatment. It was noted that fenugreek treatment is the most effective treatment in increasing oil % followed by guava treatment, while the lowest increase resulted from lantana treatment. Fenugreek treatment caused the highest decrease in (C16:0 + C18:0) accompanied by the highest increase in (C18:1 + C18:2). Regarding the total essential amino acids, it was noticed that fenugreek treatment showed a noticeable increase, whereas lantana and guava treatments

showed a decrease in total essential amino acids. Phuwiwat et al. (2012) concluded that leaves of *Melia azedarach* contained water soluble allelochemicals that caused inhibition of both water uptake and α-amylase activity of *E. crus-galli* during germination process. Prabha et al. (2016) concluded that seed priming with 2% leaf extract of *Chlorophytum*, *Agel*, *Azadirachta*, and *Vinca* were found suitable to enhance seedling vigor and reduce mortality rate in the presence of *Fusarium oxysporum* in tomato. Seed priming with these plant leaf extracts is cost-effective and easy to apply at farmer's field and is suitable under "organic farming" framework.

10.12 Seed Priming with Chitosan

Chitosan is an abundant and relatively cheaper cationic polysaccharide obtained as waste material during seafood processing (Guan et al. 2009). Chitosan application to peanut seeds increased levels of germination percentage, energy, lipase activity, gibberellic acid (GA3), and indoleacetic acid (IAA) (Zhou et al. 2002). Chitosan has been proven to act as a positive factor in enhancing shoot and root length, fresh and dry weights of shoots, and roots and leaf area in bean plants watered with the chitosan solution (Sheikh and AL-Malki 2011). Moreover, it is evident that chitosan has some potential under stressful environment. Chitosan-coated seeds have been reported to demonstrate accelerated germination and tolerance to stress in hybrid rice (Ruan and Xue 2002) and enhanced vigor of maize seedlings (Shao et al. 2005). Furthermore, chitosan priming improved maize germination and seedling growth in relation to physiological changes under low-temperature stress (Guan et al. 2009) and improved the germination of plants under drought stress (Suchada et al. 2007). Moreover, chitosan priming under stress resulted in highly improved germination index and reduced germination time to promote early seedling establishment and synchronized growth in rice (Suchada et al. 2007) and maize (Guan et al. 2009). Sadeghi et al. (2011) mentioned that the improvement in germination and vigor of soybean plant was probably due to the reserve mobilization of food material, activation and resynthesis of some enzymes, and DNA and RNA synthesis which started during osmotic priming. There is possibility that similar germination-responsive genes may be activated because of chitosan priming under osmotic stress. Hameed et al. (2014) observed that chitosan priming treatments not only improved seed germination but also enhanced wheat seedling growth under osmotic stress induced by PEG. Its beneficial effects on germination and seedling vigor provided evidence that chitosan is a promising seed priming agent for the improvement of osmotic stress tolerance in wheat.

10.13 Seed Priming with Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) as an inert material can prevent embryo toxicity problems during priming (Cantliffe 1983). The large size of PEG molecule (6000–8000 mw) also prevents its penetration into seed tissues, avoiding lowering the

osmotic potential (Brocklehurst and Dearman 1984). Osmopriming with polyethylene glycol is most commonly used to induce either osmotic stress varying between −0.5 MPa (sweet corn, Ghiyasi et al. 2008) and −2 MPa (sunflower, Bailly et al. 2000; sugar beet, Capron et al. 2000) in plants or water-deficit condition because it is not naturally produced in the plant tissue and cannot penetrate into cell from the media. Soaking seeds before sowing with PEG solution helps to initiate the membrane repairing systems and metabolic preparation for germination via controlling the water absorption rate of seeds (Jisha et al. 2013). Massarat et al. (2014) concluded that priming with PEG 6000 had beneficial effects on germination and seedling establishment of corn seeds under drought and saline conditions, whereas the major disadvantage resulting from the use of PEG is the reduction of oxygen in the solution, because of its viscosity (Mexal et al. 1975), so aerating the solution during PEG osmopriming can overcome this problem (Bujalski and Nienow 1991).

10.14 Seed Priming with Inorganic Salts

10.14.1 NaCl

It has been shown that NaCl seed priming could be used as an adaptation method to improve salt tolerance of seeds. Sivritepe et al. (2003) and Yildirim et al. (2011) concluded that NaCl seed priming improves seed germination, seedling emergence, and growth under saline conditions. The benefits of NaCl seed priming did not persist beyond the seedling stage in cucumber (Passam and Kakouriotis 1994), while Cano et al. (1991) concluded that NaCl seed priming had positive effects on mature plants and on yield of tomato. According to Cano et al. (1991), the higher salt tolerance of plants from primed seeds seems to be the result of a higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^- ions in their roots and more sugars and organic acids in leaves than plants from non-primed seeds. Moreover, priming canola seeds with NaCl solution increased seedling cell membrane stability and decreased seedling damage under salinity condition due to increased seedling K^+ and proline content (Farhoudi et al. 2007). Massarat et al. (2014) observed that priming corn seeds with NaCl had beneficial effects on germination and seedling establishment under drought and saline conditions. Omami (2005) suggested that priming of amaranth seeds with NaCl improve cell membrane stability and decrease MDA production and increased salt tolerance by promoting K^+ and Ca^{2+} accumulation.

10.14.2 KNO3

The beneficial effects of seed priming with KNO_3 solution on seed germination under salinity conditions have been observed in sunflower (Kaya and Day 2008;

Farhoudi 2014) and muskmelon (Nascimento 2003). Singh and Rao (1993) reported that KNO_3 priming effectively improved germination, seedling growth, and seedling vigor index of the seeds of sunflower varieties with low germination. Improving seedling sunflower growth by KNO_3 priming under salinity condition can suggest nontoxify of KNO_3 due to ion accumulation in the embryo (Kaya et al. 2006). Mauromicale and Cavallaro (1996) mentioned that seed priming of herbage grasses with KNO_3 solution decreased the mean germination time as compared to PEG solution because of KNO_3 did not have toxicity and did not prevent water uptake. Farhoudi (2014) showed that priming of sunflower seeds with 0.6 and 0.9 MPa KNO_3 solution was more effective than non-priming seeds under salinity condition and demonstrated its potential effect in improving tolerance to salinity by increasing seedling fresh weight and POX enzyme activity accompanied by decrease in seedling MDA concentration compared to non-priming seeds.

10.14.3 Sodium Silicate

Evidences have proved that sodium silicate treatment improved the cell membrane stability by reducing the lipid peroxidation in different plants under abiotic stresses (Liang et al. 2007; Pei et al. 2010; Wang et al. 2011). Sodium silicate resulted in improved germination, growth, and antioxidant enzyme activities and reduced lipid peroxidation during drought stress in wheat (Pei et al. 2010; Ali et al. 2012). Moreover, sodium silicate treatment lowered down the oxidative stress by enhancement of antioxidant production (glutathione reductase, catalase, peroxidase, and superoxide dismutase) during drought stress in wheat, barley, and soybean plants (Liang et al. 2003; Gong et al. 2005; Miao et al. 2010; Wang et al. 2011). Moreover, sodium silicate application has been reported to enhance the germination and nutrient use leading to better seedling development in soybean (Miao et al. 2010). Hameed et al. (2013) mentioned that seed priming with sodium silicate not only improved the seed germination and seedling vigor but also enhanced the wheat seedling growth under water-deficit stress induced by PEG.

It is worthy to mention that the accumulation of salts in the seed could be toxic (Bradford 1995), reduce the osmotic potential, and induce a high water absorption during treatment (Parera and Cantliffe 1994), resulting in a more likely radicle protrusion. In addition, effects of inorganic salts on germination are different from those carried out by PEG, depending on seed species. For instance, osmopriming with inorganic salts was toxic to sorghum seeds (Haigh and Barlow 1987), whereas it was effective as PEG in asparagus (Pill 1995) and performed better as PEG in tomato (Mauromicale and Cavallaro 1997). The difference in the response of different species to salts or PEG may be due to a selective semipermeable layer that surrounds the embryo: when this layer is present, it allows the absorption of water, but prevents salt diffusion; when it is absent, ions can be absorbed and cause embryo damages (Welbaum et al. 1998). For example, tomato (*Solanum lycopersicum* L.), melon (*Cucumis melo* L.), lettuce (*Lactuca sativ*a L.), and *Capsicum annuum* seeds possess this layer and may be safely subjected to osmopriming with inorganic salts

(Welbaum and Bradford 1990). Conversely, this treatment is harmful to broccoli and cabbage seeds (*Brassica oleracea* L.), which lack this layer (Taylor et al. 1997).

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Part II

Case Studies on Priming

Seed Priming: A Low-Cost Technology for Resource-Poor Farmers in Improving Pulse Productivity

11

Malay K. Bhowmick

Abstract

Pulses continue to become an integral component of sustainable crop production system for their ability towards biological nitrogen fixation, low water requirement, comparatively shorter duration and capacity to withstand abnormal weather conditions. Average pulse productivity in India often becomes low at famers' fields due to a number of constraints. Moreover, these crops are mainly grown under energy-starved conditions by small and marginal farmers, who do not have sufficient resources with regard to fine seedbed preparation before sowing as well as post-sowing management practices along with appropriate plant protection measures. Hence, poor seed germination, slow and insufficient seedling emergence and inappropriate stand establishment are not uncommon in stress-prone areas. Seed priming is a simple, inexpensive, highly effective and risk-averting tool for improving plant acclimatization under both biotic and abiotic stresses, besides ensuring uniform seed germination, rapid emergence, better stand establishment, improved crop growth, and higher productivity of pulses. The possible technological options for seed priming in pulses include hydro-priming, osmo-priming, bio-priming, nutri-priming, solid matrix priming, hormo-priming, halo-priming, nano-priming and ultra-priming. The present chapter highlights different aspects, techniques and importance of seed priming with particular reference to pulse crops.

Keywords

Crop establishment · Pulse productivity · Seed priming

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11.1 Introduction

Belonging to the Fabaceae family, pulses represent the world's third largest group of plant life. Humans have cultivated pulse crops since the dawn of farming as one of the first plants in the world to be domesticated. Pulses are one of the most important sources of high-quality dietary protein, especially for a large part of the population who are vegetarian. These crops are hardier than most other crops, require less quantity of water and withstand severe weather extremes like droughts and floods, where other crops usually fail, thereby playing an important role in mitigating, adapting and reducing the adverse effects of climate change. Besides, their unique attributes, especially their ability to biologically fix nitrogen, also have a direct and positive impact on soil biodiversity. Being legumes, they become an ideal and integral component of conservation agriculture (Siddique et al. 2012). As recently reported by the Food and Agriculture Organization of the United Nations (FAO) on World Soil Day, 'climate smart' pulses are essential to global food security by delivering high-nutrition protein to people and critical nutrients to soil (FAO 2016a).

Globally, India becomes a home to about 24% of undernourished people. About 15.2% of people in the country are undernourished. This signifies the importance of pulses in food and nutrition security for Indian population. However, India is the largest producer, importer and consumer of pulses, accounting for 25% of global production from 35% of global area under pulses (Ahlawat et al. 2016; FAO 2016b). More than a dozen pulses crops are grown in different parts of the country. Among them, chickpea (Bengal gram: *Cicer arietinum*), pigeon pea (redgram, *arhar*, *tur*: *Cajanus cajan*), mung bean (green gram, *moong*: *Vigna radiata*), urdbean (black gram, *biri*, mash: *Vigna mungo*), lentil (masur: *Lens culinaris* subsp. *culinaris*) and field pea (*mator*: *Pisum sativum*) are the most common ones. Other pulses of regional or local importance include *Lathyrus* (grass pea, chickling pea, *khesari*: *Lathyrus sativus*), rajmash (French bean, kidney bean, common bean: *Phaseolus vulgaris*), moth bean (moth, Turkish gram: *Vigna aconitifolia*), horse gram (*kulthi*: *Macrotyloma uniflorum*), cowpea (lobia, *barbati*: *Vigna unguiculata*), soybean (*soya bean*: *Glycine max*), broad bean (faba bean, *bakla*: *Vicia faba*) and ricebean (climbing mountain bean, red bean: *Vigna umbellata*). Still the on-farm productivity of pulses as a whole in India is less than half of the productivity levels in the USA and Canada, because of the fact that pulse crops are mostly confined to marginal areas under stress-prone environments, getting subjected to a number of constraints. Moreover, these crops are mainly grown by small and marginal farmers, who do not have sufficient resources, unlike progressive farmers, in investing much towards fine seedbed preparation since sowing along with agronomic management practices including plant protection measures till crop harvest.

Since pulse seeds tend to absorb water vapour in humid atmosphere, the decline in germination and viability of seeds becomes a major problem upon ageing of seeds, especially under hot and humid climatic situations. In many agricultural areas, the major cause of poor stand establishment and low crop yield is due to prevalence of unfavourable environmental conditions for seed germination and seedling emergence, especially during *kharif* season. Poor crop establishment is a major constraint

for pulse production, particularly in drought-prone environment, exhibiting irregular (asynchronous) emergence trends that can extend over a long period of time (Naseem et al. 1997; Rahmianna et al. 2000; Nayban et al. 2017). Such an unreliable, irregular and uneven germination as well as delayed emergence is also of common occurrence during pre-*kharif* season. In case of *rabi* season, pulses are mostly grown on the residual soil moisture in rice fallows as *utera* (*paira* or relay) crop (Bhowmick et al. 2005, 2010, 2011, 2014a; Gupta and Bhowmick 2005, 2012a, b, 2013; Mondal and Ghosh 2005; Biswas and Bhowmick 2015). Low productivity especially under *utera* system is a major problem associated with *rabi* pulses (Bhowmick et al. 2005). Even there is a limited scope for agronomic manipulation under rice-*utera* system, there is a huge potential for increasing cropping intensity in considerable areas that remain idle after *kharif* (*aman*) rice (Rautaray 2008).

There are many reports towards improving seed germination as well as crop performance in the field, involving a wide range of techniques, which may be categorized into physiological (seed priming, coating and pelleting), physical (magnetic, radiation and plasma) and biological (seed enhancement) aspects (Afzal et al. 2016). Of these, seed priming (interchangeable with on-farm seed priming or invigoration) is a simple, effective and low-cost technology towards improving seed germination, seedling emergence, stand establishment, crop growth, nodulation and productivity of pulses (Ali et al. 2005; Gupta and Bhowmick 2012a; Sujatha et al. 2013; Ghosh et al. 2016; Bhowmick et al. 2017). Not only that, it can also be an appropriate tool for resource-poor small and marginal farmers towards better managing climate risk crop husbandry, besides guaranteeing the full expression of crop yield potential (Padgham 2009). The present chapter is intended to illustrate different aspects, techniques and importance of seed priming with particular reference to pulses.

11.2 Basis of Seed Priming

The theory of seed priming was proposed by Heydecker in 1973 (Heydecker 1973). Seed priming is a controlled hydration process, used widely for enhancing seed performance by improving the rate and uniformity of germination and decreasing seed sensitivity to external factors (Corbineau and Côme 2006; Sharma et al. 2015; Afzal et al. 2016). Once sown in the fields, seeds are to spend a significant period of time for absorbing water from the soil. By reducing this time to a minimum, seeds can be made to germinate, and the germinating seeds to emerge more quickly and uniformly in the way, known as seed priming. This technique is based on the progress of germination in three phases: imbibition (phase I), transition (the true germination process) or germination *stricto* sensu (phase II), and growth (phase III). In particular, water uptake follows this triphasic pattern with an initial rapid imbibition phase (phase I), followed by a lag period (phase II) referred to as germination *stricto* sensu and finally a second water uptake phase (phase III) associated with radicle growth (Côme 1980; Bewley 1997; Hadas 2004). There is a rapid initial water uptake due to the seed's low water potential during imbibition that results in the resumption of respiratory activity and protein synthesis using extant messenger ribonucleic acid (mRNA). Most

important one is the phase II, which is associated with a slow increase in seed water content along with initiation of physiological activities related to germination (Varier et al. 2010). Various cellular and biochemical events including mitochondria repair and synthesis, protein synthesis relying on the translation of new RNA, changes in soluble sugars, etc. are carried out in phase II (Bray 1995; Bewley 1997). The basis of seed priming is to allow a controlled water uptake by the seeds up to the end of phase II, before the radicle protrudes from the seed coat. Since most seeds are desiccation tolerant up to this stage, the germination process can be arrested by drying. Phase II is more sensitive to external factors than phase III (Côme and Thévenot 1982). Therefore, seeds that have passed through this phase in the priming process germinate in a wider range of environmental factors than non-primed seeds (Corbineau and Côme 2006). In phase III, the process of germination is completed, culminating in radicle emergence (Bennett et al. 2013). Varier et al. (2010) described the sub cellular basis of seed priming in detail.

11.3 Different Techniques of Seed Priming

Seed priming can be accomplished through a number of shotgun techniques such as hydro-priming, osmo-priming, bio-priming, nutri-priming, solid matrix priming, hormonal priming using plant growth regulators, halo-priming, etc. (Bose 2014; Singh et al. 2015a; Nayban et al. 2017). These techniques have been briefed hereunder.

11.3.1 Hydro-priming

As proposed by Harris (1992), it is a very low-cost technique, designated as on-farm seed priming, that involves soaking of seeds in water for a certain period of time prior to sowing (Pill and Necker 2001). It may or may not be followed by air-drying of the seeds (Nawaz et al. 2013). This pre-sowing seed treatment, known as hydro-priming, allows the seeds to imbibe water and go through the first phase of germination in which pregermination metabolic activities get started, whilst the latter two phases of germination are inhibited (Pill and Necker 2001). Another kind of hydro-priming is known as ‘on-farm steeping’ that is practiced by incubating seeds (cereals, legumes) for a limited time in warm water (Sivasubramaniam et al. 2011). A drum is used for this purpose, and the water can also be applied as humid air or as water vapour, and hence, it is also called as drum priming (Rowse 1996; Taylor et al. 1998). Seed priming in water has been found to decrease time between sowing and emergence and to improve seedling vigour (Harris 1996; Parera and Cantliffe 1994). Besides, it can reduce the effect of salinity on the morphological parameters of the plants (Rafiq et al. 2006). Any factor that facilitates rapid germination may contribute to the establishment of a successful crop. Although soaking the seeds in water and drying before sowing is the easiest way to achieve hydration, a major disadvantage is that it may result in uneven hydration and non-uniform germination (Nawaz et al. 2013). According to Swarnkar et al. (2012), seed priming (soaking seeds

overnight in water) helps germination and gives seedlings a head start. After priming, the seeds need to dry just enough to be sown easily. For example, soaking chickpea before sowing costs practically nothing, but such a simple practice can raise the seed yield of chickpea by over 40% in many places (Swarnkar et al. 2012). Significant increase in seed yield of *Lathyrus* has been reported in sowing of sprouted seeds under rice-*utera* system in rice fallows (Bhowmick et al. 2012, 2014a, b; Bhowmick 2013). Yucel (2012) studied on priming of lentil seeds by soaking in 1% KH_2PO_4 and 1% KNO_3 for 12 h, comparing with hydro-priming in distilled water (24 h) at 24 °C and control (untreated seeds) when primed seeds were subjected to germination under six different constant temperatures (5, 10, 15, 20, 25 and 30 ± 0.5 °C). Amongst these treatments, seed priming with water for 12 h at 15 °C recorded the highest germination percentage along with the least mean germination time and synchrony. In general, soaking seeds of pulses in water for 4–8 h and subsequent sowing of these primed seeds at 8–10 days before harvest of rice proved to be a simple and effective practice to improve seed germination, crop growth, plant stand and seed yield by 10–20% under relay cropping system (Urkurkar 2012; Bhowmick et al. 2010, 2013, 2014a).

11.3.2 Osmo-priming

Osmotic priming or osmo-priming (also known as osmotic conditioning or osmo-conditioning) is a commercially and widely used standard technique, employed for improving seed germination and vigour, requiring only small quantities of seeds (Halmer 2004). It is a pre-sowing treatment in an osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination but prevent radicle protrusion through the seed coat (Heydecker et al. 1973). It involves controlled imbibition of seeds to start the initial events of germination followed by seed drying up to its original weight. Osmo-priming has many advantages including rapid and uniform germination and emergence, improved seedling growth and better stand establishment under any environmental and soil conditions (Chiu and Sung 2002; Nawaz et al. 2013; Singh et al. 2015a; Afzal et al. 2016). In this technique, seeds are soaked for a certain period of time in well aerated solutions with a low water potential and later washed and air-dried. The low water potential of the solutions can be achieved by adding osmotica like sugar, polyethylene glycol, i.e. PEG [H-(O-CH_2-CH_2)$_n$-OH], glycerol ($C_3H_8O_3$), sorbitol ($C_6H_{14}O_6$), mannitol ($C_6H_{14}O_6$) or salts like potassium chloride (KCl). Some more examples of the osmotica that can be used include potassium nitrate (KNO_3), monopotassium phosphate or potassium dihydrogen orthophosphate or potassium dihydrogen phosphate (KH_2PO_4), dipotassium phosphate or dipotassium hydrogen orthophosphate (K_2HPO_4), tripotassium phosphate (K_3PO_4), calcium chloride ($CaCl_2$), zinc sulphate ($ZnSO_4$), borax or sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), magnesium chloride ($MgCl_2$), manganese sulphate ($MgSO_4$), sodium chloride (NaCl), sodium sulphate (Na_2SO_4), and different organic compounds, viz. agrosan, cycocel, citric, furamic, succinic, malic acids, purines, pyrimidines, caffeine, uracil, xanthine and uridine diphosphate (De Chandra

1999; Sivasubramaniam et al. 2011). Sivasubramaniam et al. (2011) made an indicative list of different osmotica used for seed priming in food legumes.

Bhowmick et al. (2012, 2014b, c) made a study at Pulses and Oilseeds Research Sub-station, Beldanga, Murshidabad, West Bengal during *rabi* season to evaluate different levels of seed priming in *Lathyrus* raised under rice-*utera* system. The treatments included seed soaking in water (6 h), seed soaking in 2% KH_2PO_4 solution (6 h), use of sprouted seeds and control (no soaking). Amongst these treatments, use of sprouted seeds and 2% KH_2PO_4 soaked seeds significantly recorded higher number of pods plant^{-1}, resulting in higher seed yield. Higher plant height, more number of branches plant^{-1}, seeds pod^{-1} as well as 100-seed weight were also registered under these treatments which ultimately exhibited 17.64–24.61% yield advantages over no soaking plots. Comparatively better performance of crop plants under all the seed priming treatments except 'no soaking' could be attributed to their good establishment as well as tolerance to soil moisture stress, which might be explained due to a number of physico-chemical changes within the cytoplasm including greater hydration of colloids, higher viscosity and elasticity of the protoplasm, etc. (Solaimalai and Subburamu 2004). Values of all the growth and yield attributes along with seed yield were, however, found to be the lowest when non-soaked (non-primed) seeds were sown (Bhowmick et al. 2014b, c). Similar findings were also reported earlier in *Lathyrus* (Bhowmick 2013; Bhowmick et al. 2017), lentil (Gupta and Bhowmick 2005; Bhowmick 2010) and chickpea (Bhowmick et al. 2010, 2013) under rice-*utera* system.

Singh et al. (2014) studied on osmo-priming in cowpea. Three levels of osmo-priming duration by soaking in 1% KNO_3 salt for 6, 8 and 10 h were compared with hydro-primed control (10 h). The results showed that osmo-priming with KNO_3 for different durations were superior to unprimed treatment in terms of seed germination, emergence, plant height and dry matter accumulation in cowpea. Use of primed seeds (both osmo-priming and hydro-priming) was found to improve the performance of cowpea crop. Moreover, osmo-priming with KNO_3 salt (soaked in 1% KNO_3 salt solution and dried before sowing) for 6 h resulted greater seed germination and seedling height than hydro-priming.

11.3.3 Bio-priming

Bio-priming is a process of biological seed treatment that refers to a combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control and nutrient uptake) of seed with beneficial organism to protect seed. It integrates both the biological (seed inoculation) and physiological (seed hydration) aspects. It is an ecological approach using either bacteria or selected fungal antagonists against the soil- and seed-borne pathogens (Sivasubramaniam et al. 2011; Afzal et al. 2016; Rakshit et al. 2015). Bio-priming techniques involve the addition of beneficial rhizosphere microorganisms in the priming process, either as a method for efficient delivery to the crop or to control pathogen proliferation during priming itself (Sharma et al. 2015; Meena et al. 2016, 2017).

Bio-priming with plant growth promoting rhizobacteria (PGPR) has been an alternative to mineral fertilizers to increase crop yield by their ability to produce and/or change the concentration of plant hormones, asymbiotic N_2 fixation and solubilization of mineral phosphate and other nutrients. Some most prominent bioagents are *Trichoderma*, *Pseudomonas*, *Glomus*, *Bacillus*, *Agrobacterium* and *Gliocladium*. Inoculation of seeds with these beneficial microorganisms in combination with priming (bio-priming) potentially can promote rapid and more uniform seed germination and plant growth, improve the versatility of crop performance and stabilize the efficacy of biological agents in the present fragile agricultural ecosystem by reducing dependency on chemical inputs (Sivasubramaniam et al. 2011; Rakshit et al. 2014; Sharma et al. 2015) and by ameliorating a wide variety of biotic, abiotic and physiological stresses to seed and seedlings (Mastouri et al. 2010; Rajendra Prasad et al. 2017). Seed bio-priming process has been discussed in detail (Sivasubramaniam et al. 2011; Rakshit et al. 2014). According to Vishwas et al. (2017), seed priming with *Rhizobium* + *Pseudomonas* at 10% for 12 h recorded significantly higher percent and speed of germination, and seedling vigour, and was followed by seed priming with *Rhizobium* at 10% for 12 h in chickpea. Rakshit et al. (2014), however, made an indicative list of bioagents used for different food legumes.

11.3.4 Nutri-priming

Nutrient priming or nutri-priming means soaking of seeds in nutrient solution of a specific concentration, instead of pure water, for a certain period of time or duration prior to sowing (Shivay et al. 2016). Seed priming with nutrients (macro or micro) can increase seed nutrient content and improve seed quality for better germination, seedling establishment, plant growth, nutrient uptake and water use efficiency of several crop species. Rakshit et al. (2013) discussed in detail about the usefulness of micronutrient seed priming in integrated nutrient management. In micronutrient seed priming, micronutrients are used as osmotica (Imran et al. 2004; Singh 2007). For example, seed priming with 0.02% and 0.04% sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$) for 5 h has been reported to improve productivity of mung bean (Umair et al. 2011).

Swarnkar et al. (2012) reported improvement in germination due to water soaking of chickpea seeds. Adding *Rhizobium* to the soaking water further helps the seedlings form nitrogen-fixing nodules that can capture nitrogen from the air. This is especially true for acidic soils, often encountered in rice fallows, where many legumes become relatively unproductive because of limited nodulation owing to poor availability of molybdenum (Mo). Large yield increases in chickpea are possible at farmers' fields following seed priming with tiny amounts of Mo because it is an essential micronutrient for chickpea. Priming seeds with both Mo and *Rhizobium* can raise seed yield by a third compared to priming with just water. According to Swarnkar et al. (2012), 0.5 g sodium molybdate (Na_2MoO_4) and 5.0 g *Rhizobium* inoculum can be added in 1 L of water required for each kilogram of chickpea seed.

Alkaline soils are often deficient in zinc (Zn). In moderately Zn-deficient soils, zinc priming is an effective tool, whereas it may not fulfil the zinc requirement of the plant under severe deficiency. For example, seed priming alone is not sufficient to fulfil the requirement of kidney bean (Harris et al. 2008). However, seed priming with Zn is helpful in improving crop emergence, stand establishment, plant growth, seed yield and nutrient concentration (Shivay et al. 2016). Seed priming of chickpea seeds in a 0.05% solution of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) has been found quite effective to exhibit 19% higher seed yield and 29% more Zn concentration in seeds over that of non-primed seeds (Harris et al. 2008).

Dey et al. (2014) studied on chickpea with four levels of seed priming (control, hydro-priming, 0.05% zinc sulphate priming and 0.1% ammonium molybdate priming) under rainfed condition. In case of priming treatments, seeds were fully immersed in priming solution at room temperature for 8 h. All seeds were rinsed thoroughly with tap water to remove excess salts from seed coat and lightly hand-dried using blotting paper. Afterwards, primed seeds were allowed to dry back to their original moisture content under shade for 3 days and in sun for 1 day. Significantly higher values of major growth and yield attributes (plant height, branches plant^{-1}, pod number plant^{-1} and pod weight plant^{-1}) were recorded in plots having seed primed with 0.05% $ZnSO_4.7H_2O$ and was followed by seed priming with 0.1% ammonium molybdate [$(NH_4)_2MoO_4$] and simple water (hydro-priming). The positive effect of seed priming on plant growth might be due to earlier emergence of seedlings (Passam and Kakouriotis 1994), leading to uniform crop stand and more vigorous plants. Moussavi-Nik et al. (1997) reported the stimulatory effect of Zn on crop germination and establishment as well as the final yield, especially in Zn-deficient soils. Use of zinc sulphate might play the role in protein synthesis, cell membrane function and cell elongation (Cakmak 2000). Being an important element for nitrogenase enzyme, Mo helped in nitrogen fixation as well as crop productivity when seeds were primed with 0.1% ammonium molybdate (Dey et al. 2014).

11.3.5 Solid Matrix Priming

Solid matrix priming (SMP) or matriconditioning is a process in which seeds are mixed with a solid carrier material (e.g. ground leonardite shale, agro-lig, vermiculite, expanded calcined clay, synthetic calcium silicates, etc.) and water in known proportion (Khan 1992; Parera and Cantliffe 1992; Harman and Nelson 1994; Rogis et al. 2004; Hacısalihoglu 2007; Ermiş et al. 2016). This mixture allows the seeds to imbibe and attain the threshold moisture content but prevents radicle emergence (Taylor et al. 1988). Both the holding capacity and density of solid carriers and the amount of carrier relative to seed and water used for optimum conditioning have differed greatly (Khan et al. 1992). Bioprotectants and/or chemical pesticides may be used in conjugation with SMP. These materials may be added first to seeds as slurry, followed by the addition of solid particulate and water (Taylor and Harman 1990). SMP can increase germination at suboptimal temperatures and increase

anti-oxidative enzyme activity and levels of antioxidants in treated seeds (Wang et al. 2003; Kepczynska et al. 2007). Chitosan is a large cationic polysaccharide mainly obtained from waste materials from seafood processing. As an abundant and comparatively cheap organic compound in China, chitosan can be used for seed priming (Zhou et al. 2002). For example, SMP with 0.1% chitosan at 5% moisture level and 0.2% chitosan at 10–20% moisture level can enhance seed germination and seedling invigoration that synchronize with emergence of radicle and salinity stress tolerance up to the level of 6 dS m^{-1} in mung bean (Sen and Mandal 2016).

11.3.6 Hormo-priming

Hormonal priming or hormo-priming is the pre-seed treatment with different hormones, viz. salicylic acid (SA), abscisic acid (ABA), ascorbate, kinetin, gibberellic acid (GA_3), etc., which promote the growth and development of seedlings (Nawaz et al. 2013; Singh et al. 2015a). Plant growth hormones or their derivatives contained by several products are indole-3-butyric acids (IBA), an auxin and kinetin type of cytokinin (Afzal et al. 2016). Cytokinins are known to play a vital role in all phases of plant development starting from seed germination up to senescence. These cytokinins can be used as priming agents, which are mainly involved in the breakdown of dormancy of some seeds (Arteca 1996). For example, GA_3 treated soybean seeds recorded better field performance due to its stimulation effect in the formation of enzymes which are important in the early phase of germination and emergence (Riedell et al. 1985; Maske et al. 1997; Assefa et al. 2010). Bassi et al. (2011) reported that priming with 50 ppm GA_3 for 2 h enhanced emergence, germination percentage and speed of germination as compared to non-primed seed lots in soybean.

Various naturally occurring growth promoting substances such as moringa (*Moringa oleifera*) leaf extract, chitosan, sorghum (*Sorghum bicolor*) water extract and seaweed extract are also commonly used for seed priming (Afzal et al. 2016). Seed soaked with chitosan increased the energy of germination, germination percentage, lipase activity and GA_3 and indole acetic acid (IAA) levels in peanut (Zhou et al. 2002).

11.3.7 Halo-priming

Halo-priming refers to soaking of seeds in solution of inorganic salts such as sodium chloride (NaCl), potassium nitrate (KNO_3), calcium chloride ($CaCl_2$), calcium sulphate ($CaSO_4$), etc. A number of studies have shown a significant improvement in seed germination, seedling emergence and establishment and final crop yield in salt-affected soils in response to halo-priming (Nawaz et al. 2013; Singh et al. 2015a). Halo-priming not only promotes seed germination but also stimulates subsequent growth, thereby enhancing final yield of certain food legumes (Eleiwa 1989; Sallam 1999). In an evaluation study on the response of seeds primed with NaCl solution at different salinity levels (0, 3, 6 and 9 dS m^{-1}) in relation to early growth stage, seed priming with NaCl proved to be better treatment as compared to

non-primed seeds (Khan et al. 2009). Seed treatment with $CaCl_2$ or KNO_3 generally exhibited improvement in proteins, free amino acids and soluble sugars during germination under salt stress in pigeon pea (Jyotsna and Srivastava 1998). Beneficial effects of KCl have been reported in redgram (Rajandran 1982) and green gram (Basha 1982). Major yield attributes (pods plant^{-1} and seeds plant^{-1}) along with seed yield plant^{-1} in soybean were significantly enhanced by seed priming particularly with KNO_3. On contrary, seedling emergence percentage and germination time were significantly adversely affected by seed priming (KH_2PO_4 and KNO_3) as compared to non-primed seeds (Golezani et al. 2011). Pradhan et al. (2017) reported higher germination percentage, energy of emergence, seedling length, seedling dry weight and seed vigour index when seeds of black gram were soaked in 1% KCl solution (12 h), compared with control (unsoaked seed), and it was followed by priming in 1% KNO_3 solution (12 h).

11.3.8 Nano-priming

It is a new method of seed priming with nanoparticles for increasing germination percentage and improving seedling vigour. Maroufi et al. (2011) reported significant effect of nano-priming with the spray of titanium dioxide (TiO_2) nanoparticles (0.02%) in terms of improved seed germination percentage, seedling dry weight and seedling vigour in mung bean. However, comprehensive studies on nano-priming in pulses have not been elucidated.

11.3.9 Ultra-priming

Ultrasonic priming or ultra-priming involves the use of ultrasound energy for achieving more complete and faster seed germination as well as higher crop yields. Ultrasound energy (or simply ultrasound) is a type of mechanical energy called sound characterized by vibrating or moving particles within a medium. Ultrasound is distinguished by vibrations with a frequency higher than 20,000 Hz, which is not audible to humans. These ultrasounds only impose mechanical pressure on seeds to break the dormancy; therefore, no chemicals would contaminate the seeds (Nazari and Eteghadipour 2017). Water is used as a medium for treating the seeds by ultrasounds (Nazari et al. 2014). Seeds of the desired species are placed into an ultrasonic wave emitting apparatus containing water (Sharififar et al. 2015). Ultrasonication enforces the uptake of water, nutrients and/or other beneficial substances into the seed grains. Furthermore, the ultrasonic waves help in activating enzymatic and other biological reactions within the plant cell, resulting in a faster and more uniform germination. Even weak seeds/grains can be activated so that the overall seed performance gets improved (Hielscher 2017). The ultrasound technology has been successfully used in many mass transfer processes in food, such as in drying, extraction, osmotic dehydration, desalting and hydration (Miano et al. 2016). For example, use of ultrasound in mung bean seed priming was found to reduce ~25% of the

hydration process time, causing an acceleration of seed germination (Miano et al. 2016). Ultrasonic priming is a simple low-cost method to improve seed germination percentage, seed vigour index and root and shoot length of seedlings.

11.4 Importance of Seed Priming in Pulse Cultivation

In seed priming, seeds are partially hydrated to allow metabolic events to occur without actual germination and then redried to permit routine handling. Such a simple, inexpensive and economically viable technology offers a handful of benefits in pulse cultivation by resource-poor farmers. These are discussed hereunder.

11.4.1 Enhancement in Seed Germination/Seedling Emergence

Soil moisture is the most serious constraint for cultivating *rabi* pulses in rice fallows. Because, growing of *rabi* pulses solely depends upon the availability of soil moisture in the field after rice harvest when availability of soil moisture becomes low, followed by a fast decline in water table with the advancement of *rabi* season, resulting into mid- and terminal drought at flowering and pod filling stages, thereby adversely affecting pulse productivity in rice fallows (Ali et al. 2014; Ghosh et al. 2016). Hence, timely sowing of *rabi* pulses is very crucial in view of the moisture deficit during critical periods under rice fallow conditions (Kumar et al. 2012; Ali et al. 2014). Germination is an important aspect of seedling establishment under these conditions. Rice fallows affect seed germination, seedling emergence and crop establishment due to disruption of soil structure, soil water deficit, poor aeration and mechanical impedance of the seed zone. Of these, soil hardiness is the major limiting factor, followed by low organic matter content in the soil. Soil hardiness in the puddle rice fields deteriorates the hydraulic properties of the soil, which adversely affects the distribution of soil moisture as well as root growth of deep-rooted pulse crops. Such a hostile environment creates potential threat to microbial activity, nutrient availability, root growth (mostly confined to top soil layer) and water and nutrient uptake, which together make the subsoil resources unutilized in rice fallows. Even under relay (*paira*) cropping, plant population often becomes low due to low seed rate, poor contact of seed with soil, seed rotting as well as dryness of soil in patches (Ali et al. 2014; Ghosh et al. 2016).

Seed priming techniques have been found to improve germination characteristics including germination process, germination rate, germination uniformity (synchronized germination), germination percentage, etc., owing simply to less imbibition time (Brocklehurst and Dearman 2008; McDonald 2000; Taylor et al. 1998) and build-up of germination-enhancing metabolites (Basra et al. 2005; Farooq et al. 2006), thereby obtaining sufficient emergence followed by vigorous seedling growth at early stage, adequate crop stand (Arif et al. 2005; Ali et al. 2007; Diniz et al. 2009; Tiwari and Shivhare 2016; Nayban et al. 2017) as well as seed yield under abiotic stress conditions (Farooq et al. 2006, 2009; Harris 1996, 2006; Iqbal

and Ashraf 2007; Kaya et al. 2006; Khan et al. 2008; Patade et al. 2011; Saglam et al. 2010; Ansari et al. 2012; Dey et al. 2014). Simple overnight soaking of seeds can hasten seed germination and establishment under relay cropping (Ali et al. 2014; Singh et al. 2016). Pre-sowing soaking of seeds with KH_2PO_4, Na_2HPO_4, etc. has also earlier been reported to improve seed germination, seedling vigour and root growth early in the season, resulting in good establishment, better drought tolerance and more yield of crop plants (Solaimalai and Subburamu 2004). Osmo-priming not only improves seed germination but also enhances general crop performance under nonsaline or saline conditions.

11.4.2 Improvement in Root and Shoot Length of Seedling

Seed priming ensures rapid seed germination and seedling emergence, helping in faster emergence, producing deep roots before the upper layers of the soil are dried and crusted and finally leading to better crop establishment and higher crop yield (Ashraf and Foolad 2005). Healthy plants with well-developed root system can more effectively mobilize limiting nutrients from the soil and can also better withstand adverse conditions (Nayban et al. 2017). Seed priming (osmo-conditioning) with KH_2PO_4 (200 mM) and mannitol (20 g L^{-1}) has been found to significantly increase the root as well as shoot length of mung bean seedlings possibly due to priming-induced nuclear replication in root tips of fresh seedlings (Umair et al. 2013). Vishwas et al. (2017) reported significantly higher root and shoot length of chickpea seedling due to seed priming with *Rhizobium* + *Pseudomonas* at 10% for 12 h. Likewise, Sajjan et al. (2017) reported an improvement in different growth parameters including root and shoot length of seedling due to seed priming with mesquite (*Prosopis juliflora*) leaf extracts (2%) for 1 h in pigeon pea.

11.4.3 Improvement in Nodulation

Seed treatment with beneficial microbes is becoming increasingly important (Howell et al. 1997). Treatment of leguminous seeds with *Rhizobium* spp. is well known for many years for nitrogen fixation. *Azospirillum* and other nitrogen-fixing bacteria are also in use. There are reports on improvement in nodulation, besides imparting a good control of seed-borne diseases with the use of microbiological products for seed priming in pulses. SMP, osmo-priming and hydro-priming methods have all been employed to increase beneficial microbial populations on the seed (Sharma et al. 2015), helping better root nodulation in pulses. Dugesar et al. (2017) evaluated four different methods of seed priming (hydro-priming with distilled water; osmo-priming with 20% PEG 6000; halo-priming with 1% NaCl; halo-priming with 1% $CaCl_2$; organic priming with 5% tulsi leaf extract; organic priming with 5% curi leaf extract) for 12 h in black gram. They reported significant differences among priming treatments with the control (no priming). The highest values of field emergence (%) and plant nodulation characters were observed under osmo-priming, whilst the highest nodulation was observed under the seeds primed with

PEG and $CaCl_2$. Mishra et al. (2017) reported better root nodulation in pigeon pea due to osmo-priming with PEG (20%), compared with control.

11.4.4 Increased Nutrient Uptake and Improved Crop Nutrition

There is an opportunity to improve crop nutrition through seed priming with both macro and micronutrients (Harris 2006). Seed priming is an easiest way of supplying starter nutrient(s) for seedling establishment and early growth and to integrate mineral nutrients into food legumes (agronomic biofortification). In particular, micronutrient deficiency (hidden hunger) is a global issue in food crops, causing reduced yields and nutritional quality of the produce (Dey et al. 2014). Micronutrient application through seed priming not only improves germination, stand establishment, plant vigour and yield but also micronutrient concentration (enrichment) in the grain (Singh et al. 2015b). For example, priming chickpea seeds in solutions of optimal concentration of micronutrients resulted in an increase in Zn content from about 40–60 (unprimed) to 500–800 mg kg^{-1} (Zn-primed). Priming lentil seeds exhibited similar results. There was no difference in Zn content of seeds primed for 8 h, compared to those primed for 12 h in solutions of similar nutrient concentration. However, an increase in concentration of Zn in the priming solution increases the amount of nutrient taken into both chickpea and lentil seeds (Johnson et al. 2005). Nutrient priming has been found more economical and convenient as compared to soil application in low fertile soils (Slaton et al. 2001). Umair et al. (2013) reported an improved nutrient uptake and crop nutrition through seed priming in mung bean.

11.4.5 Increased Crop Yield

Since pulses are commonly grown under energy-starved conditions with suboptimal agronomic management, seed priming can be effective for legumes. There are many reports that yields of a number of legumes can be increased considerably by priming seeds before sowing (Parera and Cantliffe 1994; Musa et al. 2001; Harris et al. 1999, 2004; Rashid et al. 2004; Grant et al. 2005; Gupta and Bhowmick 2005; Khan et al. 2008; Bhowmick 2010; Umair et al. 2011, 2013; Bhowmick et al. 2013). However, yield increases due to priming are not always only due to increased stand density but also may be due to a combination of population density and improved individual plant performance (Musa et al. 2001; Rashid et al. 2004; Harris 2006). According to Afzal et al. (2016), hydro-primed seeds produced healthy seedlings, which resulted in uniform crop stand, drought resistance, early maturity and somewhat improved yield.

11.4.6 Increased Pest Resistance

Seed priming can be a viable option (Rakshit et al. 2013) for improving plant acclimatization not only under abiotic stresses but also it is quite effective for the

management of biotic stresses (insect pests, diseases and weeds), besides sustaining agricultural production without endangering natural resource base (Rakshit et al. 2014; Nayban et al. 2017). There are several field reports that primed crops suffer less from insect pests and diseases (Harris 2006). Musa et al. (2001) reported that seed priming significantly reduced the seed-borne diseases like collar rot (*Sclerotium rolfsii*) and *Fusarium* wilt in chickpea. Likewise, the primed chickpea crop suffered less damage from pod borer damage in India (Musa et al. 2001; Harris 2006). Seed priming in water for 8 h has been reported to significantly minimize the incidence of viral disease caused by *Mungbean yellow mosaic virus* in mung bean (Rashid et al. 2004). Rakshit et al. (2014) reported that seed bio-priming with bioagent (*Trichoderma harzianum* and/or *Pseudomonas fluorescens*) at the rate of 10 g kg^{-1} seed might provide protection against seed- and soil-borne plant pathogens and improve seed germination and seedling growth. Seed priming with zinc salts is used to increase growth and disease resistance of seedlings (Afzal et al. 2016). Seed priming ensures good crop establishment, which can increase the competitiveness of crop plants, enabling them to suppress the weeds also (Nayban et al. 2017).

11.4.7 Seasonal Climate Risk Management

Greater uncertainties under changing climatic situation often combined with the use of poor quality seeds and pulse cultivation in marginal lands lead to slow the germination and emergence, causing patchy stand as well as multiple and delayed replanting of seeds. Seed priming is a simple, effective and climate-resilient technology for addressing this problem. It has been found that primed seeds usually emerge faster, produce more vigorous seedlings with better-developed root systems, reach flowering and maturity earlier and result in higher yield than non-primed seeds, which is important for avoiding or escaping terminal drought and heat stress (Uddin et al. 2005; Padgham 2009). When crop planting gets delayed due to adverse climatic conditions such as low temperature or high rainfall at sowing and high temperature at reproductive stage, seed priming may be beneficial in avoiding detrimental conditions by earlier crop maturity without compromising yield. In fact, earlier and vigorous crop stand usually captures more resources of water and nutrients through better root system, owing to larger leaf area and duration with enhanced photoassimilation that subsequently contributes towards better yield (Afzal et al. 2016). Seed priming with beneficial microorganisms promotes plant growth and increases abiotic stress tolerance in arid or semi-arid areas.

11.5 Conclusion

Pulses continue to occupy a prominent place from the view point of food and nutritional security and also become well suited to any cropping system owing to their ability towards biological nitrogen fixation, low water requirement, comparatively shorter duration and capacity to withstand weather abnormalities. Since these crops

are mainly raised in the hands of small and marginal farmers, their productivity levels often become low due to suboptimal agronomic management practices. Seed priming is an easily applicable, practicable, low-cost, risk-averting and economically viable technology for the resource-poor farmers towards ensuring uniform seed germination, rapid emergence, successful crop establishment and better yield performance of pulse crops under stress-prone conditions, including adverse soil temperature, variable soil moisture, soil hardiness, etc. Several research accomplishments clearly show that priming pulse seeds before sowing can increase the rate and extent of emergence, improve seedling vigour, produce deeper roots with better nodulation and nutrient acquisition, hasten flowering and maturity, impart pest resistance and ultimately enhance crop yield in most of the cases. But the longevity and viability of primed seeds during storage may be reduced. There may have certain other instances, where priming does not benefit farmers, but negative effects of seed priming are uncommon. Further studies are, however, needed for developing and fine-tuning different alternative seed priming options and their effective combinations, based on easily available natural resources, crop seasons, growing situations and prevailing soil and environmental conditions. Moreover, location-specific selection of suitable pulse crops as well as priming modalities (appropriate priming material, its solution concentration and/or soaking duration, as applicable) need to be standardized and validated through on-farm testing before making any solid recommendation to the resource-poor farmers.

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Studies on Seed Priming in Pepper (*Capsicum annuum* L.)

12

Nusret Ozbay

Abstract

Plants are regularly exposed to various adverse environmental conditions such as drought, salinity, chilling, and high temperatures. These abiotic stresses adversely affect the plant growth and productivity and, in extreme cases, cause plant death. One feature expected of high-quality seeds is to be able to germinate under adverse growing conditions. Rapid germination and emergence are essential for successful crop establishment, for which seed priming could play an important role. Seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigor, leading to better stand establishment and yield. Over the past 50 years, there has been extensive literature published on seed priming and related pre-sowing seed treatments. These have been particularly promising for high-value small-seeded vegetable crops such as pepper where rapid, uniform germination is at a premium either in the cell transplant production or where accurate and uniform plant population density is required from direct sowing. The aim of this chapter is to review some of the current ideas and recent work on physiological priming treatments designed to enhance germination and emergence performances of pepper seeds.

Keywords

Pepper · Seed priming · Germination · Emergence · Seedling growth and establishment · Vigor · Environmental stress

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12.1 Introduction

Pepper is one of the most important vegetable crops in the world. Peppers are grown worldwide because of their adaptation to different agroclimatic regions and their wide variety of shapes, sizes, colors, and pungencies of the fruit (Qin et al. 2014). Bell pepper or sweet pepper belongs to the genus *Capsicum*, a member of the Solanaceae family that includes tomatoes, potatoes, and eggplants. Pepper is a cultivar group of the species *Capsicum annuum* L. Cultivars of the plant produce fruits in different colors, including red, yellow, and orange, but more exotic colors include purple, white, and lime green. The fruit is also frequently consumed in its unripe form, when the fruit is still green. In the United States, Canada, the United Kingdom, Ireland, and some other parts of the world, in addition to the terms "bell pepper" and "sweet pepper," the fruit is often referred to simply as a "pepper," whereas in many Commonwealth of Nations countries, such as Australia, India, Malaysia, and New Zealand, they are called "capsicum."

Peppers are native to Mexico, Central America, and northern South America. Pepper seeds were later carried to Spain in 1493 and from there spread to other European, African, and Asian countries (Andrews 1993; Abdel-Kader and El-Mougy 2014). According to FAO statistical database, in 2014, pepper is cultivated on 3,625,452 ha in the world, with an annual production of 36,143,113 metric tons (MT) representing an annual yield of 9.97 MT ha^{-1}. Today, China is the world's largest pepper producer, followed by Mexico, Turkey, and Indonesia (FAO 2017).

Peppers grow better in evenly moist and evenly warm soil and can be grown year round in frost-free conditions. If moisture levels or temperature levels fluctuate, too much growers will have problems related to some diseases and yield. It has also been reported that pepper seed germination and emergence are slow and nonuniform under normal as well as stressful conditions (Belletti and Quagliotti 1988; Chartzoulakis and Klapaki 2000; Demir and Okcu 2004; Khan et al. 2009a). Low ability of seed germination during seed production and rapid deterioration during storage of pepper seed are also the major problems that affect seed quality (Siri et al. 2013). Rapid germination and emergence are essential for successful crop establishment. Various seed treatments have been suggested to improve seed germination and emergence, and one of them is priming. Seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigor, leading to better stand establishment and yield (Singh et al. 2015; Ibrahim 2016).

During priming, the seeds are partially hydrated in such a way that pregermination metabolic activities start; however, radicle protrusion is prevented followed by drying of seeds to the original moisture content (Sharma et al. 2015). Seed is primed to obtain faster and more uniform germination resulting in a stronger crop stand. Yadav et al. (2011) reported that priming treatments improved germination percentage as well as rate of seed germination in pepper under normal as well as the stressed conditions. Maiti et al. (2009) studied the effect of priming on seedling vigor and productivity of pepper, during post-rainy seasons demonstrating that

priming improved germination and seedling development and yield pepper. Another primary benefit of priming is the extension of the temperature range at which a seed can germinate (Valdes and Bradford 1987; Ellis and Butcher 1988). This has particular importance for crops such as pepper, in which seedlings are produced in the early spring at low temperatures for planting in the open field and in late summer at higher temperatures for glasshouse production. It has been reported that priming improved seed germination at suboptimal temperatures (Pandita et al. 2007; Korkmaz and Korkmaz 2009). Nascimento et al. (2011) reported that priming improved the physiological quality of hot pepper seeds by improving germination at high temperature (35 °C).

12.2 Seed Priming Methods

In seed technology, different pre-sowing treatments have been utilized to reduce the time from sowing until germination or emergence and to shorten the interval between the first and last seed germination. One of the most important pre-sowing treatments is known as seed priming. The principle of priming is based on the fact that it is possible to hydrate seed in some ways at a moisture level sufficient to initiate the early events of germination but not sufficient to permit radical protrusion through the seed coat (Sivritepe and Sivritepe 2016). Seed priming enhances seed performance by rapid and uniform germination, more uniform emergence, normal and vigorous seedlings, greater tolerance to environmental stress, and reduced dormancy in many species (Khan 1992; Desai et al. 1997; Cantliffe 2003). Additionally, this physiological treatment provides invigoration treatment to partially aged seed lots. Excellent review studies have considered the importance of seed priming and the factors that affect its success (Khan 1992; Parera and Cantliffe 1994; Bray 1995; Pill 1995; Taylor et al. 1998; Welbaum et al. 1998; McDonald 2000). Beneficial effects from priming have been reported for several vegetable seeds including pepper (Gomes et al. 2012). Improvement of germination in pepper plant by priming with water and NaCl has been reported (Smith and Cobb 1991a). The results of Kaya et al. (2010) showed that priming pepper seeds can increase germination percentage and accelerate germination at suboptimal temperatures. Priming pepper seeds in PEG and KNO^3 solutions significantly increased emergence percentage and decreased mean time to emergence of pepper seedlings (El-Shatoury 2010). Various priming treatments have been developed to enhance seed germination, seedling growth, and yield in most of the crops under normal and stress conditions and also to avoid biotic and abiotic stresses during germination and emergence phases (Bradford 1986; McDonald 2000). These priming methods can be listed as follows (Ashraf and Foolad 2005; Lutts et al. 2016; Sivritepe and Sivritepe 2016): hydropriming, osmopriming, solid matrix priming, halopriming, drum priming, hormopriming, thermopriming, biopriming, nutripriming, and organic priming.

12.2.1 Hydropriming

It is a simple and low-cost priming technique in which seeds are soaked in water (aerated distilled water is preferred) for a certain period of time to a point where pre-germination metabolic activities start without actual germination and subsequently dried back to storage moisture contents before sowing (Singh et al. 2015). This process is especially useful in economically disadvantaged, arid crop-growing areas. Hydropriming has been considered as a simple and cost-effective strategy for improving germination and emergence of pepper. For example, Demir and Okcu (2004) reported that aerated hydration treatment of pepper and eggplant seeds significantly increased final germination of both species over a wide range of temperature (18–35 °C). Hydropriming with normal water or warm water improved the radicle protrusion of pepper seeds significantly as compared to the control treatment (Yadav et al. 2011). Similarly, hydropriming of pepper seeds for 24 h enhanced the germination and emergence percentage, ensured early germination and seedling emergence, increased uniformity of emergence, and increased seedling vigor compared to unprimed seeds (Uche et al. 2016).

12.2.2 Osmotic Priming

This method is originally developed and described by Heydecker et al. (1973, 1975). Osmotic priming technique, also called osmopriming or osmoconditioning, is based on the controlled hydration of seeds to a level that allows pre-germination metabolic activity but inhibits radicular emergence. It is achieved by immersing seeds in an aqueous solution of a chemically inert but osmotically active compound such as polyethylene glycol (PEG) for a specific period at a specific temperature (Heydecker et al. 1975; Khan 1992). At the end of the process, seeds are rinsed before further processing. Taylor and Harman (1990) and Gray (1994) provide access to the very large literature on osmotic priming. Some of the compounds that are used for osmotic priming include sugars, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol. The improvement of germination and emergence performance of seeds of many plant species through a pre-sowing treatment with a chemically inert osmoticum have been reported by many researchers (Bennett et al. 1992). The effect of seed priming on field emergence of pepper seeds has ranged from no improvement to some advancement in mean germination time (Yaklitch and Orzolek 1977; Bradford et al. 1990). Aljaro and Wyneken (1985) reported that osmoconditioning of sweet pepper seed did not affect the germination percentage but shortened germination time and gave more uniform germination. Pepper seeds primed in mannitol had improved final percent germination and produced larger seedlings than nonprimed seeds (Georghiou et al. 1987; Passam et al. 1989). Kikuti et al. (2005) reported that sweet pepper seeds primed with PEG 6000 performed better in all the vigor tests assessed, except the germination test. Demirkaya (2006) studied osmotic conditioning with polyethylene glycol to enhance the germination percentage and to

reduce the mean germination time of pepper seeds. He found that osmoconditioned seed enhanced germination percentage and reduced the mean germination time. Cortez-Baheza et al. (2007) indicate osmopriming treatments using PEG in combination with GA_3 or KNO_3 have shown to be good approaches to revigorate pepper seeds for commercial purposes. Siri et al. (2013) reported that seed germination was improved in osmoprimed pepper seeds primed in a PEG 6000 solution with the osmotic potential of −1.5 MPa for 6 days.

12.2.3 Halopriming

In halopriming, the seeds are immersed in different inorganic salt solutions (NaCl, KNO_3 $CaCl_2$, $CaSO_4$, etc.) which facilitate the process of seed germination and subsequent seedling emergence even under adverse environmental conditions. Halopriming is a simple and cheap agro-technique and therefore found suitable to be recommended to the farmers owing to better synchrony of emergence and crop stand under various conditions of environment (Sedghi et al. 2010). A number of studies have shown a significant improvement in seed germination, seedling emergence and establishment, and final crop yield under unfavorable conditions such as suboptimal temperature and salinity as a result of seed halopriming. For example, Smith and Cobb (1991a) reported that priming with water and NaCl improved germination in pepper plant. Carter (1994) initiated a work to examine the effects of NaCl priming on total germination and the rate of germination under suboptimal temperature conditions. Seeds of "Tam Veracruz" pepper were primed at 23 °C for 5 days in double-distilled H_2O, or 0.2, 0.4, or 0.6 M NaCl solution (equivalent to an osmotic potential of −0.89 MPa, −1.77 MPa, and −2.66 MPa, respectively). He suggested that priming "Tam Veracruz" pepper seeds for 5 days at 23 °C in 0.2 M NaCl increased the germination rate at temperatures from 15 to 23 °C. A previous study on pepper (Amjad et al. 2007) showed that halopriming improved seed germination, seedling emergence, and growth under saline and drought conditions. Khan et al. (2009a) observed that priming of seeds using NaCl improved seedling vigor and establishment under salt stress conditions. It has been reported that halopriming with salt (NaCl) improved the rate of pepper seed germination (Yadav et al. 2011). In a study comparing hydropriming and halopriming with dehydration treatments for physiological enhancement of pepper seeds, both hydro- and halopriming treatments with and without dehydration conditions caused increases in normal germination percentage and germination index of pepper seeds compared with the control (Sivritepe and Senturk 2011). Maiti et al. (2013) reported that halopriming in 3% KNO_3 solution for 40 h at normal room temperature increased speed of emergence, seedling vigor index, root length and shoot length over hydropriming, and control in pepper. They also reported that, at field level, the halopriming treatment increased yield compared to the control and hydropriming treatments. Dutta et al. (2015) also reported that pepper seeds primed in 1% KNO_3 recorded the highest germination percentage as compared to nonprimed control.

12.2.4 Drum Priming

Drum priming is a special seed priming method in which seeds are hydrated to predetermine humidity content by placing them inside a horizontal rotating drum. Drum priming has the capability to accurately hydrate seeds without concern for spent solution or solid carrier disposal (Rowse 1996). In this novel method, the supply of water to the seeds is controlled by physical rather than osmotic means or solid matrix materials, limiting the amount of water introduced to the seed. Specially designed apparatus enables monitoring of the seed weight, precise regulation of time, and water amount during hydration process, what ultimately results in an appropriate and uniform moisture level of the seeds (Warren and Bennett 1997). The method enables the priming of much larger quantities of seed than previously been practical and avoids the environmental problems of disposal of the used priming solution. Unlike other priming methods, it does not require an oxygen enrichment of the atmosphere because of the nature of the process. Drum priming systems are among the most common methods of seed priming for commercial treatment. Da Silva et al. (2015) combined drum priming with 24-epibrassinolide (24-EpiBL) for enhancement of bell pepper seed performance. Several advantages were verified in the drum priming technique with added 24-EpiBL compared with the traditional procedure (water alone). Drum priming with 24-epibrassinolide showed positive effect on germination time and seedling growth of bell pepper.

12.2.5 Solid Matrix Priming

Solid matrix priming (SPM), also known as matriconditioning, is a process in which seeds are mixed with a solid material and water in known proportions and then incubated for a given duration at constant temperature. Solid matrix priming utilizes carriers like vermiculite, diatomaceous earth, or another highly water-absorbent polymer (Celite® or Micro-cel®) possessing characteristics such as high water holding capacities, low osmotic potentials, and low bulk density (Taylor et al. 1988, 1998; Khan et al. 1990; Khan 1992; McDonald 2000). In order to improve the control of imbibition, pure water may be replaced by an osmotic solution, as in osmotic priming (Khan 1991). After incubation the extraneous solid material is sieved off. Solid matrix priming is similar to osmotic priming, allowing the seed to imbibe and attain threshold moisture content and pre-germination metabolic activities but preventing radicle emergence. However, it has the advantages of allowing aeration, incorporation of biological agents to combat soilborne pathogens, and improved ease of handling (Taylor et al. 1988; Harman et al. 1989; Paparella et al. 2015). This technique has been used to improve seed germination and rate in many species including peppers. Kubik et al. (1988) compared SPM with commercially osmoprimed tomato and pepper seeds under growth chamber conditions. They used expanded calcine clay as a solid material. SMP-treated seed performed equally as well as, or better than, commercially osmoprimed seed. Ilyas et al. (2002) reported that matriconditioning improved quality and protein level of medium vigor hot

pepper seed. Kang et al. (2003) compared osmotic with solid matrix priming to determine the more effective treatment for improving seed germination in pepper and tomato and reported that solid matrix primed pepper seeds germinate faster than osmotic primed seeds at all temperatures (15, 20, 25, and 30 °C). On the other hand, they also reported that early growth was not significantly influenced by osmotic priming or SMP treatment of pepper and tomato seeds. Hacisalihoglu and White (2006) conducted an experiment to determine the optimum duration, temperature, and ratio (seed/carrier/water) of matriconditioning "long red cayenne" pepper seeds for improved germination percentage and mean germination time. The results showed that matriconditioning, which is carried out in 453 g glass jars by mixing seeds, water, and synthetic calcium silicate at 25–30 °C for up to 5 days, increased final germination percentage to 96.7%, compared with 82% in the control seeds. Furthermore, matriconditioning decreased mean germination time tenfold, compared with a nonprimed control. Pandita et al. (2007) found that solid matrix priming improved germination of hot pepper seed by 10–16% depending on temperature, and this effect enhanced when SMP was followed by halopriming and osmopriming. QianQian et al. (2009) indicated that priming treatment with vermiculite could improve the vigor of hot pepper seeds and the salt tolerance of hot pepper seedlings.

12.2.6 Biopriming

Biopriming seed treatment is gaining importance in seed germination and management of plant pathogens as another alternative to chemical fungicides in recent years. The biopriming procedure first described by Callan et al. (1990) for biological control of *Pythium* preemergence damping-off of sh2 sweet corn integrated imbibition at an optimal temperature with protection by a biocontrol agent. Biopriming is a process of biological seed treatment that refers to combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organisms to protect seed (Rakshit et al. 2015). The seeds are removed before radicle emergence (Callan et al. 1990). Because seeds can fail to germinate due to a number of reasons, both biological and physiological seed treatments used in combination seem to provide the best seed protection (Bennett et al. 1992). The leakage of seed exudates during biopriming may supply nutrients and energy for biocontrol agents (Wright et al. 2003). This favorable environment contributed to colonization and proliferation of biocontrol agents over the seed surface to facilitate water uptake and nutrients during biopriming. Biopriming with different beneficial microbes may not only enhance seed quality but also boost seedling vigor and ability to withstand abiotic and biotic stressors and thus offer an innovative crop protection toll for the sustainable improvement of crop yield (Rakshit et al. 2015). Biopriming is recently used as an alternative method for controlling many seed- and soilborne pathogens (Reddy 2012). For example, combined effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* as seed biopriming resulted in significant growth of pepper seedlings (Kumar et al. 2010).

In another work on bell pepper, Da Silva and Filho (2012) compared the efficiency of biostimulant priming (10 mL of Stimulate® in 100 mL of distilled water) to hydropriming in bell pepper seeds as evaluated by SVIS® analysis and recommended vigor tests. They indicated that hydroprimed and biostimulant primed seeds performed better than control. An investigation was carried out using "PKM 1" chili (*Capsicum annuum* L.) seed to standardize biopriming with the biocontrol agents *Trichoderma viride* or *Pseudomonas fluorescens* in order to improve seed germination and seedling vigor (Ananthi et al. 2014). They have suggested that seed biopriming with 60% (w/v) preparations of *T. viride* or *P. fluorescens* for 3 h or 12 h, respectively, can therefore be adopted to improve seed germination and seedling vigor in chili. Ilyas et al. (2015) reported that biopriming with the biofungicide and clove oil 0.06% or 0.1% was an effective seed treatment to improve the vigor and relative speed of germination while reducing the percentage of *Colletotrichum capsici*, a seed-borne pathogen causing anthracnose disease in hot pepper seeds (Ilyas et al. 2015). However, it still provides inconsistent results. Copeland and McDonald (1995) indicate that, in many cases, the biological treatment of seeds is not persistent in soil, seed, or plant, under natural conditions. Therefore, a deeper research concerning the methodology of application, doses, storage of coated seeds, responses from different species to the materials, types of products, and polymers should be performed to standardize.

12.2.7 Priming with Plant Growth Regulators and Other Organic Sources

Soaking or treating seeds in optimal concentrations of plant growth regulators (PGRs) improves germination, stand establishment, growth, and yield of crop plants under both normal and stress conditions. Carter and Stevens (1998) determined that when primed with gibberellic acid (GA3), pepper seeds had higher germination (91%) as compared to nonprimed seeds at high temperature (40 °C). The direct benefits of seed priming with PGR were reported by Mendoza et al. (2002), who demonstrated that that imbibing pepper seeds in 0.1 mM salicylic acid prevented seedling from subsequent chilling-induced damage. Yogananda et al. (2004) found that bell pepper seeds invigorated with GA_3 (200 ppm) recorded higher germination, root and shoot length seedling dry weight, rate of germination, and seedling vigor indices over control. A laboratory study was performed by Khan et al. (2012) to explore the benefits of seed priming with polyamines on seed germination and seedling growth of hot pepper. Hot pepper seeds were primed in aerated solution of putrescine, spermine, or spermidine (25, 50, 75, and 100 mM) for 48 h at 25 ± 2 °C. Results showed that polyamine priming resulted in earlier and synchronized germination via improving final germination percentage, time to 50% germination, mean germination time, germination energy, germination speed, and germination index compared with control. Seed performance of various crops including pepper can also be improved by inclusion of plant growth regulators and hormones during priming and other pre-sowing treatments (Lee et al. 1998;

Korkmaz and Korkmaz 2009). For example, priming with PEG and GA_{4+7} increased early germination in pepper especially at low temperature (15 °C), 3.8 days as compared 4.7 days in nonprimed seeds (Watkins and Cantliffe 1983). Andreoli and Khan (1999) reported that matriconditioning pepper seeds at 25 °C in the presence of gibberellic acid hastened seed germination and improved stand establishment of pepper. Korkmaz (2005) reported that inclusion of acetyl salicylic acid and methyl jasmonate into the priming solution improved low-temperature germination and emergence of sweet pepper.

Several new compounds are also being tested for their potential use as priming agents. For example, priming pepper seeds in 3% KNO_3 with the presence of 5-aminolevulenic acid (ALA) improved final germination percentage (FGP) and germination rate at 15 °C compared to nonprimed seeds (Korkmaz and Korkmaz 2009). Ozbay and Susluoglu (2016a) reported that sweet pepper were treated in 3% KNO_3, 2% KH_2PO_4, and 10% PEG solutions containing 0, 25, 50, and 100 mg L-1 prohexadione-calcium (Pro-Ca) in darkness at 25 °C for 3 days. Priming pepper seeds, in the presence or absence of plant growth regulator, improved final germination percentage, mean time to germination, and germination index, final emergence percentage, and mean time to emergence compared to nonprimed seeds. The highest final germination percentage and the lowest mean time to germination were obtained from KH_2PO_4 + 25 mg L-1 Pro-Ca treatment.

Priming with some organic agents could also be used in pepper seeds for physiological enhancement. In an earlier study conducted by Sivritepe and Sivritepe (2008), possibility of using seaweed extract (as an organic material) in priming technique was examined in pepper seeds. The seeds (cv. California Wonder) were subjected to priming treatments performed by the use of 1:1, 1:5, 1:10, 1:25, 1:50, 1:100, 1:250, 1:500, and 1:1000 dilutions of seaweed extract (Maxicrop) and also H_2O, for 1, 2, and 3 days at 20 °C. They concluded that seaweed extract could be used as an osmotic agent in organic priming of pepper seeds for physiological enhancement. They also commented that seaweed extracts caused physiological enhancements and increases in performance of seeds due to their hormonal components such as cytokinins and amino acid contents and also their hygroscopic characteristics. Demirkaya (2010) demonstrated that seaweed extract could be used in osmotic conditioning treatments of onion seeds as well as pepper seeds. Similarly, Sivritepe et al. (2015) reported that organic priming with continuously aerated seaweed extract (1000 ppm) for 2 days at 20 °C and dehydration treatments gave the best results in terms of physiological enhancement of pepper seeds. An experiment was conducted to improve the germination and quality of seedling through seed priming of bell pepper cv. California Wonder using 16 botanicals and animal by-products/wastes for 12 and 24 h. The best germination and quality of seedlings were obtained through pre-sowing seed priming treatments of *Melia azedarach* leaf extract 10% followed by Eucalyptus leaf extract 10%, garlic clove extract 5%, cow urine 5%, and cow dung extract 5% at seed soaking duration of 24 h (Mehta et al. 2010). Dutta et al. (2015) carried out an experiment to find out efficacy of organic (200 g/Kg seed neem leaf powder) and inorganic priming (3% KNO_3 and 2% KH_2PO_4) on germination and seedling vigor of bird's eye chili (*Capsicum*

frutescens L.) seeds. Among all priming treatments, 1% KNO3 (39.68% higher) recorded the highest germination percentage as compared to nonprimed control. In a recent study conducted to investigate effect of organic priming with marigold herbal tea on seed quality in Aji pepper (*Capsicum baccatum* var. *pendulum* Willd.), the herbal teas obtained from flowers of the *Tagetes patula* and *Tagetes erecta* species were used as an organic priming solution. It has been concluded that organic priming effectively increased germination, emergence, and the seedling fresh weight at different maturity levels of pepper (Mavi 2016). On the other hand, Teksan and Kavak (2016) reported that organic priming treatments with marigold and rose flower herbal teas did not significantly affect germination and emergence of pepper seeds compared to the control. Demirkaya (2016) found that organic priming treatments with seaweed extracts and methyl jasmonate improved the germination percentages and reduced the mean germination times of pepper seeds at low temperatures (15 and 20 °C).

12.3 Factors Affecting Priming Success

Priming efficiency is affected by many factors such as plant species (even cultivars within a species), priming technique, priming media, its concentration, water potential, oxygen availability, priming duration, temperature, seed condition, and storage conditions (Parera and Cantliffe 1994; Bray 1995). Optimal priming conditions may vary among cultivars and even seed lots of a given species. Bradford et al. (1990) indicated that there was a strong interaction between pepper seed lots and priming response, with the slowly germinating lots exhibiting the greatest benefit from priming. It has been reported that the germination response of two pepper varieties, viz., California Wonder and Yolo Wonder, was investigated under the osmotic potential of −5, −10, and −15 bar created by aqueous solution of PEG 6000 and KNO_3. Variety California Wonder appeared to be more responsive to preconditioning treatments as compared to the variety Yolo Wonder (Thakur et al. 1997). Priming in salt solutions leads to faster germination than priming in mannitol or PEG (Passam et al. 1989). Similar results were also observed for pepper seeds primed in KNO_3 and $KNO_3 + K_3PO_4$ solutions (O'Sullivan and Bouw 1984). $KN0_3$-primed jalapeno pepper seeds resulted in significantly earlier germination and accelerated vegetative seedling development, whereas priming in PEG appeared to retard vegetative seedling development (Rivas et al. 1984). In a study conducted by Jones and Sanders (1987), pepper seeds were soaked in water, a 1% KNO_3 + 1% K_2HPO_4 solution or a 1.5% KNO_3 + 1.5% K_2HPO_4 solution at 21 °C for either 72 h or 96 h. Seeds were air-dried and germinated at 15 °C, 20 °C, or 25 °C. All soaking treatments hastened germination and resulted in more uniform germination. Soaking seed in water or a 1% KNO_3 + 1% K_2HPO_4 solution gave similar results and was slightly better in promoting germination than the 1.5% KNO_3 + 1.5% K_2HPO_4 solution. Hydropriming (48 h) for tomato and sand matrix priming (80% water holding capacity, 3 days) for eggplant and chili were established as best methods of priming treatment capable of improving seed vigor (Venkatasubramanian and Umarani 2007).

12.4 Effect of Priming on Seed Germination and Seedling Establishment

High yield of the vegetable crops can only be obtained with high stand establishment of seedlings so that they can compete with the environment and can produce best plantation, ultimately increased yield and quality (Grassbaugh and Bennett 1998; Cantliffe 2003). The nonuniformity of pepper seed germination is one of the main problems faced by growers, since these seeds usually show low germination rate and low vigor (Silva et al. 2012). High germination and uniform stand establishment for chili pepper production are essential to maintaining profitable yields. However, pepper has non-starchy endosperm, and this offered a mechanical barrier to the growing embryo resulting in poor germination and emergence (Andreoli and Khan 1999). Nonuniform crop emergence results in plants of variable size and competitive ability. Subsequent management practices may be less effective on fields with nonuniform emergence. If stand establishment is poor, yields and quality of once-over machine-harvested crops will be poor. An increasing number of investigators are becoming interested in seed biology with the objective of understanding and controlling the many aspects of seed germination and seedling establishment (Bradford 1995). Seed priming is an alternative to achieve this objective. A number of processes stimulating germination are activated by seed priming and persist after the dehydration of the seed (Asgedom and Becker 2001). Therefore, upon sowing, the primed seeds can rapidly imbibe and restore the seed metabolism, resulting in an increased germination rate, decreased nonuniform germination, and better seedling development (Rowse 1995). Many evidences have shown seed priming could improve germination and early seedling growth under normal and stress conditions compared to plants grown from untreated seed (Bradford 1986; Chen et al. 2012). There are several investigations reporting either no effect or negative or beneficial responses of seed priming for pepper. Ghate and Phatak (1982) reported a significant decrease in germination rate when pepper seeds were primed with K_2HPO_4 plus $(NH4)_2HPO_4$ solution. It has been also reported that seed priming has not proved beneficial effect for tabasco pepper (*Capsicum frutescens* L.) field stand establishment (Sundstrom et al. 1987). In a study carried out by Jones and Sanders (1987), pepper seeds were primed in water, a 1% KNO_3 + 1% K_2HPO_4 solution, or a 1.5% KNO_3 + 1.5% K_2HPO_4 solution at 21 °C for either 72 h or 96 h. Seeds were air-dried and germinated at 15 °C, 20 °C, or 25 °C. All priming treatments hastened germination and resulted in more uniform germination. They also reported that priming seed in water or a 1% KNO_3 + 1% K_2HPO_4 solution gave similar results and was slightly better in promoting germination than the 1.5% KNO_3 + 1.5% K_2HPO_4 solution. Sundstrom and Edwards (1989) reported that an increased rate of germination was observed when jalapeno (*Capsicum annuum* L.) and tabasco (*Capsicum frutescens* L.) peppers were primed in a 3.0% or 2.75% KNO_3 solution, respectively. In bell pepper, priming of seeds enhanced the rate of germination (Bradford et al. 1990; Khan et al. 1992). Cooksey et al. (1994) compared non-treated seed, primed seed, and transplants for effects on stand establishment, plant morphology, and yield of paprika pepper. They concluded that non-treated seed was satisfactory

for stand establishment, although primed seed had the potential to provide greater initial stands. They also concluded that transplanting is not recommended for stand establishment of paprika pepper intended for mechanical harvest. Lanteri et al. (1994) revealed that priming of pepper seeds in −1.1, −1.3, and −1.5 MPa PEG solution for 14 days at 25 °C reduced the mean time to germination. Lee et al. (1997) found that the rate of germination and improvement of seedling stands were also accelerated as a result of seed priming in pepper. Pepper seeds osmoprimed with PEG 6000 and KNO_3 germinated more rapidly than the control or water presoaked ones. In addition to enhanced germinability, osmopriming treatment was found to significantly improve emergence index and vigor index (Thakur et al. 1997). It was also observed that aerated hydration treatment of pepper seeds produced larger seedlings and better stands in the field or greenhouse compared to untreated (Demir and Okcu 2004). It was noticed by Yogananda et al. (2004) that bell pepper seeds invigorated with GA_3 (200 ppm) or KNO_3 (1.0%) recorded higher germination, root and shoot length, seeding dry weight, rate of germination, and seedling vigor index over control. Korkmaz (2005) for sweet pepper reported that priming treatments generally improved the germination synchrony. In capsicum, Pandita et al. (2007) observed that osmo- and solid matrix priming improved seed germination over nonprimed seeds at 15 °C, 20 °C, and 25 °C temperatures. They also observed that priming also reduced mean days to germination significantly over control. It was reported by QianQian et al. (2009) that after priming treatment of the seeds with vermiculite, the germination rate, the germination energy, the germination index and vigor index of hot pepper seeds, and the fresh and dry weights of seedlings were significantly higher than those of control. Yadav et al. (2011) noticed that germination percentage of primed pepper seeds was increased to as compared to control and also tolerated cold and salt stress for 10 days with 100% survival, whereas control seedling could not survive. Ameri et al. (2011) conducted a laboratory experiment on seed priming of pepper "California Wonder" with 1% NaCl, 1% $CaCl_2$, 3% KNO_3, 3% $FeSO_4$, and control. All treatments resulted in a higher germination rates compared to the control. They also indicated that seed priming with $FeSO_4$ was the best treatment resulting in the maximum radicle dry weight, germination percentage, and germination rate with values of 0.126%, 70%, and 5.07%, respectively, while in the control values were 0.063%, 36.81%, and 0.83%, respectively. In a work investigating effects of osmotic conditioning and humidification applications on emergence percentage and mean emergence time of pepper seeds (Demirkaya 2012), the seeds were osmoprimed with PEG-6000 and −1.0 MPa for 1, 2, and 3 days and hydroprimed for 1, 2, and 3 days. The results of the study indicated that humidification and priming applications with PEG-6000 positively affected mean emergence time and emergence percentages in pepper seeds.

12.5 Effect of Priming on Performances of Pepper Seeds at Low Temperature

Low temperature is one of the most important environmental factors affecting pepper seed germination or seedling emergence. Most of the pepper cultivars are extremely sensitive to chilling stress particularly during emergence and early stages of seedling development. However, different varieties of the same species can tolerate low temperature differently. Peppers are very sensitive to germination temperatures, tolerating 16 °C on the lower end, with an optimum range between 18 and 35 °C and a mean optimum of 29.5 °C (Nonnecke 1996). The rate of germination and emergence of pepper seeds is markedly reduced at a temperature ranging from 15 to 20 °C. Pepper plants experience chilling injury with prolonged temps of 0–10 ° C. Methods to bypass the slow germination of pepper seed at low temperatures have been studied by researchers. Seed priming is an effective and practical technique that enables the seed to germinate and emerge faster even at suboptimal temperatures and also known to reduce the imbibitional damage associated with planting seeds in cold soils (Bennett and Waters 1987; Jisha et al. 2013). The effect of priming on low-temperature performance of pepper seeds has ranged from no improvement to some advancement in germination and emergence percentages and rates. For example, imbibition at 30 °C for 48 h in water or in aerated KNO_3 solutions for 6 or 8 days enhanced the germination at 15 °C when the seeds were not redried after treatment (Sachs et al. 1980). It has been reported that field emergence percentages of pepper at 20 °C generally were unaffected by priming (Bradford et al. 1990). Carter (1994) initiated to examine the effects of NaCl priming on total germination and the rate of germination under suboptimal temperature conditions. Seeds of "Tam Veracruz" chili were primed at 23 °C for 5 days in double-distilled H_2O or 0.2, 0.4, or 0.6 M NaCl solution (equivalent to an osmotic potential of −0.89 MPa, −1.77 MPa, and − 2.66 MPa, respectively). He suggested that priming "Tam Veracruz" chili seed for 5 days at 23 °C in 0.2 M NaCl increased the germination rate at temperatures from 15 to 23 °C. Matriconditioning pepper seeds at 15 °C for 7 days or at 25 °C for 4 days reduced the time needed for germination on filter paper at 15 °C, and conditioning at 25 °C was more effective than conditioning at 15 °C in reducing the germination time. The priming treatment also improved the performance of pepper seeds in early field plantings at suboptimal temperatures (averaged over 10 days after planting) ranging from 12 to 18 °C (Khan et al. 1995). Increasing evidence suggests that benzoic acid derivatives such as salicylic acid (SA) or ASA regulate stress tolerance in plants (Lopez-Delgado et al. 1998). Mendoza et al. (2002) reported that priming pepper seeds in 0.1 mM SA prevented seedlings from subsequent chilling-induced damage. The incorporation of plant growth regulators into the priming solution has been shown to be effective to enhance germination and seedling emergence of crops under adverse condition. For example, incorporation of acetyl salicylic acid (ASA) or methyl jasmonate (MeJA)

into the priming solution improved sweet pepper germination and emergence at low temperature (Korkmaz 2005). Priming sweet pepper seeds in KNO_3 supplemented with 0.1 mM acetyl salicylic acid resulted in 91% germination and 85% emergence at 15 °C which are significantly higher than the germination and emergence of nonprimed and seeds primed in KNO_3 only (Korkmaz 2005). In the same study, it has been also reported that the primed seeds stored for 1 month at 4 °C still exhibited improved germination performance at 15 °C. Similarly, Korkmaz and Korkmaz (2009) indicate that priming seeds in 25 ppm and 50 ppm ALA (5-aminolevulinic acid) incorporated into the KNO_3 solution improved low-temperature performance of red pepper seeds. They also indicated that primed seeds stored for 1 month at 4 °C or 25 °C still exhibited improved germination and emergence performance at 15 °C. Chemical seed priming treatments imparted tolerance to subsequent cold (4 °C) or salt stress (NaCl, 200 mM) exposure at later growth stages. Among the treatments, seed priming with thiourea (TU, 1.3 mM) was effective in imparting cold as well as salt stress tolerance (Yadav et al. 2011). In a recent work carried out by Sharma et al. (2015), the effect of seed priming using PEG-6000, gibberellic acid, KH_2PO_4, Na_2HPO_4, distilled water, and cow urine on seedling vigor of bell pepper seeds was studied. The seeds were primed at 20 °C for 24 and 48 h. They reported that although all the priming treatments significantly improved seed performance over control, seed priming with 100 ppm GA_3 for 48 h was more effective in improving seed performances under low temperature than the other treatments. The addition of prohexadione-Ca into the priming solution significantly improved sweet pepper germination and emergence at low temperatures compared to unprimed seeds and the seeds primed in KNO_3, KH_2PO_4, and PEG only (Ozbay and Susluoglu 2016a). Samarah et al. (2016) compared hydropriming and natural compounds that could be used as seed treatments to increase seed germination rate and improve cold tolerance of bell pepper. They reported that treatments with nanochitin, chitosan, acetic acid, or hydropriming improved low-temperature germination in soil by 17–39% compared with untreated seeds, being hydropriming or nanochitin more effective in reducing mean time to germination than chitosan or acetic acid treatments. Result of the study conducted by Korkmaz et al. (2017) revealed that seed application of 1 μM or 5 μM melatonin significantly improved pepper seed germination and seedling emergence at chilling temperatures compared to seeds not treated with melatonin.

12.6 Effect of Priming on Performances of Pepper Seeds at High Temperature

High temperature is also one of the most important environmental factors affecting seed germination and seedling emergence of many vegetable crops including pepper. Pepper seeds for fall production are sown when summer greenhouse temperatures can reach 40–45 °C (Vavrina 1994). This range is far above the optimum temperature (29 °C) for pepper germination (Maynard and Hochmuth 1997). At supraoptimal temperatures, pepper seeds may enter into the state of thermoinhibition. The inability

to germinate at higher than optimal temperatures is attributed to a condition called thermoinhibition or thermodormancy (Carter and Varina 2001). Most of the pepper cultivars are extremely sensitive to high temperature during germination and emergence and also later stages of plant development such as flowering and fruit set (Aloni et al. 2001). However, different varieties of the same species can tolerate high temperature differently as in low temperature mentioned previously. Cultivar differences in final germination pronounced at 35 °C. Therefore, growers should consider this, when choosing cultivars for fall transplants. However, no cultivar germinated well at temperatures higher than 35 °C (Carter 2000). Priming can also help alleviate high-temperature inhibition of germination and improve seedling emergence of pepper and other species (Ellis and Butcher 1988; Cantliffe 1991). For example, pepper seed germination was improved under supraoptimal conditions if the seeds were first soaked in solutions of ethephon or gibberellic acid (GA_3) (Carter and Stevens 1998). Nascimento et al. (2011) reported that osmoconditioning improves the physiological quality of hot pepper seeds by improving germination at high temperature (35 °C). In a study conducted by Silva et al. (2012), hot pepper "Mari" seeds were osmoconditioned in aerated solution of polyethylene glycol (PEG 6000) for 7 days and subjected to germination test at 15, 20, 25, 30, and 35 °C. The total seed germination decreased with increasing temperature. The osmotic conditioning was effective in improving seed germination at all temperatures, especially at high temperatures. Ozbay and Susluoglu (2016b) reported that priming pepper seeds in the presence or absence of plant growth regulators improved final germination and emergence percentage, mean emergence time, emergence index, and emergence rate (E_{50}) at high temperature (35 °C) compared to control seeds.

12.7 Priming to Overcome Salt Stress

Among the abiotic stresses, salinity is a major limiting factor in the crop productivity all over the world. Almost 20% of cultivated area of the world and half of the world's irrigated lands are stressed by the salinity (Chinnusamy et al. 2005). Soil salinity, if not properly managed, often results in poor stand establishment, reduced plant growth, and reduced yield of many horticultural crops such as peppers (Flynn et al. 2003; Niu et al. 2010). When exposed to high salt concentration, growth of pepper, which is not a salt-tolerant crop, can be negatively affected, and yield and fruit quality are diminished (Ibn Maaouia-Houimli et al. 2011). Salinity may reduce crop yield by upsetting water and nutritional balance of the plant (Khan et al. 2007). The salinity delays or prevents the seed germination through various factors, such as a reduction in water availability by high osmotic potential and toxicity of Na and Cl ions, changes in the mobilization of stored reserves, and affecting the structural organization of proteins (Kumar 1995; Ibrahim 2016). Salt concentrations affected percentage and rate of germination as well as the opening of cotyledonary leaves in *Capsicum annuum* L. cv. California Wonder. The 100 mM or higher salt concentrations reduced the rate of radicle protrusion significantly (Yadav et al. 2011). Similarly, Chartzoulakis and Klapaki (2000) reported the significant reduction in

the germination as well as growth of two bell pepper hybrids on exposure to 100 and 150 mM NaCl. Furthermore, pepper yield is reduced up to 14% for every increase in unit of salinity above its threshold (Rhoades et al. 1992). To alleviate this problem, a number of studies were conducted with the aim of removing the inhibitory effect of salt stress on plant growth. The beneficial effect of seed priming has been used for improving germination, emergence, and stand establishment of peppers under salt conditions (Hassen et al. 2014). Plants derived from primed seed have a higher adaptation capacity to salinity originating in the osmoregulation process (Levitt 1980). In studies conducted by Smith and Cobb (1991a), Amjad et al. (2007), and Yadav et al. (2011) with pepper, it was concluded that seed priming improves seed germination, seedling emergence, and growth under saline conditions. Priming of seeds with NaCl was found to be effective in alleviating the adverse effects of salt stress on pepper plants. Khan et al. (2009a) observed that priming of seeds using 1 mM NaCl improved seedling vigor and establishment under salt stress conditions. It has been reported that hormonal priming with salicylic acid (0.8 mM) and acetylsalicylic acid (0.2 mM) showed significantly better results over the control by improvement in time taken to 50% emergence, final emergence percentage, root and shoot length, seedling fresh and dry weight, and seedling vigor under normal as well as saline conditions. It has also been reported that acetylsalicylic acid exhibited superiority over salicylic acid (Khan et al. 2009b). Pepper seeds treated with 10 mM glyciebetaine (GB), an organic osmolyte accumulated in variety of plants in response to abiotic stress, for 24 h in darkness improved germination and synchrony of germination under salt stress. Glyciebetaine decreases the melonoldehyde (MDA) and increases the proline content and SOD activity in seeds. The enhanced tolerance to salt stress may be due to reduced lipid peroxidation and elevated SOD enzyme activity (Korkmaz and Sirikci 2011). Osmopriming with $CaCl_2$, KCl, and NaCl improved germination rate, chlorophyll content, proline, and protein accumulation in pepper under salt stress conditions (Hassen et al. 2014). Hassen et al. (2017) also conducted a study to assess the effects of priming with concentrations of KCl, NaCl, and CaCl2 on morphological and biochemical parameters of the pepper cvs. Beldi, Baklouti, and Anaheim chili. They reported that seedlings developed from primed seed had improved biomass, water content, carotenoid content, soluble sugar, polyphenols, and soluble proteins at salt concentrations of 6 $g \cdot L^{-1}$.

12.8 Reversal of Seed Deterioration by Priming

Another area of practical concern of priming is the interaction between priming treatments and seed deterioration. Seed deterioration may be defined as the loss of seed viability and vigor due aging effects and adverse environmental factors particularly higher temperature, relative air humidity, and oxygen/carbon dioxide ratio (McDonald 1999; Jyoti and Malik 2013). Seed deterioration is associated with various cellular, metabolic, and chemical alterations including lipid peroxidation, membrane disruption, DNA damage, impairment of RNA, and protein synthesis and causes several detrimental effects on seed (Jyoti and Malik 2013). Seed storage

causes a decrease in the protein content which may be related to oxidation of the amino acids due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds (Manonmani et al. 2014). Poor storage conditions may accelerate seed deterioration in primed and unprimed seeds (Georghiou et al. 1987). It is a serious problem particularly in developing countries where seeds are stored in places usually without a proper control of air humidity, temperature, and O_2/CO_2 concentration (Khan et al. 2016). As seed deterioration increases, seed performance progressively decreases. Plants that have originated from deteriorate seed can also reduce growth rate (Kapoor et al. 2010). Pepper is a warm climate crop, and seeds are sensitive to storage conditions and lose germination potential within a short period of time, particularly under adverse storage conditions (Priestly 1986). Decline in germination potential due to seed aging may result in low seedling emergence and stand establishment (Ermis et al. 2016). Accelerated aging of sweet pepper seeds at 42 °C and 100% R.H. for 0, 5, 10, 15, 20, 25, and 30 days resulted in decreased germinability in terms of radical emergence (%) that was also differed significantly among different aging duration (Kaewnaree et al. 2011). According to Li et al. (2005), the main mechanism for aging of pepper seeds is associated with increased peroxidation of lipid membranes. The aging of pepper seeds, during long-term storage, deteriorated their vital status which was expressed in changes in their moisture content, decreasing of their sowing qualities, and development of weaker seedlings with higher water content (Panayotov and Aladjadjiyan 2014). Priming can reverse some of the aging-induced deteriorative factors and thus improve seed performance (Taylor et al. 1998). The beneficial effects of priming are associated with the repair and building up of nucleic acid, increased synthesis of proteins, as well as the repair of both mitochondria and membranes (McDonald 1999, 2000). Under invigoration, metabolic repair processes in deteriorated seeds occur before onset of seed germination process (Srinivasan et al. 2009). Many seed priming treatments have been used to reduce the damage of aging and invigorate their performance in many crops including pepper (Farooq et al. 2009; Ermis et al. 2016). In pepper, osmoconditioning was found to be also an effective treatment for protecting seeds stored under high temperatures by increasing longevity of seeds. However, osmoconditioning after storage did not seem to have any significant effect on seed viability, though it enhanced the germination rate (Georghiou et al. 1987). Osmoconditioning of controlled deteriorated sweet pepper seeds exerted various effects on seed germination depending on deterioration rate (Lanteri et al. 1996, 1997). Lanteri et al. (1996) reported that effects of aging pepper seeds may be partly reversed by priming and part of priming effects is related to the induction of nuclear replication. In a study conducted by Siri et al. (2013), sweet pepper seeds were artificially aged by exposing to high temperature (42 °C) and high humidity (100% relative humidity) and then primed with PEG 6000 solution with the osmotic potential of −1.5 MPa for 6 days. They concluded that seed germination was improved in primed seeds due to accumulation of antioxidants and the improvement of cell membrane integrity. Ermiş et al. (2016) indicate that priming is more useful for enhancing germination of low-quality seed lots than higher-quality ones which indicates that repair of aging is one of the primary advantages of the priming treatments.

While in general it is clear that the best commercial potential for priming is the enhancement of performance of seeds of the highest quality (Perkins-Veazie and Cantliffe 1984), such repair treatments may be appropriate in the cases of valuable deteriorated stock or rare genetic material.

12.9 Storage Life of Primed Seeds

It would be highly advantageous if the promotive effects of priming on the germination of seeds were retained after drying and storage. In general, storing primed seeds reduces germination or seedling emergence, and there are conflicting results on the effect of storage life of primed seeds. As far as it concerns the storage of pepper seeds following priming treatments, Bruggink et al. (1999) reported that for pepper seeds, the desired longevity was obtained by keeping the seeds, after a priming treatment, under a mild water and/or temperature stress for a period of several hours to days. O'Sullivan and Bouw (1984) reported that a promotive effect on the germination of pepper seeds, when primed in 15% salt solution, was retained for 2 days after treatment. Perl and Feder (1981) showed a retention of seed vigor and seedling development of pepper seeds for 2 months following priming. Aljaro and Wyneken (1985) reported the effects of priming of "Y010 Y" pepper seeds to be apparent for up to 140 days prior to sowing. In pepper, osmoconditioning was found to be also an effective treatment for protecting seeds stored under high temperatures (Georghiou et al. 1987). Thanos et al. (1989) reported that primed sweet pepper seeds retained their improved vigor after a storage period of 6 months at 5–8 °C. It was determined that primed pepper seeds with PEG could be packaged in original packages and stored for 12 months under controlled conditions without any loss of effect on emergence time (Cetin and Duman 2005). They also reported that air- and moisture-proof aluminum materials were the best seed storage packaging material for the primed seeds. Korkmaz (2005) reported that 1 month storage at 4–8 °C did not reduce the performance of pepper seeds that were primed in the presence of salicylic acid. Korkmaz and Korkmaz (2009) indicate that red pepper seeds primed in 25 ppm and 50 ppm ALA (5-aminolevulinic acid) incorporated into the KNO_3 solution can be stored for 1 month at 4 °C or 25 °C and still exhibit improved germination and emergence performance at 15 °C. On the contrary, in other experiments, the promotive effect of priming was reduced rapidly after drying of the seeds (Sachs et al. 1980; O'Sullivan and Bouw 1984). The response of primed seeds to storage is species and variety dependent. Therefore, it is important that for the beneficial effects of priming to be retained, new techniques and markers have to be used to monitor the priming progress.

12.10 Effect of Priming on Physiological and Biochemical Changes in Pepper Seeds

The positive effects of priming on the germination performance of many species might be attributed to the induction of physiological and biochemical changes such as DNA replication, increased RNA and protein synthesis, greater ATP availability, increased respiratory activity, faster embryo growth, reduced leakage of metabolites, and decrease in lipid peroxidation and increased in the antioxidant activities.

12.10.1 Seed Priming and Cell Cycle Regulation

Some of the hypotheses proposing explanation for priming-induced improvement of seed performance are based on its effect on DNA in relation to activation of DNA repair mechanisms, synchronization of the cell cycle in G2, and preparation to cell division (Lutts et al. 2016). For example, Stofella et al. (1992) indicate that the increase in root/shoot ratio with hydropriming treatments may be due to the fact that priming induced nuclear replication in root tips of fresh seeds. By incorporating DNA-specific fluorescent dyes, nuclear DNA contents expressed as C values have been quantified in pepper (Lanteri et al. 1993) seeds during germination and priming. Lanteri et al. (1994) reported that priming of pepper and tomato seeds in −1.1, −1.3, and −1.5 MPa PEG solution for 14 days at 25 °C reduced the mean time to germination. Lanteri et al. (1996) reported that effects of aging pepper seeds may be partly reversed by priming and part of priming effects is related to the induction of nuclear replication. It has been reported that priming of seed promotes germination by repair of the damaged protein, RNA, and DNA (Koehler et al. 1997). An induction of 4C signals was also found after priming, indicating that during priming the cells of the embryonic root tip had replicated their DNA and arrested at the G2 phase of the cell cycle. Flow cytometric determination of nuclear DNA contents in embryos of dry, fully matured pepper seeds revealed only 2C signals. Therefore, pepper belongs to those species in which the quiescent embryo arrests nuclear division in the presynthetic G1 phase. 4C nuclei appear 1–2 days after imbibition in water, while radicle emergence starts 2 days later; at this time the proportion of the 4C nuclei is over 50% (Lanteri et al. 1992, 1993; Saracco et al. 1995). Thus, DNA replication precedes pepper seedling growth, and the 4C/2C ratio can be used to predict seedling performance of pepper (Sliwinska 2009). Using flow cytometry, Lanteri et al. (1997) observed that priming treatments in PEG solutions might induce DNA replication in the embryo root tips of pepper seeds. Lanteri et al. (1998) have also reported that the improvement of performance in pepper seeds after conditioning osmotic activity has been correlated with macromolecular repair that occur during the treatment. Changes in nuclear replication stages upon priming have been studied by flow cytometry on pepper seeds. The author observed that significant correlations were found between the frequency of priming-induced nuclear replication and the improvement of pepper seed vigor, as measured by the reduction in mean germination time (Sliwinska 2009). Flow cytometric data

published by Varier et al. (2010) also reveal that the improvement of germination associated with priming is accompanied by increase in 4C nuclear DNA. This indicates that priming enhances DNA replication allowing the advancement of the cell cycle from G1 to G2 phase.

12.10.2 Effect of Priming on Seed Ultrastructure

The seeds of some species including peppers are prevented from completing germination because the embryo is constrained by seed coats and surrounding structures (Watkins and Cantliffe 1983; Bradford 1995; Bewley 1997a). After imbibition of the seeds, however, the endosperm tissue enclosing the embryo restrains the germination process acting as a physical barrier, which restricts radicle emergence (Bino et al. 1998; Lutts et al. 2016). It is necessary to reduce the resistance of these enclosing structures for germination to be completed. Therefore, weakening of the endosperm, particularly in the micropylar region adjacent to the radicle, is a prerequisite for the completion of germination (Gong et al. 2005). Priming may also contribute to rapid seed germination by reducing the mechanical restraint of endosperm on developing embryo (Mayer and Mayber 1989). In studies with pepper seeds, Watkins and Cantliffe (1983) and Watkins et al. (1985) found that the mechanical constraint by endosperm in the tip region of the radicle contributed to slow germination rate and that the weakening of the endosperm by externally applied gibberellic acid (GA_{4+7}) occurred prior to germination. The reserves in endosperm are degraded by specific enzymes after the initiation of germination to facilitate the developing seedling until photosynthesis is initiated (Bewley and Black 1994; Homrichhausen et al. 2003). In most cases, endo-beta-mannanase degrades endosperm cell walls in endospermic seeds and can be detected during germination in the seeds (Bewley 1997b; Cantliffe 2003). Andreoli and Khan (1999) indicate that the matriconditioning seed treatment provides a means to efficiently digest the endosperm cell by GA-induced enzymes and reduce the mechanical restraints of endosperm thus providing energy to start and sustain embryo growth of tomato and pepper. Lanteri et al. (2000) investigated the expression of β-tubulin in the root tips of pepper seeds as a complementary marker for priming de novo synthesis of β-tubulins in response to priming was observed prior to DNA replication after osmopriming for different durations at two water potentials. Ethylene also directly influences germination speed and percentage. Increase in ethylene production during priming may promote endo-β-mannase activity facilitating endosperm weakening and post-priming germination (Chen and Arora 2013). Priming was reported to modify the kinetics of ethylene synthesis from its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Wu et al. 2014). It has been reported that the primed pepper seeds had higher ACC level, greater ACC-oxidase activity, and greater embryo growth potential than the nonprime seeds (Khan et al. 1995).

12.10.3 Effect of Priming on Reserve Mobilization

It is proposed that germination-related processes such as respiration, energy metabolism, and early reserve mobilization can also occur during priming (Varier et al. 2010). Higher respiratory activity is required to cover energy pool for speed up germination. Increased respiratory activity has been reportedly associated with pre-sowing treatments. For example, Halpin-Ingham and Sundstrom (1992) indicate that priming increases respiratory activity of pepper seeds. They also reported that seed priming generally reduced the oxygen-time constant and increased the standard deviation of germination responses. During seed germination, storage proteins, which provide a source of reduced nitrogen, and inorganic minerals need to be mobilized to support seedling growth (Lutts et al. 2016). Pepper seeds were examined during priming to determine if seed treatments which accelerate the rate of germination could be correlated with specific physiological changes within the seeds. Smith and Comb (1991b) found that soluble protein content increased to 109% and 120% in pepper seeds primed in −0.90 and −1.35 MPa NaCl solutions, respectively, after 12 days of priming and also revealed that there was no significant difference in the soluble protein content between two priming treatments. Khan (1992) found that two amino acids were incorporated in proteins during the first 24 h of imbibition of sweet pepper seeds in PEG solutions. QianQian et al. (2009) reported that the priming treatment with vermiculite significantly resulted in significantly higher contents of soluble protein in the pepper seedlings under NaCl stress. Hassen et al. (2017) reported that pepper seedlings developed from primed seed had improved soluble proteins at salt concentrations of 6 $g \cdot L^{-1}$. In contrary, in a study conducted to determine the relationship between physical and metabolic changes and germination/emergence time and rates both under optimum and stress conditions during osmotic conditioning process in pepper seeds, Ozpercin et al. (2005) reported that no significant changes were found in total protein levels in primed pepper seeds. Osmopriming induced accumulation of stress proteins, such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSP) (Gallardo et al. 2002). As LEA proteins accumulate at a high level in response to cell/tissue dehydration, they may contribute to acquisition of tolerance to drought and related stresses such as osmotic, salt, and cold stress (Lutts et al. 2016). Cortez-Baheza et al. (2007) have shown that several late embryogenesis abundant (lea) genes are strongly induced in pepper seeds when osmoprimed with PEG incorporated with GA3. A new LEA protein of 73 amino acids (Calea 73 gene) was highly induced in osmoprimed treatments in which KNO_3 was used in combination with PEG on *C. annuum* cv. Caballero seeds. Besides being induced by PEG + GA_3, the Calea 73 gene was also stimulated by PEG + KNO_3, which indicates that this gene is expressed during osmopriming regardless of the osmotic solution used (Cortez-Baheza et al. 2008).

12.10.4 Effect of Priming on Osmoprotectants

The osmoprotectants are compounds produced in plants during osmotic stress condition. Osmoprotectants are chemically small, electrically neutral molecules, which play important roles in the protection and stabilization of proteins and membranes against abiotic stresses without disrupting plant metabolism (Yancey 1994). Osmoprotectants or compatible solutes include proline, mannitol, D-ononitol, trehalose, sucrose, fructane, glycine betaine, and polyamines (Khan et al. 2015; Lutts et al. 2016). Accumulation of osmoprotectants such as proline and sugars has been reported in various plant species including peppers during a wide range of abiotic stresses. Proline is a proteogenic amino acid and accumulates both under stress and non-stress conditions as a beneficial solute in plants (Kavi Kishor et al. 2015). The main function of proline in plants is to restrain the osmotic effects by stabilizing protein structures and scavenging free radicals (Smirnof and Cumbes 1989; Biedermannova et al. 2008). Accumulation of sugars in various plant species is related to high tolerance to stress conditions (Bohnert and Jensen 1996). Sugars protect membranes and enzyme complexes from reactive oxygen species mainly by interacting with enzymes of the glutathione–ascorbate cycle (Khan et al. 2015). There are several reports showing that the osmoprotectant content increases after seed priming. For example, the priming pepper seeds with vermiculite significantly increased content of free proline and soluble sugar in the seedlings under NaCl stress (QianQian et al. (2009). Korkmaz and Şirikçi (2011) reported that pepper seed treated with glyciebetaine exhibited an enhancement in proline content under saline conditions. Hassen et al. (2014) showed that priming pepper seeds with NaCl, KCl, and $CaCl_2$ caused a marked increase in proline accumulation. Seedlings developed from primed pepper seeds had improved biomass, water content, carotenoid content, soluble sugar, and polyphenols at salt concentrations of 6 $g \cdot L^{-1}$ (Hassen et al. 2017).

12.10.5 Effect of Priming on Management of Oxidative Status

Management of oxidative status is also an important part of primed seed physiology (McDonald 2000; Kibinza et al. 2011). The priming could activate the response of the antioxidant system, becoming the primed seeds more prepared for possible stresses. During seed imbibition and early stages of germination, reactive oxygen species (ROS) production occurs mainly through respiratory activities of mitochondria, activities of β-oxidation pathways and enzymes such as NADPH oxidases, extracellular peroxidases, and oxalate oxidases (Wojtyla et al. 2016). Antioxidants, by scavenging the excessive ROS during early imbibition, play an essential role in ensuring successful germination, especially under stress conditions (Bailly 2004). An increased activity of antioxidant enzymes like ascorbate peroxidases (APX), catalase (CAT), peroxidase (POX/POD), glutathione reductase (GR), and superoxide dismutase (SOD) as a consequence of seed priming has been reported in various crops including peppers. For example, the priming treatment with vermiculite

significantly improved the activities of superoxide dismutase (SOD) and peroxidase (POD) in the leaves of seedlings and significantly decreased the rate of producing superoxide anion (O2-) and the content of malondialdehyde (MDA), resulting in lower membrane lipid peroxidation in the seedlings under NaCl stress (QianQian et al. 2009). Kaya et al. (2010) reported that the most important effects of priming on enzymatic activities of pepper seeds were recorded in catalase which increased remarkably with priming. They further stated that, even though not the same extent, priming also increased ascorbate peroxidase and superoxide dismutase activities (Kaya et al. 2010). Korkmaz et al. (2010) reported that 5-aminolevulinic acid (ALA) pretreatment increased relative water content, stomatal conductance, and superoxide dismutase (SOD) enzyme activity and reduced membrane permeability in pepper. Pepper seeds treated with glyciebetaine exhibited a significant decrease in MDA content and enhancement in SOD enzyme activity under saline conditions (Korkmaz and Sirikci 2011). In a study focusing on the effect of priming process on quality and biochemical changes in sweet pepper seed, Siri et al. (2013) reported that osmopriming with −1.5 MPa PEG-6000 for 6 days of artificially aged seeds of sweet pepper (42 °C and 100% relative humidity) resulted in an improved germination with decreased levels of malondialdehyde (MDA) and total peroxide concentration. They further stated that accumulation of total antioxidant activity (TAA), total ascorbate, dehydroascorbate, and catalase (CAT) activity in primed seeds enhanced the defense mechanism in protecting the cell membrane damage from reactive oxygen species. Da Silva et al. (2015) showed that priming improved superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities.

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13 Effect of Different Seed Priming Treatments on Germination and Seedling Establishment of Two Threatened Endangered Medicinal Plants of Darjeeling Himalaya

Dhiman Mukherjee

Abstract

Present investigation was conducted under the aegis of Uttar Banga Krishi Viswavidyalaya, in Algarah (1900 m asl) under Darjeeling Himalaya. The aim was to study the effects of priming with different concentration of growth hormone, etc. on seed emergence characteristics and seedlings vigour of two endangered plant species of *Swertia chirayita* and *Valeriana jatamansi*. An experiment as randomized block design in 14 pretreatment seeds of *S.chirayita* and 15 pretreatment seeds of *V. jatamansi* with 3 replications was carried out in the field condition during November 2011 to November 2014. The pretreatments include osmo-priming with GA_3 50, 100, 200, 400 and 800 ppm; IAA 50, 100, 200, 400, 800 ppm and KNO_3 1, 2, 3 and 4% and hydro-priming (control with distilled water) for *S.chirayita*. In another experiment *V. jatamansi* seed osmo-priming with IBA 50, 100, 150, 200, 250 ppm; GA_3 50,100, 150, 200, 250 ppm, kinetin 50, 100, 150, 200, 250 ppm and hydro-priming (control with distilled water). The maximum seed germination was observed with GA_3 400 ppm and was at par with the GA_3 200 ppm. Onset of germination was earliest, i.e. 19.00 days after sowing as registered by GA_3 800 ppm for 12 h, which was statistically at par with IAA 200 ppm (20.33 days) and IAA 50 ppm (22 days). Mean germination time (MGT) 44.11 days in untreated (control) seeds which were significantly reduced to 27.34 days in seeds pretreated with IAA 400 ppm, which was 38.01% lower than the control and was at par with 29.63 and 30.67 days in seed pretreated with GA_3 400 ppm and KNO_3 (2%). Seeds treatment with GA_3 400 ppm for 24 h showed maximum SV-I (71.11), which was 207.71% higher than the control. The highest SV-II of 54.18 was registered by KNO_3 (2%), which was 68.16% higher than the control and was statistically at par with GA_3 200 ppm. Germination energy of 1.16 was found in seeds pretreated with

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GA_3 400 ppm, which was statistically at par with GA_3 200 ppm, GA_3 800 ppm, KNO_3 (1%) and KNO_3 (2%). Highest germination percentage seed of *V. jatamansi* was observed with the kinetin 200 ppm treated seeds and significantly better to all other treatments measures. Days require for onset of germination was earlier recorded with the IBA 200 and kinetin 250 ppm and was at par with the kinetin 200 and IBA 150 ppm. MGT was 33.03 days in untreated (control) seeds which were significantly reduced to 22.95 days with GA_3 250 ppm, which was 31.88% lower than the control and was at par with kinetin 200, kinetin 250 and IBA 200 ppm primed seeds. Kinetin 100 ppm showed maximum SV-I (303.33), which was 484.15% higher than the control and was significantly better to all other 14 seed priming treatments. The highest SV-II of 190.06 was registered with kinetin 200 ppm, which was 362.32% higher than the control and was statistically better to other treatments. Maximum emergence index and germination energy were exhibited by kinetin 200 ppm.

Keywords
Conservation · Endangered plant · Gibberellic acid · IAA · Himalaya · *Swertia chirayita* · *Valeriana jatamansi*

Indian Himalaya has 270 endemic (62 endemic and 208 near-endemic) plant species that are used for medicinal purposes (Samant et al. 1998) most of which are harvested indiscriminately for preparation of herbal drugs and serve as important medicine to our society (Mukherjee 2009). Among these, the genus *Swertia* (family Gentianaceae) with over 32 species in IHR (15 in North East Himalaya) occupies an important place (Gupta 1987). Darjeeling-Sikkim range of Himalaya is one of the native places of different kinds of *Swertia* species and is situated between the 87°59′–88°53′E and 28°31′–27°13′N in the Eastern Himalayan region of India (Mukherjee et al. 2010). *Swertia chirayita* (Roxb. ex Fleming) Karsten, syn *S. chirata* (Wall.) Clarke, so-called authentic 'Chirayita', is considered a critically rare medicinal plant in the Himalayan region (Samant et al. 1998; Chakraborty et al. 2008). In general, Gentianaceae is reported to have physiological dormancy, so seed germination is under question (Kandari et al. 2007). It occurs in the temperate Himalaya at an altitude of 1500–3000 m (Kala 2006). The bitter infusion of the plant is used as a blood purifier, for skin disease and as a bitter tonic for fever and indigestion (Mukherjee 2009a). The presence of xanthones in the species is reported to remedy tuberculosis (Bhattacharjee 2001). The bitterness and anthelmintic, hypoglycaemic and antipyretic properties are attributed to amarogentin (most bitter compound till date) (Keil et al. 2000). Because of these medicinal uses, demand has increased so much in the pharmaceutical industry that annually 95,000–160,000 kg of chirayita is imported to India (Dubey et al. 2014). Germination of chirayita becomes a problem for quite some time, and its germination percentage is very low (Raina et al. 1994; Mukherjee 2008), and even some time seed germination totally

failed. *Valeriana jatamansi*, which is another important endangered plant in northeastern and western Himalaya, also face problem of seed germination due to poor viability of seed and lack of dormancy breaking technology (Mukherjee et al. 2009). Seed germination, one of the most critical phases of plant growth and development, is adversely affected under hill conditions (Ghavami and Ramin 2007). Germination of seeds, one of the most critical phases of plant life (El-Keblawy and Al-Rawai 2005), is greatly influenced by environment and local condition (Mukherjee 2014). *Valeriana jatamansi* (family, Valerianaceae) is another endangered plant, which grow at an altitude of 1200–2000 m asl. It is distributed from Afghanistan to Southwest China, Burma and few part of Indian Himalayan range (Chakraborty et al. 2015a). Like many other non-timber forest products (NTFPs), this vulnerable plant (*Valeriana jatamansi* Jones; Syn. *Valeriana wallichii*) is taken as forest gift, and hence there is neither any control system in its harvest nor its domestication (Mukherjee 2009). Locally it is being used for medicinal purpose especially for headache and eye trouble. In Ayurvedic medicine, it is used as aromatic, stimulant, carminative and antispasmodic. It is also used for the treatment of epilepsy and hysteria (Polunin and Stainton 1987; Mukherjee 2016). Although the economic value of above mentioned two endangered herbs was reportedly unknown to the local people until recent past, the herb has now been widely known for its market potential. Thus, the exploitation of this plant is increasing leading to its rapid decline from its natural habitat in Himalayan range (Mukherjee 2013). Suboptimal and imbalanced disturbance of soil are the main reasons for low availability of this valuable medicinal plant species in Himalayan range and Darjeeling-Sikkim hills in particular. However, no concrete step had been taken till date regarding various possibilities of seed germination for proper conservation of this species. Low germination percentage and viability of the seeds, long gestation period and delicate field handling are some of the factors which discourage commercial cultivation of the plant (Mukherjee 2015). Similarly reckless harvesting from its natural habitat created lot of pressure on their population. In CAMP workshop chirayita and jatamansi have been listed under 'critical' category (Sharma et al. 2005). Hence according to the International Union for Conservation of Nature and Natural resources (IUCN) criteria, *Swertia chirayita* and *Valeriana jatamansi* have been categorized as critically endangered (Mukherjee et al. 2015; Chakraborty et al. 2015b). Viable seed production potential was very low in both above-mentioned plants. Seeds mature late in the season and therefore fail to produce sufficient number in both the species (Chakraborty et al. 2016). Poor germination percentage and ruthless harvesting from forest range of Himalaya, the *Swertia chirayita* and *Valeriana* plant becomes wipeout day by day, and now come in category of endangered plant. Conservation of these plants becomes a question mark to our research wings and other R&D sectors throughout the world (Chakraborty et al. 2015b). Seed priming becomes an effective tool to break through this problem up to certain extent. Seed priming is seen as a viable technology to enhance fast and uniform germination and emergence, high vigour of seedling and better yields (Basra et al. 2002). Priming can be more efficient on poorer quality seed lots, and it is an important way for enhancing of seed germination and is a helpful tool particularly for valuable medicinal plant conservation. Seed priming enhances seed

performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination in different crops (Ashraf and Foolad 2005). There are several priming techniques used for seeds. Effects of priming or pretreatment of seeds persist under suboptimal field conditions, such as acidity (Foti et al. 2008) and low soil moisture availability (Du and Tuong 2002). Seed priming mainly involves uptake of water to initiate the early events of germination but not sufficient to permit radicle protrusion, followed by drying (McDonald 1999). Priming or pretreatment of seeds persist under suboptimal field conditions, such as acidity (Foti et al. 2008) and low soil moisture availability (Du and Tuong 2002), and are very helpful for improvement of germination of valuable temperate herbs. Presowing chemical treatments can be used to enhance and improve seed germination in Himalayan medicinal plants (Mukherjee 2013a). Seed germination studies on several Himalayan medicinal plants have proved useful in developing appropriate conservation strategies (Chakraborty et al. 2015a). Gibberellins are most directly implicated in the control and promotion of germination. This chemical helps to promote growth by increasing extendibility of the cell wall followed by the hydrolysis of starch to sugars which reduces the potential in the cell, resulting in the entry of water into the cell causing elongation (Arteca 1996). Few other growth hormones such as IAA and IBA are known to stimulate germination in many species by playing a major role in cell division and differentiation (Choudhary et al. 1996). Potassium nitrate is well documented as a compound which increases the germination of photo-dormant seeds (Shanmugavalli et al. 2007). According to Bewley and Black (1994), potassium nitrate raises ambient oxygen levels by making less oxygen available for citric acid cycles. Keeping the above aspect in mind, present work was conducted to restore our valuable natural herb resource for future generation. The objective of this research was to determine the effects of best seed priming treatments on enhanced seed germination parameters and seedling vigour and other physiological parameters of two endangered medicinal plants in Darjeeling Himalaya.

13.1 Materials and Methods

Present research work is carried out in Algarah (Darjeeling hill), under the aegis of Uttar Banga Krishi Viswavidyalaya, Kalimpong (1900 m asl), during November 2011 to November 2014 (Fig. 13.1). The soil sample used for germination was analysed for physico-chemical properties as per standard procedure (Jackson 1973). Soil samples were taken before actually conducting the experiment from the depth of 0–15 cm, taking all possible precautions prescribed for soil sampling. The soil of the experimental site was sandy loam having pH 5.1, with moderate available nitrogen, phosphorus and potassium (Table 13.1). The purpose of this research was to evaluate the efficiency of employing different priming (Osmo- and hydro-priming) to mitigate germination barriers and improve seed viability and seedling establishment of two endangered plants on earth, viz. *Swertia chirayita* and

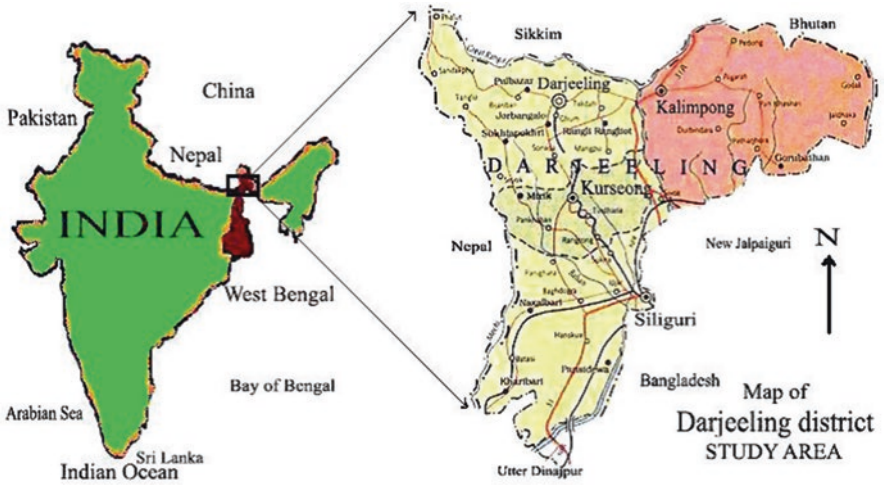

Fig. 13.1 Location of Darjeeling district (study area) of West Bengal, India

Table 13.1 Physico-chemical status of soil sample for germination study (pooled value of 2-year data)

pH	ECE (inch/cm)	Available (kg/ha) N	P_2O_5	K_2O	Total N (%)	Organic C (%)	Organic matter (%)	C/N ratio
5.1	0.17	297	26.6	226	11.16	2.71	5.08	16.01

Valeriana jatamansi. Mature seeds of *S. chirayita* and *V. jatamansi* were collected from the Sandhapukh (2200 m asl) and Lava (2000 m asl) of Darjeeling district.

The fruit of chirayita are superior bicarpellate ovoid pointed capsules with minute reticulate and irregular ovoid seeds, weighing 100 seeds to a mg (±0.96), size about 0.23–0.50 mm, length 0.15–0.50 mm, broad. Seeds of *S. chirayita* and *Valeriana* were collected during end of November 2011 and last week of February 2012, respectively, and kept in paper bags of convenient size after proper labelling. The fruit were shed dried in the open air and gently hand shaken while inside the paper bag. The minute clean seeds fall and settle in the bottom of paper bag. These fallen seeds were collected and stored in an open container. This process was repeated till the seed were almost fully collected. Finally the remaining fruits (some left over seeds) were hand crushed and passed through a fine nylon sieve and stored in a separate but similar container. This contains impurities like vegetative, clay particles and other foreign materials of almost similar size. This seed material was kept in desiccators with silica gel to avoid moisture absorption. During the investigation, seeds were surface sterilized by dipping them in 0.5% aqueous solution of $HgCl_2$ for 2 min to remove bacterial and fungal contamination and then rinsed thoroughly (four times) with distilled water and then soaked in different concentration of growth regulators for 24 h and placed in a glass plate for shed dry. Two experiments were made for seed germination investigation under field

condition, with 200 seeds taken with triplicate in bed size 1.50 × 1.50 m^2 inside polyhouse under field condition. Sowing of seed in a nursery bed was done under careful observation with finally sieved and well-sterilized (in autoclave) clay soil in the 25th and 21st of April, respectively, in 2012 and 2013. Earlier work indicated that months of April and May were favourable for seed sowing in the field at high altitude regions (Anon 1997). Meteorological data indicate that during these months, day-night temperature of the experimental sites varied between 6 and 23 °C, probably meeting the optimum temperature requirement of this species and resulting in higher germination with less time for completion of germination of seeds. Two investigation programmes were conducted under different polyhouse. First one was on germination of *S. chirayita* under different hormonal treatments which includes control along with prechilled seed (−4 °C) of 15 days dipped in 14 different levels of seed priming measures mainly GA_3 (50, 100, 200, 400 and 800 ppm), IAA (50, 100, 200, 400 and 800 ppm) and KNO_3 (1, 2 , 3 and 4%) for 12 h. After this treatment seeds were washed with distilled water, shed dried and then sown under polyhouse. In the second experiment, seeds of *V. jatamansi* under different treatments include control along with different levels of IBA (50, 100, 150, 200 and 250 ppm), GA_3 (50, 100,150, 200 and 250 ppm) and kinetin (50, 100 ppm, 150, 200 and 250 ppm). Both the experiments were conducted consecutively 2 years in randomized block design with three replications in the same field.

Germination percentage was evaluated by counting the numbers of normal seedlings at the end of standard germination test. For onset of germination, seeds kept for germination were observed daily, and the day when the first seed showed germination was considered as time taken for start of germination. Completion of germination mainly includes daily count of seeds for germination and the day when the last seed showed germination. Mean germination time (MGT) was calculated according to the following formula (Sfairi et al. 2012):

$$\mathrm{MGT}\left(\mathrm{days}\right) = \sum\left(ni \times di\right) / N$$

where *ni* is the number of germinated seeds on day *i*, *d* is the incubation time (day) and *N* is the total number of seeds germinated.

Emergence index and germination energy calculated as per standard procedure advocated by ISTA (1965). Seedling fresh weight was measure by weighing five normal seedlings from each replicate was taken. The weight of fresh seedling samples was recorded and expressed in grams. For seed dry weight, measured seedling weight from each replicate were kept in the paper bag and dried in a hot air oven at 40 ± 10 °C temperature for 24 h (Chakraborty and Mukherjee 2010). Thereafter, seedlings were cooled for 30 min, and the weight of dried samples was recorded and expressed in grams. Seedling vigour index-I and index-II were calculated as per the following formula given by Abdul Baki and Anderson (1973). Results were analysed statistically by analysis of variance (ANOVA), using SAS software (SAS Institute Inc. 1988). Pooled analysed data of 2 years have been presented in table form. When analysis of variance showed significant treatment

effects, the least significant difference test (LSD) was applied to make comparisons among the means at the 0.05 level of significance.

13.2 Results and Discussion

Seed germination is one of the most critical phases of plant life (Davies 2004) and is greatly influenced by various stresses and local governing biotic and abiotic factors (Dezfuli et al. 2008). These can affect germination and seedling growth either by creating an osmotic pressure that prevents water uptake or by toxic effects of available ions in soil (Khan et al. 2009). Epigeal type of germination of *S. chirayita* seeds was observed under field condition. The data pertaining to germination percentage of 15 invigoration treatments have revealed that all treatments under field condition showed significant variation in germination percentage (Table 13.2). The maximum seed germination percentage (61.00%) was registered in the treatment GA_3 400 ppm (Plates 13.1 and 13.2) and was at par with the GA_3 200 ppm (55.87%) and statistically better than the rest of the chemical treatment measures of seeds. This might be because GA_3 increases the synthesis of hydrolytic enzymes at aleurone layer, and by the activity of these enzymes, storage compounds convert to transferable ones (sucrose and glucose) and transfer to embryo (Sedghi et al. 2008; Mukherjee 2013a). The main factor in transferring of reservoirs is their solubility in water that helps to remobilization to embryo (Khan et al. 2009). Nearly 28–62% reduction was observed in germination when the concentration of GA_3 decreases. This corroborates with earlier finding that gibberellic acid is the most commonly used hormone for promoting seed germination and is particularly considered responsible for mobilization of nutrients (Basnet 2001). Also, gibberellic acid is used to release dormancy of seeds in many species for reducing inhibitor level or by activation GA_3 synthesis or both. On the contrary, treatment of 800 ppm GA_3 sowed inhibitory effect on seed germination and reducing germination percentage significantly over rest of the levels. Similar result also observed by earlier workers (Zheleznov et al. 1997). Lowest seed germination was observed with IAA 800 ppm; this might be due to phytotoxic effect of higher concentration of IAA on seeds. This was followed by control (Plate 13.1) and showed very poor and scanty germination of seeds. Onset of germination was earliest, i.e. 19.00 days after sowing as registered by seeds pretreated with GA_3 800 ppm for 12 h, which was statistically at par with IAA 200 ppm (20.33 days) and IAA 50 ppm (22 days) treated seeds under high altitude of Darjeeling hills. Invigoration of seeds with KNO_3 (3%) for 12 h (36.33 days) resulted in most delayed onset of germination which was significantly higher than all the other treatments except control and IAA 100 ppm treated seeds. Days required for completion of germination failed to give any statistical difference with various treated seeds; moreover maximum days required for germination was registered with the IAA 200 ppm and less time requirement for completion of germination was observed with GA_3 200 ppm. Mean germination time (MGT) of *S. chirayita* was 44.11 days in untreated (control) seeds, which was significantly reduced to 27.34 days in seeds pretreated with IAA 400 ppm. This was 38.01% lower than the control and was at

Table 13.2 Effect of seed invigoration treatments on seed germination characteristics of *Swertia chirayita* (pooled value of 2-year data)

Treatments	Germination (%)	Days require for onset of germination (days)	Days required for completion germination (days)	Mean germination time (days)	Seedling vigour		Seedling vigour index-I	Seedling vigour index-II
					Fresh biomass (g)	Dry biomass (g)		
Control	26.66 ± 2.11	36.66	44.66	44.11	1.17[a] (0.87)	0.78 (0.11)[b]	23.11	2.93
GA_3 50	36.00 ± 1.31	27.33	47.00	36.36	1.49 (1.74)	0.95 (0.40)	35.21	13.64
GA_3 100	40.44 ± 2.33	30.33	50.33	39.37	1.84 (2.89)	0.94 (0.39)	35.98	15.36
GA_3 200	55.87 ± 3.44	28.00	50.00	37.11	2.32 (4.94)	1.22 (0.98)	64.08	53.74
GA_3 400	64.00 ± 5.91	27.00	55.33	29.63	2.01 (3.54)	0.95 (0.41)	71.11	26.53
GA_3 800	33.66 ± 1.72	19.00	30.66	31.00	1.20 (0.95)	0.79 (0.12)	34.33	4.37
IAA50	30.32 ± 2.16	22.00	45.33	33.77	1.19 (0.92)	0.84 (0.21)	29.71	6.67
IAA100	44.11 ± 1.56	33.66	55.66	40.34	2.03 (3.65)	1.19 (0.91)	48.96	39.63
IAA200	34.00 ± 3.81	20.33	57.66	39.97	2.14 (4.12)	1.22 (0.98)	36.04	32.65
IAA400	30.15 ± 5.11	24.33	40.66	27.34	1.74 (2.54)	1.04 (0.59)	29.24	17.18
IAA800	2.66 ± 3.03	28.33	40.33	31.45	1.77 (2.66)	0.85 (0.23)	2.97	0.64

KNO_3 (1%)	44.11 ± 1.16	34.00	41.33	39.32	1.92 (3.21)	0.96 (0.43)	38.88	19.86
KNO_3 (2%)	53.65 ± 3.33	26.66	46.66	30.67	2.19 (4.32)	1.23 (1.01)	57.99	54.18
KNO_3 (3%)	23.54 ± 2.19	36.33	54.66	34.17	2.27 (4.66)	1.17 (0.87)	24.95	20.47
KNO_3 (4%)	32.92 ± 4.11	29.66	43.33	36.13	1.61 (2.11)	0.87 (0.25)	25.34	8.69
LSD ($P = 0.05$)	9.60	3.07	N.S	3.65	0.22	0.16	4.98	5.33

N.S Nonsignificant

[a]Values marked are square root transformed values

[b]Original values

Plate 13.1 *Swertia chirayita* under control (untreated seed) condition

Plate 13.2 *Swertia chirayita* under GA_3 400 ppm treated seeds (Best treatment)

par with 29.63 and 30.67 days in seed pretreated with GA_3 400 ppm and KNO_3 (2%). By definition, MGT is associated to the time length (day) that radicle exits. Least mean germination time was at control level because of no seed germination enhancement technique and the high with IAA 400 ppm pretreated seeds due to better mobilization of reserve food material. Seedling vigour varies significantly with various treatments, and it's showed quite promising response. Observation

Plate 13.3 Layout of the experiment

revealed that highest fresh biomass of seedling at 2-month stage registered with the GA_3 200 ppm and showed parity with the KNO_3 (3%), KNO_3 (2%) and IAA 200 ppm treated seeds. Dry biomass of seedling was highest found with the KNO_3 (3%) and was statistically similar with the KNO_3 (2%). Moreover, lowest dry biomass of seedling was observed with control and was followed by GA_3 800 ppm treated chirota seeds. The data pertaining to the effect of seed invigoration treatments on seedling vigour index-I (SV-I) in Table 13.2 reveals that IAA800 ppm and untreated (control) seeds registered minimum SV-I of 2.97 and 23.11 which was statistically poor to rest of the seed priming treatments. Seeds pretreated with GA_3 400 ppm for 12 h showed maximum seedling vigour index-I (71.11), which was 207.71% higher than the control and was significantly better results compared to the rest of the treatments. Seedling vigour index-II (SV-II) of *S. chirayita* exhibited that IAA 400 ppm treated seeds gave lowest this parameter and was followed by control. The highest SV-II of 54.18 was registered by seeds pretreated with KNO_3 (2%), which was 68.16% higher than the control and was statistically similar with GA_3 200 ppm used seeds (Plate 13.3).

Seedling length was maximum registered with the pretreatment of seeds with GA_3 200 ppm (1.16 cm) and was at par with the GA_3 400 ppm, IAA 100 ppm, KNO_3 (2%) and KNO_3 (3%) and significantly better to other treatments (Table 13.3). Lowest seedling length was observed with KNO_3 (4%) and was statistically similar with control (Fig. 13.2). Effect of seed invigoration treatments on emergence index

Table 13.3 Effect of seed invigoration treatments on seedling length, emergence index and germination energy of seeds of *Swertia chirayita* (pooled value of 2-year data)

Treatments	Seedling length (cm)	Emergence index	Germination energy
Control	0.87	0.73	0.61
GA_3 50	0.98	1.32	0.77
GA_3 100	0.89	1.33	0.81
GA_3 200	1.16	2.14	1.12
GA_3 400	1.11	2.37	1.16
GA_3 800	1.02	1.77	1.12
IAA50	0.98	1.38	0.67
IAA100	1.11	1.31	0.79
IAA200	1.06	1.67	0.59
IAA400	0.97	1.24	0.74
IAA800	0.86	0.09	0.07
KNO_3 (1%)	0.88	1.3	1.07
KNO_3 (2%)	1.08	2.01	1.15
KNO_3 (3%)	1.06	0.65	0.43
KNO_3 (4%)	0.77	1.11	0.76
LSD	0.16	0.14	0.14

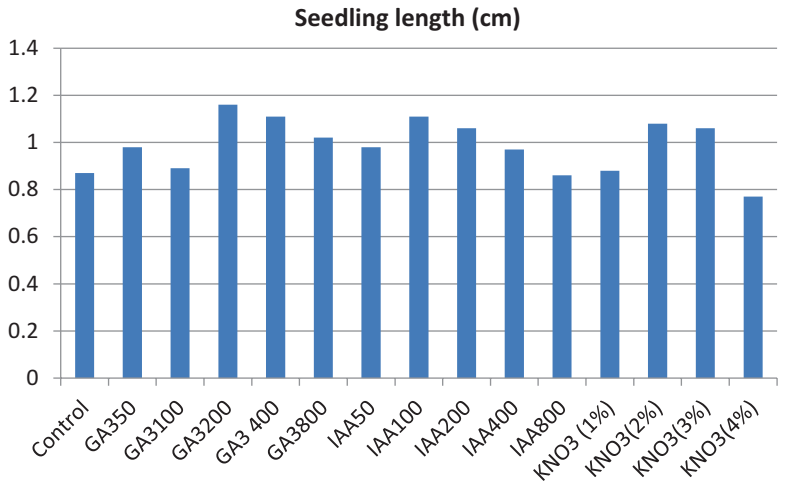

Fig. 13.2 Effect of various treatments on seedling length at 2-month stage of seedlings of *Swertia chirayita*

(EI) in chirota plant revealed that (Table 13.3) pretreated with GA_3 400 ppm gave maximum emergence index (2.37) which was significantly higher than other mentioned treatments except GA_3 200 ppm (2.14). It was followed by seeds invigored with KNO_3 (2%) and GA_3 800 ppm followed by IAA 200 ppm for 12 h (1.67) as compared to control (0.73). Further, germination energy (GE) of seeds reveals that maximum GE of 1.16 was found in seeds pretreated with GA_3 400 ppm, which was

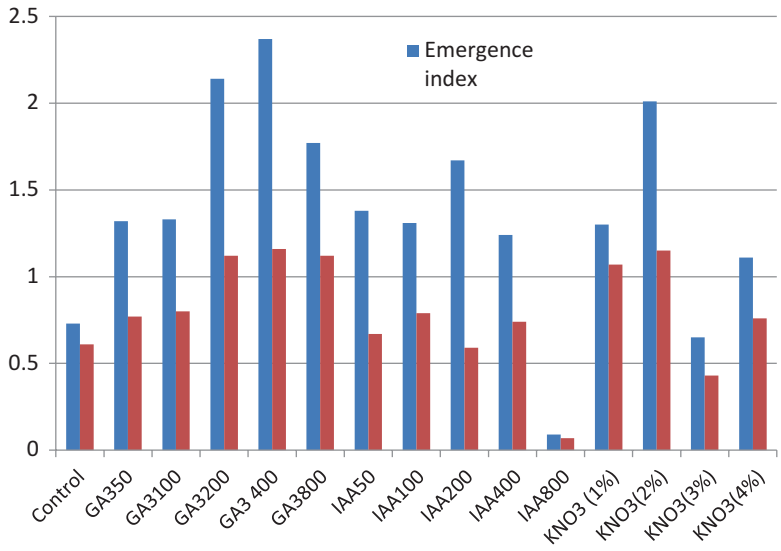

Fig. 13.3 Effect of various treatments on emergence index and germination energy of seeds of *Swertia chirayita*

statistically at par with GA_3 200 and 800 ppm, KNO_3 (1%) and KNO_3 (2%) (Fig. 13.3). EI is a parameter which reflects the seed vigour. The higher the EI the more vigorous the seed will be. The possible reasons for faster germination and higher germination energy in above-mentioned treatments in *S. chirayita* seem to be the completion of pre-germinative metabolic activities which might have resulted in attainment of the status seeds to enable them ready for radicle protrusion as compared to the control.

Valeriana jatamansi showed significant response with various seed priming measures (Plates 13.4, 13.5, 13.6 and 13.7), and highest germination percentage was observed with the kinetin 200 ppm (Plate 13.7) and significantly better to all other chemical exposed seed for priming (Table 13.4). This was followed by GA_3 200 ppm (Plate 13.6), which was at par IBA 150, GA_3 150, and 250 ppm treated seeds of *V. jatamansi*. GA_3 and kinetin were most effective chemicals that showed good response in many medicinal plant species conservation (Mukherjee 2014a). The results of our work are similar to those of Baskin and Baskin, where they found positive effect of soaking of *Osmorhiza claytonia* seeds in GA_3 solution (Baskin and Baskin 1991). Two functions for gibberellins (GA) during seed germination have been reported from various literatures. First, GA increases the growth potential of the embryo. Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed covering layers, by weakening of the tissues surrounding the radical (Kucera et al. 2005). Days required for onset of germination was earlier recorded with the IBA 200 and kinetin 250 ppm and was at par with the kinetin 200 and IBA

Plate 13.4 Seeds pretreated with distill water (control)

Plate 13.5 Seeds pretreated with kinetin 100 ppm

150 ppm. More time for initiation of germination took place by control (untreated seeds). Out of 16 treatments, untreated seeds are recorded with maximum time of 29.33 days of sowing for onset of germination. Onset of germination was earliest, i.e. 20.33 days after sowing observed by seeds pretreated with IBA 200 ppm, which

Plate 13.6 Seeds pretreated with GA_3 100 ppm

Plate 13.7 Seeds pretreated with kinetin 200 ppm

was 9 days earlier as compared to control. Further Table 13.4 depicts that days required for completion of germination was least observed with the kinetin 250 ppm (23.33 days) and showed parity with the kinetin 200 (24.33 days) and GA_3 250 ppm (25.66 days) pretreated seed. In the present study, percentage of seed germination,

Table 13.4 Effect of different treatments on seed germination of *Valeriana jatamansi* (pooled value of 2-year data)

Treatments	Germination (%)	Days require for onset of germination (days)	Days required for completion of germination (days)	Mean germination time (days)	Seedling vigour		Seedling vigour index-I	Seedling vigour index-II
					Fresh biomass (g)	Dry biomass (g)		
Control	51.33 ± 6.16	29.33	36.33	33.03	2.03 (3.64)	1.02[a] (0.54)[b]	51.87	41.11
IBA 50	54.00 ± 2.12	26.00	39.00	31.59	2.22(4.43)	1.24 (1.03)	76.22	53.66
IBA 100	79.33 ± 1.33	23.33	33.33	27.83	2.37(5.12)	1.35 (1.32)	173.08	104.21
IBA 150	85.66 ± 4.13	20.66	44.66	30.56	2.85(7.65)	1.43 (1.54)	260.83	133.92
IBA 200	70.00 ± 3.11	20.00	34.33	25.15	2.13(4.02)	1.27 (1.11)	153.52	72.71
IBA 250	63.66 ± 3.92	22.66	30.66	25.66	1.81(2.79)	0.93 (0.36)	121.66	36.65
GA_3 50	61.66 ± 3.21	27.33	32.00	29.05	2.05(3.69)	1.07 (0.65)	91.19	43.07
GA_3 100	72.66 ± 3.91	27.33	30.66	27.91	2.27(4.67)	1.22 (0.98)	161.33	71.07
GA_3 150	80.15 ± 3.12	27.66	31.33	28.35	2.49(5.69)	1.26 (1.09)	156.31	86.36
GA_3 200	86.66 ± 5.60	24.00	28.33	25.16	2.61(6.32)	1.47 (1.65)	202.45	137.99
GA_3 250	80.15 ± 2.66	22.33	25.66	22.95	2.51(5.77)	1.39 (1.44)	196.66	112.42
Kinetin 50	50.21 ± 8.62	25.33	36.33	31.93	2.03(3.61)	1.13 (0.77)	74.81	33.66
Kinetin 100	58.98 ± 7.32	25.00	36.00	31.15	2.41(5.32)	1.22 (0.98)	101.83	56.81
Kinetin 150	72.36 ± 3.63	23.33	30.33	25.33	2.78(7.25)	1.48 (1.69)	220.95	121.24
Kinetin 200	94.66 ± 10.66	20.33	24.33	23.33	2.94(8.15)	1.59 (2.03)	303.33	190.06
Kinetin 250	81.33 ± 5.33	20.00	23.33	23.65	2.71(6.87)	1.32 (1.23)	201.66	100.66
LSD ($P = 0.05$)	7.21	1.98	2.05	2.11	NS	0.08	8.98	9.11

N.S Nonsignificant

[a]Values marked are square root transformed values

[b]Original values

days required for onset and final germination showed difference under different treatments. Such difference was common for many mountain species (Nautiyal et al. 2001). MGT of seeds of *V. jatamansi* which were subjected to 16 invigoration treatments showed that this was 33.03 days in untreated (control) seeds which were significantly reduced to 22.95 days in seeds soaked with GA_3 250 ppm, which was 31.88% lower than the control and was at par with 23.33, 23.66 and 25.15 days in seeds pretreated with kinetin 200, kinetin 250 and IBA 200 ppm. Fresh biomass of seedling failed to produce any statistic difference among them. However, maximum fresh weight was registered with the pretreated seeds with kinetin 200 ppm and was followed by IBA 150 ppm. Moreover, lowest fresh weight under high altitude was observed with the IBA 250 and kinetin 50 ppm treated seeds. Dry weight produced significant difference at 2-month-old seedling stage, and highest dry biomass was registered with the kinetin 200 ppm and was significantly better to all other sets of treatments. Lowest dry biomass recorded with the control followed by IBA 250 ppm. The data pertaining to the effect of seed invigoration treatments on seedling vigour index-I in *V. jatamansi* reveals that untreated (control) seeds were registered minimum SV-I of 51.87, which was statistically poor to rest of the treatments (Table 13.4). Seeds pretreated with kinetin 100 ppm showed maximum SV-I (303.33), which was 484.15% higher than the control and was significantly better to all other 14 seed priming treatments. Other treatments which gave better results on SV-I were IBA 150 ppm and kinetin 150 ppm. Further, observation reveals that kinetin 50 ppm recorded lowest SV-II and was statistically similar with IBA 250 and untreated (control) seeds registered. The highest SV-II of 190.06 was registered by seeds pretreated with kinetin 200 ppm, which was 362.32% higher than the control and was statistically better to other treatments (Fig. 13.4).

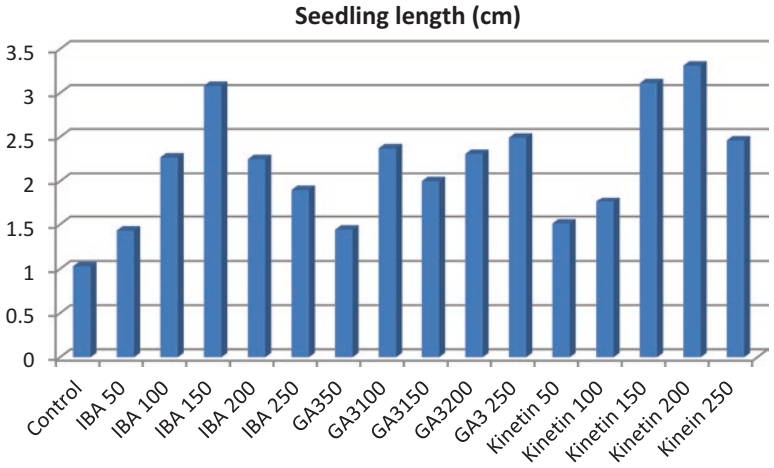

Fig. 13.4 Effect of various treatments on seedling length at 2-month stage of seedlings of *Valeriana jatamansi*

Table 13.5 Influence of different seed invigoration treatments on seedling length, emergence index and germination energy of seeds of *Valeriana jatamansi* (pooled value of 2-year data)

Treatments	Seedling length (cm)	Emergence index	Germination energy
Control	1.03	1.75	1.41
IBA 50	1.43	2.08	1.38
IBA 100	2.27	3.40	2.38
IBA 150	3.08	4.15	1.92
IBA 200	2.25	3.51	2.04
IBA 250	1.90	2.81	2.08
GA_3 50	1.44	2.26	1.93
GA_3 100	2.37	2.66	2.37
GA_3 150	2.00	2.92	2.56
GA_3 200	2.31	3.61	3.06
GA_3 250	2.49	3.59	3.12
Kinetin 50	1.51	1.98	1.38
Kinetin 100	1.76	2.36	1.64
Kinetin 150	3.11	3.10	2.38
Kinetin 200	3.31	4.65	3.89
Kinetin 250	2.46	4.07	3.49
LSD ($P = 0.05$)	0.26	0.58	0.77

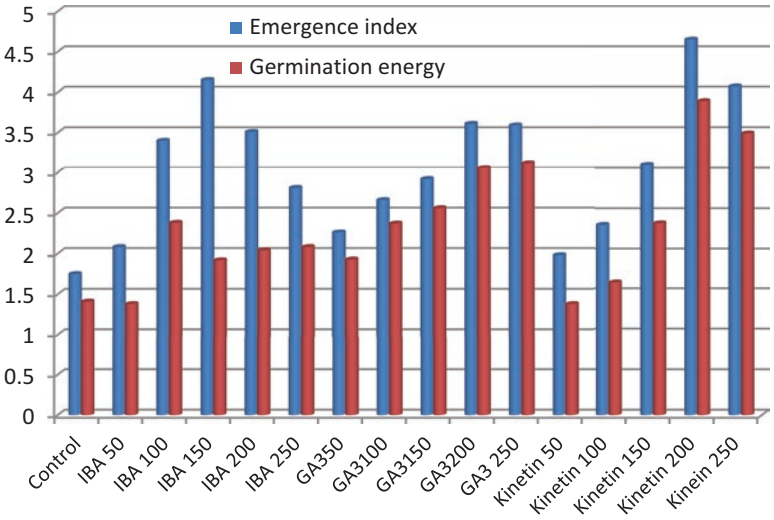

Fig. 13.5 Effect of various treatments on emergence index and germination energy of seeds of *Valerian jatamansi*

Seedling length of *V. jatamansi* maximum was registered with kinetin 200 ppm (3.11 cm) and was at par with the kinetin 200 and IBA 150 ppm pretreated seeds (Table 13.5). However, lowest seedling length was registered with the control (1.03 cm) and was significantly poor to rest all other treatments (Fig. 13.5). EI is a

Table 13.6 Effect of treatments on growth parameters of *Valeriana jatamansi* (pooled data of 2 years)

	Plant height (cm)		Fresh aerial biomass (g/plant)		Fresh underground biomass (g/plant)	
Treatments	3 months	6 months	3 months	6 months	3 months	6 months
Control	10.11	20.12	10.66	23.52	1.02	4.66
IBA 50	12.54	20.31	11.98	29.32	2.11	6.32
IBA 100	14.33	32.28	12.24	39.23	2.86	8.77
IBA 150	14.41	30.23	17.98	43.91	3.79	10.94
IBA 200	11.11	29.03	15.93	37.50	2.78	7.22
IBA 250	12.09	28.65	17.63	35.69	2.81	8.66
GA_3 50	13.65	22.11	14.83	32.65	1.88	6.11
GA_3 100	13.28	28.07	15.08	35.17	2.26	7.05
GA_3 150	15.69	30.66	13.61	40.11	2.89	8.96
GA_3 200	14.85	29.65	18.36	47.36	3.44	10.68
GA_3 250	13.36	30.01	15.25	37.39	3.03	9.11
Kinetin 50	14.23	28.02	17.06	37.12	3.29	5.91
Kinetin 100	14.69	26.09	15.89	36.01	3.20	10.81
Kinetin 150	16.82	30.78	18.53	43.95	3.87	10.99
Kinetin 200	19.35	32.66	19.11	45.32	3.98	12.01
Kinetin 250	17.32	30.40	12.76	27.33	3.11	8.24
LSD ($P = 0.05$)	NS	2.34	1.49	3.86	0.29	6.11

NS* = Nonsignificant

parameter which reflects the seed vigour and highest recorded with kinetin 200 ppm and showed parity with kinetin 250 and IBA 150 ppm pretreated seeds. Lowest value of emergence index was found with control and was followed by kinetin 50 ppm used seeds (Fig. 13.5). Effect of seed invigoration treatments on GE reveals that maximum value of 3.89 observed with kinetin 200 ppm, which was statistically at par with kinetin 250 ppm and GA_3 200 ppm (Fig. 13.5). This corroborates with the earlier finding of Sharma et al. (2005). Lowest GE was observed with the control and statistically poor to all other treatments.

Effect of various treatment measures on *V. jatamansi*, after transplanting in main field at 3-month stage, showed that plant height failed to produce any statistical difference at this stage (Table 13.6). However, at 6 months, maximum plant height was observed with the IBA 100 ppm and was at par with kinetin 200, GA_3 150 and IBA 150. GA_3 250, kinetin 150 and kinetin 250 ppm were significantly better to other treatments. Fresh plant aerial biomass also gave significant response with various seed treatment measures, and highest fresh biomass at 3-month stage was observed with the kinetin 200 ppm and was at par with the kinetin 150 and GA_3 200 ppm. At 6-month stage maximum aerial biomass was registered with the GA_3 200 ppm and was at par only with kinetin 200 ppm and statistically superior to rest of the treatments. Pretreatment with GA_3 accelerates some metabolic processes even in low potential. This causes improvement at metabolic activities in germination, especially under stress conditions, and, subsequently, the weight of radicle and plumule

increases in less time, which ultimately helps to better vegetative growth of this endangered plant at 6-month stage. Fresh underground biomass, which includes root and rhizomes, was more observed with the kinetin 200 ppm and was at par with the IBA 150 and kinetin 150 ppm used seeds.

13.3 Conclusion

The present study recommends GA_3 as the best treatment for improving seed germination. Higher percentage of germination by GA_3 400 ppm indicates that it is the best source and helps to conserve this endangered plant sp. from Himalayan range. Further, pretreatment of *Valeriana jatamansi* seeds with GA_3 200 ppm and kinetin 200 ppm accelerates some metabolic processes even in low potential. This causes improvement in metabolic activities in seeds, especially under high altitude conditions and, subsequently, the weight of seedling dry and fresh weight increases in less time. Since seed priming is simple and cheap, we can propose this method to *S. chirayita* and *V. jatamansi* growers of Darjeeling-Sikkim Himalaya, so they can increase percent and homogeneity of emergence and good germination for better economic harvest of these endangered plants and help to conserve from total extinction from our earth ecosystem.

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Seed Priming on Germination, Growth and Flowering in Flowers and Ornamental Trees

14

Anjana Sisodia, Minakshi Padhi, A. K. Pal, Kalyan Barman, and Anil K. Singh

Abstract

Seed dormancy is an emerging problem related to germination which is common in many species of ornamental trees and flowers. Poor seed germination and subsequently poor field establishment are a common phenomenon at adverse conditions of environment. The most important problems faced are the heterogneity and lack of suitable conditions in soil that causes decrease in germination percent. Priming is a water-based technique that consents metabolic processes necessary for enhancing germination rate and seed quality by managing the temperature and seed moisture content in which the seed is taken through the first biochemical processes within the initial stages of germination but preventing the seed transition towards full germination. This is a successful way through which plants would be able to complete their growth on or before the stresses arrive (Subedi KD, Ma BL. Agron J 97(1):211–218, 2005). Seed priming technique has been practised in many countries including India, Pakistan, China and Australia, and more than thousand trials had been conducted to evaluate the performance of priming in a variety of crops. The principle of seed priming is to minimise the period of emergence and to protect seed from environmental stresses during critical phase of seedling establishment to synchronise emergence which lead to uniform establishment and improved yield. It reduces the effect of salinity on the morphological parameter of the plants. Various priming techniques, like osmopriming, biopriming, halopriming, thermopriming, hydropriming, hormonal priming and solid matrix priming, give favourable result in seeds of ornamental flowers as well as trees. This technique has been successfully carried out in flower crops like balsam, coneflower, cosmos, gladiolus, pansy, marigold,

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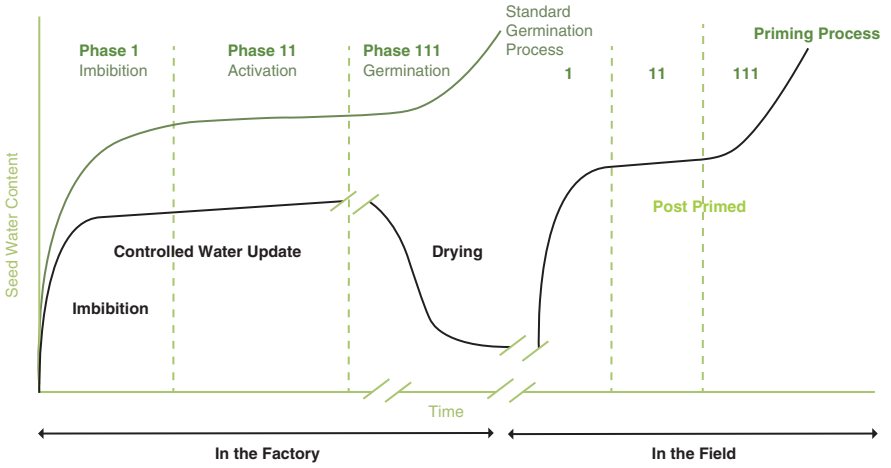
periwinkle, rudbeckia, salvia, snapdragon and zinnia and trees like cassia, cypress, senegal, eucalyptus, fig, teak, pine, almond, tamarind, oak, *karanj*, *khejri*, *siris*, *subabul*, *kapok*, *gulmohar*, *kachnar*, etc.

Keywords
Priming · Gladiolus · Periwinkle · Eucalyptus · *Kachnar*

Establishment of crop is the primary importance for optimising horticultural production. Every year, mankind awaits for the miraculous transmogrification of seeds into plants and again into seeds. Poor seed germination and subsequently poor field establishment are a common phenomenon at adverse conditions of environment. It has been reported that the major difficulty to high yield and production of crop plants is due to the lack of synchronised crop establishment and adverse weather and soil conditions (Mwale et al. 2003).

Seed dormancy is another problem related to germination which is common in many species. It is an adaptation that allows a species to regulate the timing of germination for seeds in a population. Some species use environmental cues (such as drought, rainfall or temperatures) to integrate germination for most seeds at a particular time of the year. Temperature, moisture, air and light conditions are the most important factors for seed germination. Minimum temperature is the lowest temperature at which seeds can germinate effectively (Nleya et al. 2005), and the maximum is the highest temperature at which seeds can germinate. Slight change in this temperature can damage seeds or make them go into the dormancy condition. At optimal temperatures, germination is rapid and uniform. Seeds need correct moisture to initiate internal processes leading up to germination. Osmotic adjustment or priming of seeds prior to sowing is known as a potent way to increase germination and emergence rate in some species with stepwise seed development (Sivritepe 2000).

Seed priming technique has been practised in many countries including India, Pakistan, China and Australia, and more than thousand trials had been conducted to evaluate the performance of priming in a variety of crops. The need for increased seed quality has become a priority necessary to tackle the current demand for high standards of seeds in the agricultural market. Achieving rapid and uniform seedling emergence is a key point for crop performance since slow germination rates frequently expose plantlets to adverse environmental conditions and soilborne diseases (Osburn and Schroth 1989). Priming is a water-based technique that consents metabolic processes necessary for enhancing germination rate and seed quality by managing the temperature and seed moisture content in which the seed is taken through the first biochemical processes within the initial stages of germination but preventing the seed transition towards full germination. This is the way through which plants would be able to complete their growth on or before the stresses arrive (Subedi and Ma 2005).



The above graph shows about the standard germination process and seed priming process in the factory and field condition. The green line corresponds to a standard germination process. During Phase I (imbibition), seeds take up water sufficiently under suitable availability of temperature and moisture. During Phase II (activation), the biochemical processes are activated and will eventually start the germination in Phase III (germination) where roots and hypocotyls emerge from the seeds. During priming, the seed invloves the activation processes followed by drying, before root can emerge from the seeds. Once conditions (temperature and moisture) are appropriate in the field, Phase III can continue, and germination occurs in a much shorter time (Hasegawa 2016).

The purpose of seed priming is to minimise the period of emergence and to protect seed from environmental stresses during critical phase of seedling establishment to synchronise emergence which lead to uniform establishment and improved yield. It reduces the effect of salinity on the morphological parameter of the plants. One of the priming techniques called osmopriming is a commercially used technique for improving seed germination and vigour. It controls imbibition of seeds to start the initial germination process followed by seed drying up to its original weight. Apart from germination process, seed priming also helps in growth and flowering of the crops. Various seed priming techniques like hormonal priming and chemopriming for enhancing flowering and growth of plant are commercially practised in- or on-farm basis. Where, on farm seed priming is energy intensive, high technology seed priming, seed hardening or seed conditioning process are available to farmers to help them in high input temperate agriculture and horticulture. (Harris et al. 2001).

The priming treatments which enhance seed germination include hydropriming (Afzal et al. 2004), biopriming, halopriming, solid matrix priming, chemopriming, thermopriming, osmopriming and hormonal priming (Afzal et al. 2006).

14.1 Physiological and Biochemical Aspects of Priming

A successful application of seed management technique depends upon the type of test, method of application, selection of crop, initial performance of the crop, selection of chemical, its concentration, duration of treatment and the purpose of implication. Priming method in seed management techniques is proven very essential factor for enhancing quality issues, germination rate, establishment, etc. Priming can inverse some of the ageing-induced deteriorative events, resulting in improved seed performance (Taylor et al. 1998). It has shown an immense effect to activate different processes related to cell cycle and to induce synthesis of nuclear DNA in radial tip cells in tomato (Liu et al. 1997).

Long duration seed storage caused a decline in the level of protein content which may cause oxidation of the amino acids, due to the increase in the respiratory activity and advance in the degradation process of the stored seeds. Seed deterioration causes loss of membrane integrity, changes in enzymatic activities and declines in protein and nucleic acid synthesis and lesions in DNA (McDonald 1999). Priming with 30% PEG for 24 h resulted in increase in the activity of superoxide dismutase (SOD) and peroxidise (POD) which enhance the intensity of respiration of plant and cause an increase in vigourity in germination (Jie et al. 2002). Priming is also thought to increase the activity of many enzymes involved in metabolism of carbohydrates (α- and β-amylases), proteins (proteases) and lipids mobilisation (isocitrate lyase) that are implicated in the stored reserves mobilisation (Varier et al. 2010; Di Girolamo and Barbanti 2012). These enzymes are vital in the breakdown of macromolecules for the development and growth of the embryo that ultimately result in early and higher seedling emergence (Farooq et al. 2006a, b; Varier et al. 2010). There are reports that priming facilitates the repair of chromosomal damage (Sivritepe and Dourado 1995), permits early DNA replication and repair, increases RNA and de novo protein synthesis and reduces the leakage of metabolites (McDonald 2000; Farooq et al. 2007a, b; Manonmani et al. 2014; Paparella et al. 2015). Thus, total seed protein, POD, PPO, RNA and de novo protein synthesis were increased significantly by seed priming. Among the various processes of priming, osmopriming may enhance rapid seed germination by reducing mechanical hindrance on the germinating embryo (Toorop et al. 1998).

14.2 Seed Priming Techniques

Seed priming includes various techniques which influences growth, establishment and germination of seeds and also influences yield of the crop. Techniques include the following.

14.2.1 Hydropriming (Drum Priming)

A major cause of poor establishment and low crop yield in agricultural areas is the lack of moisture content that reduces the ability of seedling to further emerge and growth. Hydropriming is a technique for enhancing germination without the emergence of the radicle and plumule which involves soaking of seeds in a priming agent solution followed by drying even if seeds are infected with pathogens (McDonald 1999). In hydropriming, distilled water plays a vital role for imbibition up to 10–20% (Pill 1995). It results into uncontrolled water uptake, since the process depends on seed affinity to water and the main critical point is to find and maintain optimal temperature and humidity conditions to avoid radicle protrusion (Taylor et al. 1998). Another limiting factor of hydropriming is the lack of homogeneous seed hydration which can lead to uneven germination (McDonald 2000). The main variant of hydropriming also called drum priming, patented by Rowse (1991) in which a drum that contains seeds is connected with a boiler generating vapour. The vapour condenses into liquid water inside the drum. The machine measures the increase in seed relative mass during the treatment. The time and volume of water required to complete seed rehydration are strictly controlled to reach gradual and even seed imbibition (Warren and Bennett 1997). Hydropriming is the most ancient type of priming, since the benefits of this pre-sowing treatment have been known for a long time; however, it is now applied less frequently in comparison with other methods.

14.2.2 Biopriming

Biopriming is a new technique of seed treatment that assimilates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) of disease control. To respond to the negative effects of pathogens, biopriming uses beneficial microorganisms to protect against pathogens and enhance plant growth. Biological seed treatments for control of seed and seedling diseases offer the grower an alternative to chemical fungicides. Storage and application conditions are more critical than with chemical seed protectants and differential reaction to hosts, and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than chemicals. Conversely, some biocontrol agents applied as seed dressers are capable of colonising the rhizosphere, potentially providing benefits to the plant beyond the seedling emergence stage (Nancy et al. 1997). Seed treatment with biocontrol agents along with priming agents may serve as an important means of managing many of the soil- and seed-borne diseases, the process often known as ‘biopriming’. It involves coating seed with a bacterial biocontrol agent such as *Pseudomonas aureofaciens* AB254 and hydrating for 20 h under warm (23 °C) conditions in moist vermiculite or on moist germination blotters in a self-sealing plastic bag. The seeds are removed before radical emergence. The bacterial biocontrol agent may multiply substantially on seed during biopriming (Callan et al. 1990). Biopriming seed treatments can

provide a high level of protection against root rot diseases of crop plants which was generally equal or superior to the control provided with fungicide seed treatment. So, it could be suggested that biopriming (combined treatments between seed priming and seed coating with biocontrol agents) may be safely used commercially as substitute for traditional fungicide seed treatments for controlling seed- and soilborne plant pathogens.

14.2.3 Halopriming

Halopriming is one of the methods of priming practices that includes salts like $CaCl_2$, $CaSO_4$ and NaCl in such a way that the pregermination metabolic activities start preventing radical protrusion followed by drying seeds to the original moisture level (McDonald 2000). In this method, the seeds are immersed in different salt solutions which facilitate the process of seed germination and subsequent seedling emergence even under adverse environmental conditions. Seeds treated with NaCl concentrations should be in a tolerable limit. Early initiation of metabolic activities and reserve breakdown and mobilisation might be the reason for faster germination in such type of primed seeds. Khan et al. (2009) reported salt priming induced salinity tolerance of hot pepper at seedling stage, wherein seed priming improved significantly the germination percentage and index, vigour index, plumule and radical length and dry weight of seedling as compared to the non-primed seeds (control). Improved stress tolerance of primed plants is thought to rise from the activation of cellular defence response due to halopriming (Beckers and Conrath 2007). This has been substantiated by reports on better antioxidant system in primed plants (Afzal et al. 2006) on exposure to stress. Conrath et al. (2006) proposed that halopriming could involve accumulation of signalling proteins or transcription factors. Halopriming is a simple and cheap agrotechnique and found suitable to be recommended to the farmers owing to better synchrony of emergence and crop stand under various conditions of environment (Sedghi et al. 2010).

14.2.4 Solid Matrix Priming

Solid matrix priming (SMP) is similar to osmotic priming that allows the seed to attain a threshold moisture content and pre-germinative metabolic activity but preventing radicle emergence. However, it has the advantages of allowing aeration, incorporation of biological agents to combat soilborne pathogens and improved ease of handling (Taylor et al. 1988; Wang et al. 1998). The matrix comprises of finely divided non-plant pathogenic water holding solid, which may be carbonaceous substance, preferably a lignateous solid which has a large eqilibrium water potential (ψ) and preferably has an osmotic potential component which is atleast about 90% or greater than 95% of the total water potential. Such materials include coal, especially soft coal, lignateous shale such as the leonardite shale, sold as Agro-Lig, and sphagnum moss. The matrix material when containing the water to prime

the seeds must be sufficiently friable, non-clumping, etc., so that it can be mechanically separated from the treated seeds after treatment without damage to the seeds when required. The process of SMP includes the admixture of a predetermined amount of solid matrix material and a predetermined amount of water and the mixture allowed to stand preferably in a container which allows entry of air but reduces evaporative losses, resulting in sufficient amount of moisture level in seeds. Matrices can be readily used in tree seed nursery operations.

14.2.5 Thermopriming

Seed treatments carried out at various intervals of time before sowing are known as thermopriming. Information exists to specify that seeds germinate better under alternating temperature conditions compared to a constant daily temperature (Felippe 1980; Shin et al. 2006; Markovskaya et al. 2007). Changing temperature can break the dormancy of seed easily. This technique has been widely adapted to improve germination efficiency under adverse climate reducing thermo-inhibition of seed germination (Huang et al. 2002). Pre-sowing seed treatments with alternating daily temperature regimes have resulted in enhanced plant development, increased cold and/or frost resistance and higher plant productivity in cucumber and melon (Markovskaya et al. 2007). Small changes in ambient temperature can regulate flowering time via a thermosensory pathway (Franklin 2009). It was shown that cold treatment at the seedling stage can modulate the flowering of some ornamental plants (Runkle et al. 1999; Garner and Armitage 2008). Although high temperature condition has been used in some species, resulting pregermination especially for plants adapted to warm climates (Khalil and Rasmussen 1983). Seeds of white spruce (*Picea glauca* L.), lettuce, etc. that were primed with combinations with other treatments resulted in beneficial effects on germination parameters (Liu et al. 2013; Ashraf and Foolad 2005). It has been suggested that priming is responsible to repair the age-related cellular and subcellular damage of low-vigour seeds that may accumulate during seed development (Bray 1995). Wang et al. (2003) reported that both thermo- and hydro-primed seeds showed significant increase in germination performance. The resultant effect of priming depends on the method used and time of treatment.

14.2.6 Osmopriming

Osmopriming, also known as osmotic conditioning, is a widespread pre-sowing priming procedure which involves treatments of seeds with osmotic solutions at low water potential facilitating the control of water uptake into the seeds. The main goal of osmopriming is to limit the reactive oxidative species, i.e. ROS-mediated oxidative injury through insufficient water absorption. Thus, the water potential of the osmotic agent used is a crucial parameter during the priming process (Heydecker and Coolbear 1977; Taylor et al. 1998). Priming with PEG provides beneficial

conditions for bacterial growth due to poor aeration (Parera and Cantliffe 1994). It shows some disadvantage when used in bulk, due to high costs and extremely high viscosity which limits oxygen transfer within the solution. Hence, research has to be done for choosing the correct chemical and its optimum dose for a crop. It is difficult to manage huge quantities of wet primed seed especially under hot tropical climate condition, while in temperate areas, maintaining the priming temperature is crucial. Some of the osmotica (osmotic compounds used for osmopriming) that can be used include potassium nitrate, potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, calcium chloride, zinc sulphate, borax, magnesium chloride, manganese sulphate, sodium chloride, sodium sulphate and organic compounds, viz. agrosan, cycocel, citric, furamic, succinic, malic acids, purines, pyrimidines, caffeine, uracil, xanthine and uridine diphosphate (De Chandra 1999).

14.2.7 Hormonal Priming

Hormone pretreatment is a commonly used priming approach to improve seed germination in stressful conditions (Atici et al. 2003; Gratao et al. 2005; Jisha et al. 2013; Masood et al. 2012; Hu et al. 2013). Hormonal priming in general consists of treatment of seeds with chemicals like growth regulators, sodium hypochlorite (NaOCl) or hydrochloric acid (HCl), natural substances and agrichemicals (e.g. fungicides, pesticides). It has reduced the severity of the effect of salinity, but the amelioration was found maximum due to the application of 50 ppm salicylic acid and 50 ppm ascorbic acid treatments and gives satisfied results on seedling growth, fresh and dry weights under non-saline and saline conditions, whereas hormonal priming with ABA was not effective in some of grass family crops (Afzal et al. 2006). In pepper (*Capscum annum* L.), Khan et al. (2009) showed that pretreatment with acetylsalicylic acid and salicylic acid resulted in greater uniformity of germination and establishment of seedlings under high salinity. In addition to these chemicals, ethylene was used to minimise the effect of high temperatures on seed germination of lettuce (Nascimento 2004, Nascimento et al. 2005).

14.3 Seed Priming in Ornamental Flower Crops

14.3.1 Balsam

Response of hormonal priming on flower quality, growth and germination rate of balsam was observed according to the basis of germination rate dry weight and shoot and root length. GA_3 at 10 ppm strikingly enhanced the germination percentage and speed of germination of balsam. The germination was higher in large seeds (grade A) which might be due to more supply of food material to the growing embryo. However, GA_3 at 30 ppm increased the length of shoot and root, dry weight and fresh weight of seedlings reported by Singh and Karki (2003).

14.3.2 Coneflower

Osmotic priming in polyethylene glycol (PEG) or matrix priming in expanded vermiculite had greater rate, synchrony and germination percentage at 20 °C than non-primed seeds of coneflower (*Echinacea purpurea*). Osmotic or matrix priming for 10 days at −0.4 MPa and 15 °C resulted in higher germination rate and germination percentage than short duration of exposure (5 days) or lower (−1.5 MPa) water potential. Seedling emergence rate, synchrony and percentage from osmotically or matrically primed seeds were similar in both cool (23–27 °C day) and warm (35–40 °C) glasshouse regimes. Emergence was faster in primed than from non-primed seeds in both regimes. Emergence percentage was higher (80%) from primed seeds than from non-primed seeds (50%) in the cool regime, but emergence synchrony was unaffected. Moistened vermiculite substituted for PEG solution as a priming medium for purple coneflower seeds benefits to seed germination or seedling emergence followed by priming (−0.4 MPa, 15 °C, 10 days of darkness) in these media (Pill et al. 1994).

14.3.3 Cosmos

In cosmos (*Cosmos bipinnatus*), hormonal priming plays a main role in enhancing flowering and quality of flowers. The triazoles, which include paclobutrazol (PB) and uniconazole, are more potent and persistent than most other growth retardants. PB is used most commonly in commercial practice, but non-uniform plant size can result from non-uniform spray application. Soaking of cosmos seeds to 1000 ppm PB reduced seedling shoot height but also reduced seedling emergence percentage (Pill and Gunter 2001). Seed treatment with PB also eliminates conventional fungicide seed coating treatment since the triazoles themselves are potent fungicides (Fletcher and Gilley 2000).

14.3.4 Fir

Seeds of true firs, including pacific silver fir (*Abies amabilis*), subalpine fir (*A. lasiocarpa*) and noble fir (*A. procera*), exhibit deep dormancy at maturity. To break the dormancy termination, seeds generally require prolonged moist-chilling condition (i.e. 3–4 months or longer) (Edwards 1981, 1986; Leadem 1986; Tanaka and Edwards 1986; Edwards 1996). In some cases, germination can be impaired by seed-borne pathogens where some of the seedlots have a high proportion of empty seed (Kolotelo 1998). This seed dormancy can be broken down with solid matrix priming when seeds are existed in most chilling temperature. Agro-Lig Greens Grade (humic acids with particle sizes between 0.212 and 1.29 mm), sand (particle size 1.29 mm), peat moss and sphagnum moss are used in matrix priming that help in breaking dormancy in seeds of Abies spp. and early germination in seeds with high seedling establishment (Ma et al. 2003).

14.3.5 Gladiolus

Gladiolus alatus is a prominent bulbous cut flower that sometimes gives less productivity due to low-quality seed, inadequate seedbed preparation, late sowing, poor sowing technique, inadequate soil moisture, adverse soil properties and high temperatures. Seed priming has been successfully demonstrated in this crop to improve germination and emergence in seeds. It is the enhancement of physiological and biochemical events in seeds during interruption of germination by low osmotic potential and negligible matric potential of the imbibing medium. Hydro-primed seeds compared to KNO_3-treated seeds (osmo-primed) were allowed to imbibe water for a longer time. Seeds treated with 0.25% KNO_3 attained the maximum (66.67%) level of germination followed by 60% in 0.75% KNO_3 (60%) and distilled water. After 30 days, the germination percentage was recorded maximum with treatment of 0.25% KNO_3, 0.75% KNO_3 and distilled water (83.33%) followed by non-priming (63%) and 0.5% KNO_3 (60%)-treated seeds (Mushtaq et al. 2012).

14.3.6 Meadow Fescue

Priming is highly useful for seeds under stress condition. NaCl priming (halopriming) and hydropriming on germination and early growth of *Festuca arundinacea* and *Festuca ovina* seeds were studied under salinity condition. It was observed that NaCl priming with concentrations of 15 and 45 dS/m in *Festuca arundinacea* seeds and NaCl priming with concentration 45 dS/m in *Festuca ovina* seeds had the highest performance of improved seed in both species at germination and early growth stages under salinity stress (Shakarami et al. 2011).

14.3.7 Pansy

Temperature stress is one of the most important factors that affect the growth and development of seeds of pansy (*Viola tricolor*). Thermo-inhibited seeds fail to germinate in high temperature but can germinate when temperature is reduced which may result in thermo-death of the plant which is mostly seen in pansy. So in such condition, there is a need for priming of seeds that may induce the germination rate by improving seed quality. Dorna et al. (2014) reported that hydropriming, halopriming and osmopriming gave significant effect on germination of pansy seeds. Osmopriming seeds in polyethylene glycol (PEG) solutions of −1.25 and −1.5 MPa osmotic potential at 15 °C and in PEG solution of −1.0 MPa osmotic potential at 20 °C increased the percentage of germinating seeds significantly at 30 °C. Osmopriming seeds, in all combinations used, improved the percentage of germinating seeds significantly at 35 °C. The best result was observed when seeds were primed in PEG solution of −1.0 MPa osmotic potential at 20 °C. At higher temperature hydropriming seeds in volume of water 600 µl H_2O g/seed for 3 days at 15 °C and osmopriming at 20 °C positively affected the germination rate. After

osmopriming of seeds at 15 °C and after osmopriming in PEG solution of −1.0 MPa osmotic potential at 20 °C, the percentage of ungerminated seeds was lower than treatment control. Both halopriming at 20 °C and osmopriming, regardless of temperature and osmotic potential of PEG, improved significantly the uniformity of germination at 30 °C compared with untreated seeds. It was also observed that osmopriming followed by halopriming improved the speed of germination in pansy at 20 °C to the largest extent. Moisture content was also increased due to priming, but drying of seeds after priming affects the moisture content (Suleman et al. 2011).

14.3.8 Periwinkle

Seed priming (biopriming) increases antioxidant activity and seedling vigour in seeds of periwinkle (*Catharanthus roseus*). Seeds were treated with diazotrophs, i.e. *Azospirillum* and *Azotobacter* as separate treatments for 30 min. The germination percentage was calculated from 8 days after sowing (DAS) to 12 DAS. There was a significant increase in germination rate and non-significant in dry matter content. There was a significant increase in SOD, POX and CAT activities under *Azotobacter* and *Azospirillum* treatments and also an increase in the germination percentage, root length, shoot length and vigour index of the *C. roseus* (Karthikey et al. 2007).

14.3.9 Pot Marigold

Calendula officinalis, commonly known as pot marigold, was positively affected by seed priming method that influences the germination percentage and quality of seeds. Hydro- and hormonal priming give paramount results in seeds of pot marigold. Water or distilled water is used as hydrating agent in hydropriming in which seeds are needed to be soaked for few hours. As a result, sufficient water gets imbibed into the cell wall of marigold seeds that enhances the germination and further growth. In hormonal priming, GA_3 results better among all the growth regulators in relation to germination rate, flowering and total sugars (Karimi and Varyani 2016), but increase in concentration adversely affects the germination rate. However, GA_3 in addition to KNO_3 results profuse seedling establishment, shooting, rooting, maximum root length and vigour index in seeds of pot marigold. It was also noticed that the catalase activity increased significantly with GA_3 application, while enzyme activity was higher in the distilled water and KNO_3 treatments compared with the untreated seeds.

14.3.10 Rudbeckia

Rudbeckia fulgida, also known as black-eyed Susan, gloriosa daisy and orange coneflower, is a herbaceous perennial plant. Seed germination in this plant is variable with variable species. Osmopriming is a process of controlled imbibition by the

seeds using different concentrations of osmotic solution containing polyethylene glycol (PEG). Accumulation of solutes and enzymatic activation during controlled seed imbibition (Bewley and Black 1978) contributed to the increased radicle emergence rate in primed seeds (Fay et al. 1994).

14.3.11 Safflower

In safflower (*Carthamus tinctorius*), hydropriming has an immense impact in increasing number of plants/m^2, capitula/plant, grains/capitulum, etc. In a field experiment, hydropriming of safflower (*Carthamus tinctorius*) seed for 12 h resulted in higher number of plants/m^2, capitula/plant, grains/capitulum, 1000 seed weight, grain yield and oil content compared to untreated seed (Bastia et al. 1999).

14.3.12 Salvia

The germination of *Salvia officinalis* L. (sage) seeds is a problem of great concern that may be overcome by employing the seed priming techniques. An effect of hydropriming positively affects the germination and seedling growth in sage (*Salvia officinalis*). Priming helps in increasing final germination percentage, improving germination rate, accelerating the synchronised seed germination, vigorous seedling establishment and stimulating vegetative growth and crop yield of many crops. Significantly, higher germination percentage and rate of germination observed in hydro-primed seeds as compared to non-primed seeds indicated a positive effect of seed priming in synchronising the seed germination process (Dastanpoor et al. 2013).

14.3.13 Snapdragon

Seed priming affects the germination growth and flowering of many flowers among which snapdragon (*Antirrhinum majus*) is one of them. There is an imperative need to work on priming techniques of these flower seeds because of the higher price and, moreover, the seeds of F_1 hybrids are difficult to germinate. The effect of biopriming influenced the germination character, shooting, rooting and flowering of snapdragon using *T. harzianum* and *B. subtilis* as bioprimers (Bhargava et al. 2015). Hormonal priming also has positive effect on biochemical changes in seeds that improves membrane integrity and metabolism of seed cell wall as well as synthesis of proteins (globulins and cruciferin) in comparison to non-primed seeds (Varier et al. 2010; Rao et al. 2009).

14.3.14 Sunflower

Biopriming in sunflower gave the utmost result for controlling fungal disease called Alternaria blight, the most important disease, and estimates yield loss up to 80%. Integration of chemicals, plant extracts and biotic agents along with priming agents for managing plant diseases has been considered as a novel approach, as it requires low amounts of chemicals, reducing the cost of control and pollution hazards while causing minimum interference with biological equilibrium (Papavizas 1973). Biopriming of sunflower seeds with *P. fluorescens* in the form of jelly can be used as an alternative method to seed treatment with chemicals (hexaconazole as foliar spray) which is eco-friendly and avoids possible residue problems.

14.3.15 Zinnia

It was found that *Alternaria zinniae* seems the most important fungal seed-borne pathogen of zinnia plants (*Zinnia elegans*), causing spotting of the petals, foliage and stems and rotting of the roots (Dimock and Osborn 1943; Richardson 1990; Łacicowa et al. 1991; Palacios et al. 1991; Wu and Yang, 1992). Łacicowa et al. (1991) reported that zinnia seeds produced in Poland were commonly infested with *A. alternata*, *A. zinniae*, *Botrytis cinerea*, *Fusarium* spp. and *Penicillium* spp. The influence of osmopriming on germination, vigour and location of pathogenic and saprotrophic fungi in zinnia seeds was studied in different varieties of zinnia (Szopinska and Tylkowska 2003) at 20 °C at 45% for 24 h that were sterilised with 1% NaOCl for 10 min at 20 °C at darkness. Disinfection of primed seeds lowered the germination capacity and increased the number of deformed seedlings in variety Jowita, Red man and Talia seeds. Seed priming considerably affected the speed of germination, regardless of sodium hypochlorite (NaOCl) treatment.

14.4 Seed Priming in Trees

Most of the tree species face the problem of extinction due to over-exploitation (Onochie 1990). Seeds of forest trees have problematic seed germination due to adverse soil and environment condition. Various salt contents in soil impaired germination of these seeds when available in excess amount, i.e. when the salt concentration increased up to 0.1%. Various salts like sodium, calcium and magnesium are the most common that contribute to salinity. High levels of fertilisation also contribute to salt accumulation and can be significant in agricultural situations (Treshow 1970). Sometimes, the lack of availability of moisture in seeds affects germination process adversely. Seed priming is an alternative way to avoid this type of problem. Some examples of seed priming of forest trees are mentioned below.

14.4.1 Baobab Tree or Senegal Tree

Baobab tree or senegal tree is scientifically known as *Adansonia digitata*, a deciduous tree. Most of the seeds of *Adansonia* fail to germinate as their propagation is adversely affected by seed coat dormancy which leads to poor growth potential. Sometimes the seeds are unable to germinate in natural condition. Osmopriming and thermopriming help in overcoming the dormancy problem in seeds of *Adansonia*. Hot water, cold water and H_2SO_4 are used for osmopriming and useful in enhancing germination percentage (70%) (Falemara et al. 2013). Different concentration of H_2SO_4 is used with variable time periods. Seeds' emergence is very fast (10 days) when treated with cold water.

14.4.2 Cassia

Cassias are ornamental plants of great beauty including more than 1000 species. Seed production is irregular with low germination percentage in natural conditions due to the integument impermeability. The vigour of stored seeds can be increased with priming, with a decrease in costs and reduction in number of collections (Vertucci 1989). Osmopriming by taking PEG with different water potentials is a helpful method for increasing germination tendency in seeds of cassia. Lowering in osmotic potential results in delaying in radicle emergence (Tarquis and Bradford 1992). Osmo-primed seeds are immediately used without drying or dehydrating as it enhances the germination ability of seeds (Heydecker and Wainwright 1976).

14.4.3 Cedar

Cedrus libani, called as Taurus cedar, is now an extinct species and difficult to grow in a large population. The temperature had the strong effect on the germination of *C. libani* seeds (Bewley and Black 1994; Schmidt 2000). The seeds of this tree exhibit seed dormancy that may be corrected by different pretreatment methods or priming methods. The seeds exhibit better germination performance at lower constant temperatures. Rise in germination temperature may develop secondary dormancy in seeds (Khan and Samimy 1982).

14.4.4 Cypress

Arizona cypress (*Cupressus arizonica*) and medite cypress (*Cupressus sempervirens*) are very important forest tree species for multiple purposes in forestry because of their ability to grow in adverse environments such as calcareous, clayish, dry and poor soils (Gallis et al. 2007). Seeds can germinate even in drought condition by using PEG-8000 as osmopriming agent. The germination percentage decreased in seeds of Arizona cypress with decreased water potential.

14.4.5 Eucalyptus

Eucalyptus has about 700 species and is commercially used for timber and pulpwood for paper and other purposes. Poor germination and seedling establishment are regarded as common problem in eucalyptus which can be corrected by pregermination treatments. Seed size is the most important factor that affects germination in eucalyptus seeds. Spring-germinated seedlings have long growing period and attain maximum size with advanced establishment. Thus, larger seeds are more likely to survive in winter frosts as their susceptible growing tips are better adapted to cold-induced photoinhibition due to more advanced foliar pigment development (Close et al. 2000).

14.4.6 Fig

Seeds are the important planting material for propagation in *Ficus* spp. through which genetically different plants can be developed. It is also easily propagated through rooting and cuttings. Osmopriming, hydropriming and hormonal priming are useful treatment in early establishment of seedlings in *Ficus*. KNO_3, GA_3 and water are used for priming purpose. Early germination due to the priming effect of GA_3 results into longest radicle, which helps in early establishment of new seedling to produce maximum food material with the help of photosynthesis that resulted into the maximum survival of seedlings (Rawat et al. 2010)

14.4.7 Gamhar or White Teak

Gmelina arborea (gamhar) is a fast-growing deciduous tree used for furniture, carriages, sports and musical instruments as its steady timber is moderately resistant to termites. Seeds of *Gmelina arborea* (white teak or gamhar) don't have any dormancy and can easily germinate. Hydropriming in seeds may influence the germination tendency and increase the seedling emergence. Also $KMnO_4$ (0.2 M) treatment increases the germination percentage when seeds are treated with different interval of time duration.

14.4.8 Gulmohar

Delonix regia also known as gulmohar exhibits seed dormancy. For breaking the seed dormancy in seeds of *Delonix*, hormonal priming is efficiently useful by using different plant hormones, i.e. ABA, BAP, GA and IBA. Hot water treatment is also useful that tends to give the germination percentage of 95% in seeds of gulmohar. ABA does not have significant effect in breaking the dormancy. Gibberellic acid will promote the endosperm breakdown and the growth of the embryo that results in the elongation of radicle cells and cause a rupture in the micropyle, and the seed

dormancy will be terminated by radicle protrusion from the seed coat. The role of temperature in breaking the dormancy to influence germination can be noticed due to the erratic nature of the germination which terms for the standardisation of both the temperature of the water and duration of cooling as suggested by Owonubi and Otegbeye (2004).

14.4.9 Henkel's Yellowwood

This ornamental tree is dioecious in nature and is used for ornamental purpose. Generally, this henkel's yellowwood tree (*Podocarpus henkelii*) is propagated through seeds. Fleshy fruit that surrounds the seed is removed as this inhibits the germination. Removal of epicuticular wax, the epidermis or the entire epimatium leads to rapid water uptake and germination (Dodd et al. 1989). Seeds stored in shade have more moisture content and reserve more starch through biochemical process. Lipids are the major reserve materials in seeds of *Podocarpus henkelii* followed by proteins, and these embryonic reserves are sufficient for early seedling establishment (Nabanyumya et al. 2015). Soaking seeds more than once a day also breaks dormancy in seeds, resulting in maximum germination percentage.

14.4.10 Whistling Pine

The whistling pine (*Casuarina equisetifolia*) is used as a windbreak in agroforestry system and also for many household uses. The wood is resistant to decomposition in soil or saltwater and is often used as roundwood for making piles, poles and fences. This is accentuated as the successful propagation of any *Casuarina spp*. by a vegetative method is very limited even with IBA hormone application (Mahmood and Possuswam 1980; Pinyopusarerk and Bolan 1990). Different concentrations of growth hormones like GA_3 and ABA, acid substances like sulphuric acid and chemical like $NaNO_3$ are helpful in promoting germination and growth in this tree. The waxy and hard seed coat in seeds of *Casuarina* prevents it to germinate which has been broken with the use of concentrated H_2SO_4 by several workers (Mayer and Poljakoff-Mayber 1963; Ballard 1973; Gill and Bamidele 1981; Etejere et al. 1982; Eze and Orole 1987).

14.4.11 Ivory Coast Almond

Terminalia ivorensis, a tropical deciduous tree, thrives in a wide range of soil and is used as multipurpose trees along with having medicinal uses. Seed germination of tropical species is influenced by several biological factors such as seed viability, seed size (Barrera and Nobel 2003) and plant growth regulators through which acts as germination stimulator (Chen et al. 2008). Seeds are very sensitive to temperature stress as it influences the germination process, and high temperature prevents the

elongation of radicle and shoot by inhibiting the synthesis of protein and nucleic acid (Sivaramakrishnan et al. 1990). Soaking seeds of *T. Chebula* (Horitaki) enhances germination rate and vigour index (Hossain et al. 2005). Gibberellic acid can overcome the dormancy in seeds of *Terminalia* spp. by inducing rapid and uniform germination due to deficit in endogenous gibberellic acids (Asomaning et al. 2011). The exogenously applied gibberellic acids help in modifying the influence of cytokinins on transport across membranes and are thus able to initiate the biochemical processes necessary for germination process (Chen et al. 2008).

14.4.12 Kachnar

Bauhinia spp. (mountain ebony) are flowering plants and propagated through seeds and cuttings. This tree has been prioritised as one of the trees for conservation to enhance its contribution to health and livelihood of communities. Various methods are there to break the dormancy in the seeds of this tree that promotes its cultivation and successful regeneration. Mainly germination in kachnar depends on the size of the seeds as larger seeds germinate more percentage than smaller one. Soaking in boiled water makes the seed coats permeable to water and water gets imbibed into the cell wall which results in swelling of the seed as water cools. Recommendation of hot water must be applied judiciously without killing the seeds with excessive heating (Phartyal et al. 2005). Chemical stimulators like KNO_3 enhance seed germination by creating a balance between hormonal ratios in the seed and reducing the growth retarding substances like abscisic acid (ABA).

14.4.13 Karanj (Indian Beech)

The use of biologically active products like biomanures and biofertilisers for the production of quality planting material helps in preventing microbial inoculation of bacteria, algae and fungi in karanj (Revathi et al. 2013). Biopriming with liquid *Azospirillum* and *Phosphobacterium* in different concentrations improves germination, seedling vigour and storability of *Pongamia pinnata* seeds by reducing microbial infections. An increase in the seed germination might be due to the increased cytokinin production which actively involved in cell division (Suma et al. 2014) and production of growth-regulating substances like auxin, GA and cytokinin (Kucey 1988) when seeds are encapsulated with biofertilisers.

14.4.14 Khejri

Prosopis cineraria (khejri) is a flowering tree of which seeds sometimes show dormancy effect which can be lessened by priming methods. Fulvic acid that is extracted from compost is beneficial in enhancing germination tendency in seeds of this tree. Fulvic acids are beneficial to increase the permeability of seed coat and plant cell

membranes and enhance enzymatic activity of the root system leading to increased root proliferation (Trevisan et al. 2010). Application of $CaCO_3$ can cause buffering capacity in soil that affects nutrient availability to plants, while sulfuric acid treatment has conventionally been used for breaking the dormancy and softens the seed coat through which the radicle can easily protrude.

14.4.15 Oak

Himalayan oak trees (*Quercus glauca*) have irregular fructification, and consumption of seeds by animals (Troup 1921) and loss of viability during storage for extended periods (Chalupa 1995) have overrated the problem of regeneration (artificial and natural). Propagation (clonal propagation) through stem cuttings is difficult in most of the oak species and has not been much successful in these species. Even the seeds' weight is also affected by germination percentage. Seed coat acts as a mechanical barrier in germination of oak seeds which prevents radicle emergence and is corrected by several scarification methods and priming treatments (Alderete-Chavez et al. 2011). The KNO_3 plays an osmotic role on water uptake with a nutritional effect on protein synthesis. KNO_3 is used for growth regeneration and also as germination-stimulating substance in many species (Ozturk et al. 1994).

14.4.16 Pine

It is the most widely distributed tree used for timber purposes and for making boxes, paper pulp and temporary electric poles. Seed germination in pine (*Pinus kesiya*) is a common problem which depends on the moisture level and substrate pH level in seeds (Verma and Tondon 2010). Hydropriming increases the imbibition rate and moisture level in seeds which enhances the germination rate. Available soil moisture also influences germination and early seedling survival. Influence in germination in pine is due to leaching of growth inhibitors and increase in endogenous level of growth regulators when seeds scarified or subjected to chilling treatment.

14.4.17 Siris

Albizia lebbeck (siris) has special place among all the forest crops. Despite its importance, the species is becoming scarce due to deep seed dormancy. Seed priming is a method used for producing numerous number of planting material by overcoming its seed coat-imposed dormancy (Baskin and Baskin 1998). Seeds are often used for propagation in Siris which is a cheapest method of propagation in many species of this tree. Its hard seed coat makes the germination process difficult in nursery and/or out in the fields. Sulphuric acid, hot water and gibberellic acid are used for breaking dormancy in this species (Alderete-Chavez et al. 2011; Baskin

and Baskin 1974). The disintegration of the seed coat increases the imbibition and subsequent germination in seeds when treated with sulphuric acid (Egley 1989).

14.4.18 Kapok

The silk cotton tree (kapok), *Ceiba pentandra*, is a fast-growing and emergent tropical forest tree species which can grow up to a height of 60 m (Gribel et al. 1999). It is one of the extinct species among forest trees, and seeds of this tree are also limitedly available. It is pollinated by hummingbirds (Gribel et al. 1999; Toledo 1977) and non-flying primate mammals, particularly by *Saimiris ciureus* (squirrel monkey), *Cebus paella* (capuchin) and *Ateles paniscus* (spider monkey) (Janson et al. 1981). Seed soaking in seeds of silk cotton tree significantly influences the germination process. But heavy rainfall may limit the process of germination and fails to break the seed dormancy.

14.4.19 Subabul

This subabul tree (*Leucaena leucocephala*) is also called 'miracle tree' due to its multipurpose nature (Ssenku et al. 2017). Germination capacity in seeds of subabul can increase by different priming or pretreatment methods. NaCl concentration in soil shows a marked effect on the germination. With the increase in salinity, the germination initiation in this species is delayed and gets ceased (Rafiq et al. 2006). The reason behind this is that the osmotic pressure of the soil solution increases with increase in salt concentration that results in the prevention of uptake of water. It also reduces the germination energy of seeds.

14.4.20 Tamarind

Tamarind (*Tamarindus indica*) has been recognised for its potential nitrogen-fixing nature (Okoro et al. 1986). Seeds in tamarind do not have self-capacity to germinate due to the lack of the factors required for breaking dormancy. Hot water treatment and sulphuric acid affect the germination rate, and seed germination increased with increasing water temperature and soaking period.

14.4.21 Teak

Teak (*Tectona grandis*) is a tropical tree species, hardy and deciduous in nature, is valued for its durability and water resistance and is used for boat building, exterior construction, veneer, furniture, carving and other small wood decorative uses. Germination of the seeds involves pretreatment to remove dormancy arising from

the thick pericarp. Pretreatment involves alternate drying and wetting of the seeds. Saline solution also affect the germination ability of seeds in teak, as increased salinity results in decreased germination ability of seeds and delayed rate of germination. This is due to the complex nature of salts that present in the solution for germination which may be sometimes having toxic effect in germination.

14.4.22 Wigandia

Wigandia caracasana, or *Caracus wigandia*, is a species of evergreen ornamental plant having purple flowers in large clusters. Sometimes, seed burial can improve the chances of establishment of seedlings through breaking the dormancy. Priming consists of a regulated hydration process that permits enhancement of some metabolic processes through the osmotic solutions or water. Burial enhanced germination process and favoured uniform and rapid germination of *W. urens* seeds (Gonzalez-Zertuche et al. 2001). This enhancement in germination is due to the increased protein synthesis in burial seeds as compared to control seeds. Burial with priming treatment in seeds increases the germination percentage, seedling growth and breaking dormancy than the priming treatment alone.

14.4.23 Willow Tree

Willow tree (*Salix babylonica*), also called sallows, is a deciduous tree as well shrub. Seeds are viable for a short period of time. Priming methods are useful in enhancing germination and growth of dormant seeds. Thermopriming gives efficient result in germination up to 100%. Seeds of willow tree results in better germination rate in lower temperature regimes (Young and Clements 2003).

14.5 Conclusion

There is a huge gap between the number of seeds sown and availability of stocky seedlings in any ornamental flower crops and several important tree species. The most important problems faced are the heterogneity and lack of suitable conditions in soil that cause decrease in germination percentage. Heterogeneous emergence, unbalanced seedling growth and competition for environmental resources such as light, nutrients and water subsequently make difference in biomass and performance of plants. Priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. The purpose of these treatments is to shorten the emergence and to protect seed from biotic and abiotic factors during critical phase of seedling establishment so as to synchronise emergence, which lead to uniform stand and improved yield.

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15 Role of SNP-Mediated Nitric Oxide Priming in Conferring Low Temperature Tolerance in Wheat Genotype (*Triticum aestivum* L.): A Case Study in Indian Northern Plains

Md. Afjal Ahmad, Pravin Prakash, and H. B. Singh

Abstract

Wheat is an economically important cereal crop grown in the vast stretch along the northern, western and part of Central Indian plains serving as livelihood tool for more than half of agrarian population of the country. The crop being grown in rabi (mid-November–mid-April) faces multifaceted abiotic stress threats among which low temperature stress being one of them. Nitric oxide has been well documented to counter many of these threats as also low temperature stress. In this context, a study was conducted to evaluate the effect of nitric oxide priming on certain morphophysiological and biochemical parameters in wheat genotype HD-2329 facing low temperature regime in the laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University. The several parameters under observations were per cent germination, germination rate, shoot length, root length, α-amylase activity and soluble sugar content both under normal (25 °C) and low temperature (5 °C) conditions. Seed priming with sodium nitroprusside (SNP, a nitric oxide donor) at 100 μM concentration resulted in enhanced performance of these parameters as compared to non-primed seeds both under normal and low temperature, but the effect was more pronounced at low temperature regime. Hence, it was concluded that NO priming of wheat seeds had a statistical significance in conferring low temperature tolerance in the crop, thus making it a fit priming model in a climate resilient era.

Keywords

NO priming · Low temperature stress · Wheat

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15.1 Introduction

Wheat (*Triticum aestivum* L.) is an important food crop of the world. Among cereals, it occupies top slot because of its highest consumer acceptance owing to higher nutritive value, protein and gluten content. Among major cereals, globally wheat ranks first in area and production and contributes more calories and proteins to the world's human diet than any other cereals. The total area under wheat in India is 30.47 mha with a production of 95.85 m tonnes and productivity 2.8 tonnes ha^{-1} (Anonymous 2016). India is the second largest producer of wheat in the world after China, and the crop has provided the fastest pace of agrarian growth to the Indian agriculture.

In most of wheat growing regions of northern India, there is late sowing of crop; as a result crop faces low temperature stress which significantly reduces germination and subsequent seedling emergence. Direct result of low temperature effects is on cellular macromolecules, which leads to slowing of metabolism, reduced activity of amylase enzyme, solidification of cell membranes and loss of membrane functions (Joshi et al. 2007). Low temperature stress has also been known to severely inhibit reproductive development in wheat crop.

Nitric oxide (NO), a highly reactive, membrane permeable free radical, is a widespread intercellular and intracellular messenger with a broad spectrum of regulatory functions in many physiological processes (Arasimowicz and Floryszak-Wieczorek 2007). In the past 20 years, NO has been reported to be involved in various key physiological processes of plants, including seed germination, leaf senescence, ethylene emission, stomatal closure and various plant responses to biotic and abiotic stresses (Chen et al. 2010). Exogenously applied sodium nitroprusside (SNP), as an NO donor, can enhance elongation growth in maize root segments, and application of methylene blue (MB), which inhibits NO production and/or NO action in plants, could reverse these responses (Kumar et al. 2010).

NO has emerged as a key signalling molecule in plants during the last decade, and its role has been implicated in number of physiological and developmental processes as well as response to abiotic stresses including heat and cold stress. In recent years, NO has been shown to be involved in seed germination and reduction of seed dormancy, photomorphogenesis, leaf expansion, root growth, regulation of plant maturation, senescence, suppression of floral transition (He et al. 2004) and phytoalexin production and as an intermediate downstream of ABA signalling. NO is a free radical reactive gas with many physiological functions and alleviates the deleterious effects of ROS and establishes a stress resistance response thus conferring low temperature tolerance (Amooaghaie 2011). It has got many important functions to play in plant system like ubiquitous signal involved in diverse physiological processes that include germination, root growth, stomatal closing and adaptive response to biotic and abiotic stresses. Nitric oxide production in plants is involved in acquiring cold acclimation or cold tolerance (Chen et al. 2010). NO is produced immediately as a plant response to cold stress, and it participates in the regulation of cold-responsive gene expression (Dahal et al. 1990). The involvement of NO in imparting cold tolerance has been indicated by its exogenous application in certain

cold-sensitive plant species such as maize, tomato and wheat. Its mechanism in providing protection against cold has been attributed to its antioxidative feature and suppression of peroxidative metabolism caused by stress (Bailly 2004).

Seed priming with SNP has been proved to be a promising technology to enhance rapid germination, better seedling emergence, high vigour and better yields in rice crops (Farooq et al. 2007). This technology is commonly employed to decrease the time between seed sowing and seedling emergence and for synchronization of seedling emergence. Priming induces some biochemical and physiological changes to help better and faster germination of seeds (Chung et al. 2007).

The present investigation was carried out in the laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. A detailed account of the materials and methods used and results obtained thereafter are discussed below.

15.2 Experimental Details

15.2.1 Source of Experimental Material

Disease-free, healthy seeds of wheat variety HD-2329 were procured from Division of Genetics and Plant Breeding, IARI, New Delhi. Bold and uniform seeds were selected, whereas small, discoloured, infected seeds were discarded. The seeds were then brought to the laboratory and washed with distilled water before giving priming treatments.

15.2.2 Treatment Imposition

Healthy seeds were selected for imposing the priming treatment. There were six priming treatments, viz. hydropriming (control), osmopriming (PEG), SNP priming (NO donor), methylene blue (MB) priming and MB+PEG and MB+SNP priming. Each priming treatments was performed in three replications, and each replication contained 20 seeds in a large-sized petriplates (150 × 25 mm). The seeds of respective treatments were put in a clean beaker, and priming solutions were put in almost equal volume as of seeds. The beaker was shaken to mix the priming solution with that of seeds and kept at room temperature for 16 h of priming period. After priming period was over, seeds were shade dried for another 6 h. Twenty seeds each were placed in the petriplates containing germination sheet. Distilled water was applied to all the petriplates irrespective of treatments in such a way that half of the seeds immersed in water. As per research objectives, two BOD incubators set at 25 °C (normal temperature) and 5 °C (low temperature) were kept ready. Eighteen petriplates (each treatment in three triplicates) were put in each incubator for studying the temperature effects on germination and subsequent growth. Observation for germination and other growth parameters were taken beginning from 1 day after treatment imposition. Distilled water was periodically applied in each petriplates. One

hundred milligrams of seeds from each patriplates were taken for biochemical estimations.

15.2.3 Treatment Details

There were six priming treatments (T1, control, distilled water; T2, osmopriming PEG 6000 150 g L^{-1}; T3, sodium nitroprusside, SNP 100 μM; T4, methylene blue, MB 100 μM; T5,MB 100 μM + PEG 6000 150 g L^{-1}; and T6, MB 100 μM + SNP 100 μM) given to the cultivar HD-2329 in three replications each in optimum and low temperature regime.

15.2.4 Sampling Procedures and Observations

Observations were recorded on changes in germination percentage, germination rate, shoot length, root length, α-amylase activity and soluble sugar content in all the priming treatments under the normal and low temperature regime. Samples for α-amylase activity were taken after 18 h of inducing temperature treatment and that of soluble sugar were taken 48 h after the treatment.

15.2.5 Statistical Analysis

Factorial completely randomized design (FCRD) was followed, and analysis of variance was performed on the data as described by Panse and Sukhatme (1967). Critical difference values were calculated at 5% level of significance in order to compare the treatment means.

15.2.6 Results and Discussion

The effect of different priming treatments on per cent germination especially with respect to nitric oxide priming and osmopriming coupled with use of methylene blue (NO inhibitor) under low temperature is shown in Fig. 15.1. The maximum per cent germination was observed in SNP (100 μM) treatment at 25 °C, whereas minimum germination percentage was recorded in methylene blue (MB 100 μM) primed seeds at 5 °C. Among the priming treatments both at optimal (25 °C) and suboptimal temperature (5 °C), the maximum and minimum per cent germination was reported in SNP (100 μM) and MB (100 μM), respectively. Significant differences were observed in germination rate (Fig. 15.1) both under temperature regimes and among the priming treatments. The effects pertaining to shoot length (cm) showed favourable effect of NO priming post germination both at normal and low temperature treatments (Fig. 15.2). The effect on root length with respect to nitric oxide priming and osmopriming showed significant differences (Fig. 15.2). The

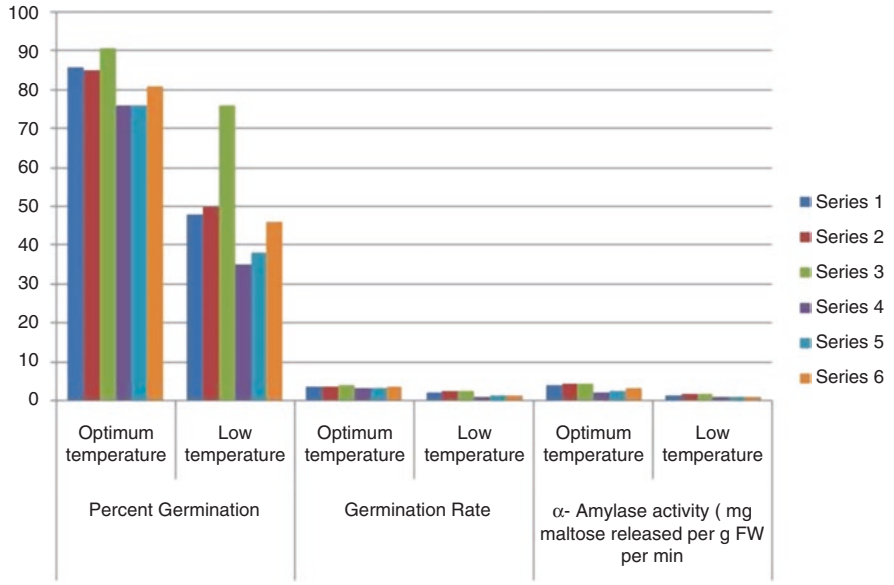

Fig. 15.1 Effect of NO priming on per cent germination, germination rate and α-amylase activity in wheat genotype both under optimum and low temperature regime. (Note: Series 1–6 refers to six priming treatments discussed in the text)

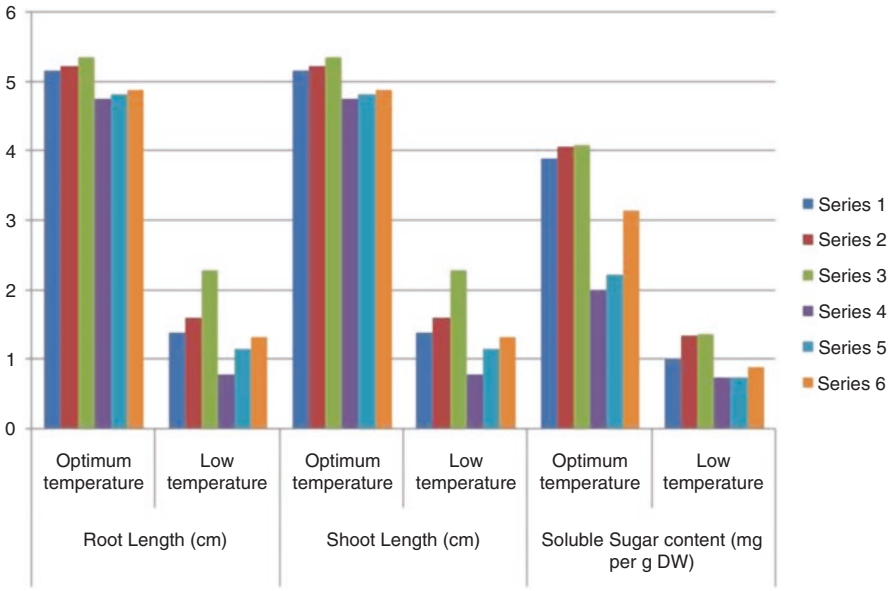

Fig. 15.2 Effect of NO priming on root length, shoot length and soluble sugar content in wheat genotype both under optimum and low temperature regime. (Note: Series 1–6 refers to six priming treatments discussed in the text)

maximum root length was observed in SNP-treated seeds at 25 °, whereas the minimum was recorded in MB (100 μM)-treated seeds at 5 °C.

Significant differences were observed in α-amylase activity both under temperature regimes and among the priming treatments (Fig. 15.1). The maximum α-amylase was observed in SNP-treated seeds at 25 °C, whereas the minimum was recorded in MB (100 μM)-treated seeds at 5 °C. The soluble sugar content (mg g^{-1} DW) showed favourable effect of NO priming post germination both at normal and low temperature treatments. The maximum soluble sugar content was observed in SNP-treated seeds at 25 °C, whereas the minimum was recorded in MB (100 μM)-treated seeds at 5 °C. Among the priming treatments both at 25 °C and 5 °C, the maximum and minimum soluble sugar content was reported in SNP (100 μM) and MB (100 μM), respectively (Fig. 15.2) (Ahmad et al. 2015).

15.3 Conclusion

Treatments to enhance seed vigour have been proven to be very effective in achieving rapid and uniform seed germination. The priming treatment may constitute a useful tool in overcoming the barriers created by abiotic stresses and, thus, assuring a high probability of successful establishment for each seed planted. Since some wheat genotypes are more sensitive to cold than others, it is possible that priming could make these genotypes germinate as fast as the cold-tolerant ones. As temperature decreased from optimum, there was a concomitant reduction in per cent germination, germination rate, shoot length and root length, as well as in α-amylase activities and sugar content. Under stress conditions, higher levels of NO are required for the maintenance of cell homeostasis, and once NO is endogenously generated or gets inside the cell from an exogenous source, it enhances stress tolerance. The tolerant genotypes might produce more NO under stress. In control, there is sufficient NO produced for signalling and germination to proceed at faster rate, and there is lesser of reactive oxygen species formed. Under stress there is increase in reactive oxygen species and oxidative damage and their effect on metabolism; henceforth, increased NO production by NO and osmoprimed seeds might be the reason for enhanced amylase activity and more soluble sugar, being able to readily support metabolic activities and consequently result in a higher rate of emergence and seedling growth under low temperature.

Our results suggest that exogenous application of NO has a profound effect on several germination attributes both under optimum and low temperature regime, but the priming treatment effects were more pronounced under low temperature, thus indicating that NO priming via SNP can be a successful priming technology for conferring low temperature tolerance in wheat.

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Seedling Bio-priming with *Trichoderma* spp. Enhances Nitrogen Use Efficiency in Rice

16

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Abstract

Present study was undertaken to investigate the effect of seedling bio-priming with *Trichoderma harzianum* (ATCC No. PTA-3701), varied N fertilizer doses (100%, 75%, 50%, and 25% RDN), and different soil type on rice plant under greenhouse condition. Results showed that the plant growth, chlorophyll content, nutrient use efficiency, and grain N content were significantly increased in various treatments. Significantly higher growth promotion, chlorophyll content, nutrient use efficiency, and grain N content were found with recommended dose of fertilizer (RDF) NPK @ 120-60-60 kg ha^{-1} followed by seedling treated with *T. harzianum* + 3/4th N and RDF of PK. Seedling bio-priming with *T. harzianum* also enhanced the plant growth and nutrient use efficiency in rice plant. Agronomic use efficiency and physiological use efficiency were maximum in alluvial soil as compared to black soil and red soil. Results indicated the suitability of seedling bio-priming with *T. harzianum* for better plant growth and nutrient use efficiency in rice plants.

Keywords

Bio-priming · *Trichoderma* · Nitrogen use efficiency (NUE)

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16.1 Introduction

Nitrogen is often considered as most essential limiting nutrient for the crop growth and yield in many of world's agriculture systems. Thus, N fertilizers are one of the most important inputs for cereal production systems worldwide, and a sharp boost has occurred in consumption of N fertilizers from 11.6 million tonnes in 1961 to 104 million tonnes in 2006 (Hoang and Alauddin 2010; Mulvaney et al. 2009). Over the past five decades, consumption of mineral N fertilizers has increased about 7.4-fold, whereas crop production has only increased by only 2.4-fold (Tilman et al. 2002) indicating a sharp decline in N use efficiency. Unfortunately, the dynamic nature of N and its susceptibility to loss from plant-soil systems creates a challenge to its efficient management. Large amounts of N fertilizers were lost due to the combined effect of surface runoff, leaching, volatilization,denitrification and gaseous plant emission. Low efficient use of N fertilizers is not only responsible for higher cost of production but also caused serious environmental problems such as soil acidification, groundwater contamination, and N_2O emission. Nitrogen emissions to the air – notably those of nitrous oxide (N_2O) – are contributing to climate change. Nitrogen use efficiency (NUE) for production of cereal crop including rice, wheat, corn, barley, sorghum, oat, and rye is approximately 33%, and the rest unaccounted 67% represents a US$ 15.9 billion annual loss of N fertilizer worldwide (Raun and Johnson 1999). Therefore, maintaining agricultural productivity in such a way that minimizes the harmful effects of fertilizers on environmental is need of the hour.

Various techniques have been advocated to increase the NUE. Incorporation of various sustainable agricultural practices, including crop rotation, fertilizer use rationalization, establishment of ground cover, green manuring, and burial of crop residues, is widely recommended. Research for new improved crop varieties, through genetic modification and breeding that take up more organic or inorganic N from the soil N and utilize the absorbed N more efficiently have been carried out since decades. Legislation aimed at protecting the environment from nutrient runoff has been enacted by some governments, and policies based on this legislation are being implemented. However, a little success has been achieved in improving nitrogen use efficiency in plants. Microbial inoculants offer promising integrated solutions to agro-environmental problems through plant growth promotion, disease protection, and enhancing nutrient availability and uptake in plants. *Trichoderma* spp. are most common filamentous free-living saprophytic fungi better known for plant disease suppression and dominate the global biopesticide market (Singh 2016; Keswani et al. 2014). However, intense investigation since last decades acknowledged various traits in *Trichoderma* that promote its applications beyond crop protection. Various species of *Trichoderma* are identified which are capable of plant growth promotion, managing abiotic stresses, and improving nutrient use efficiency and uptake (Bisen et al. 2015; Mehetre and Mukherjee 2015). Beneficial microbes mediated improvement of nutrient use efficiency in plant is gaining a lot of attention worldwide in the context of soil fertility and productivity deterioration (Rakshit et al. 2015a, b). *Trichoderma* spp. can enhance crop productivity by virtue

of both enhanced decomposition of biomass and improving uptake of inorganic fertilizers. The main objective of present work is to evaluate the response of seedling bio-priming with *Trichoderma* on nitrogen use efficiency in rice.

16.2 Materials and Methods

16.2.1 Experimental Soils

The soils of three orders, entisol, inceptisol, and alfisol, were collected from different locations. Alluvial soil sample was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The red soil was collected from the Semara village, Mirzapur, and black soil was collected from Arzilime block, Varanasi. Physiochemical properties of experimental soils were analyzed (Table 16.1).

16.2.2 Plant Material

Seeds of rice variety PHB-71 were obtained from the market. Seeds were surface sterilized with 0.1% $HgCl_2$ for 30 s, then washed with sterile distilled water thrice, and sown in nursery.

16.2.3 Trichoderma Strain

Trichoderma harzianum (ATCC No. PTA-3701) were obtained from the culture repository of Plant Health Clinic Laboratory of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

Table 16.1 Physiochemical properties of experimental soils

Physiochemical parameters	Alluvial soil	Black soil	Red soil
pH	7.2	7.4	6.4
Bulk density (g/cm^3)	1.39	1.52	1.29
Particle density	2.56	2.61	2.49
EC (dSm^{-1}) 0.39	0.44	00.61	0.31
CEC ($Cmol_{(p+)}$ kg^{-1})	28.98	30.92	18.0
Organic carbon (%)	0.37	0.39	0.31
Available N (kg ha^{-1})	229	236	173
Available P (kg ha^{-1})	17	13	8
Available K (kg ha^{-1})	230	235	109

16.2.4 Seedling Bio-priming with Trichoderma Formulation

Liquid formulation of *T. harzianum* containing approximately 10^8 spores ml^{-1} was used for seedling treatment. Roots of 40-day-old rice seedlings prepared for transplanting were dipped in liquid formulation for 30 min, and then the seedlings were kept in shade for half an hour before transplanting. Control seedlings were only treated with water.

16.2.5 Pot Experiments

The experiments have been conducted on three different soil types at the Institute of Agricultural Sciences, BHU. Seedlings of rice cultivar treated with *Trichoderma* were grown with different level of N fertilizer dose. Five treatments were applied:

T_1- Control N: P: K @ 0: 0: 0 Kg h^{-1}
T_2- Recommended dose of N: P: K @ 120: 60: 60
T_3- Seedling treated with *T. harzianum* + 3/4th N: P: K @ 90: 60: 60
T_4- Seedling treated with *T. harzianum* + 1/2 N: P: K @ 60: 60: 60
T_5- treated with *T. harzianum* + 1/4th N: P: K @ 30: 60: 60

16.2.6 Study of Effect of Seedling Bio-priming on Growth Parameter and Yield Attributes of Plants

Growth parameters including height of plants, leaf area, and dry weight were measured at 30, 60, and 90 days after transplanting. Yield attributes including number of tiller pot^{-1} and length of panicle of randomly selected plants from each treatment were recorded. Test weight and yield pot^{-1} were recorded after harvesting.

16.2.7 Estimation of Nitrogen in Plant and Grains Sample

N content in plant and grain sample was determined by modified Kjeldahl method as outlined by Peng et al. (1996). N content was calculated by the following formula:

$$\text{Percent N in plant material} = \frac{0.02 \times T \times 0.014 \times 50 \times 50}{5 \times 50}$$

T = sample reading – blank reading

16.2.8 Chlorophyll Content

The chlorophyll content of leaves was calculated as described by Arnon (1949). Leaf samples were obtained from 60-day-old rice crop. 0.5 g of leaf sample was crushed with 80% acetone in mortar and pestle and filtered through Whatman No. 1 filter. Supernatant was collected, and volume raised up to 50 ml. Absorbance was recorded at 645 nm using spectrophotometer (RayLEIGH UV-2601). Total chlorophyll content was determined as follows:

$$\text{Total chlorophyll}\left(\text{mg g}-1\,\text{fresh wt.}\right)=\left(20.2\times \text{A}\,645\right)+\left(8.02\times A\,663\right)\times\frac{\text{V}}{\text{W}}\times 1/1000$$

16.2.9 Estimation of N Use Efficiency

Nitrogen use efficiency (NUE) formulas

$$\text{Physiological use efficiency}\left(\text{PUE}\right)=\frac{\text{Yield F kg}-\text{Yield C kg}}{\text{Nutrient uptake F kg}-\text{Nutrient uptake C kg}}$$

$$\text{Agronomic use efficiency}\left(\text{AUE}\right)=\frac{\text{Yield F kg}-\text{Yield C kg}}{\text{Quantity of nutrient applied}\left(\text{kg}\right)}$$

where F is plant receiving fertilizers and C is plants receiving no fertilizer

16.3 Results (Tables 16.2, 16.3 and 16.4)

16.4 Discussion

16.4.1 Chlorophyll Content

Chlorophyll content in rice leaves was significantly affected by combination of N-fertilizer doses with seedling bio-priming by *T. harzianum*. Maximum chlorophyll content was recorded in plants shown in alluvial soil (2.64 mg g^{-1}) followed by black (2.23 mg g^{-1}) and red soil (1.5 mg g^{-1}). Treatment T2 showed significantly higher chlorophyll content in all soil type in with maximum in alluvial soil (3.46 mg g^{-1}). Singh and Singh (2011) has reported that the alluvial soil showed better

Table 16.2 Effect of soil types, seedling biopriming, graded N dose application on plant height

Treatments	30 days			60 days			90 days		
	Alluvial soil	Red soil	Black soil	Alluvial soil	Red soil	Black soil	Alluvial soil	Red soil	Black soil
T1	55.92 ± 2.17^{hi}	50.05 ± 1.65^{j}	52.0±1.95j	82.86 ± 1.5^{ef}	70.86 ± 1.5^{j}	73.81 ± 1.0^{hi}	89.36±2.13d	80.83±1.29h	85.85 ± 2.0^{ef}
T2	80.55 ± 2.15^{a}	74.22 ± 1.45^{bc}	77.42 ± 1.3^{ab}	98.15 ± 1.1^{a}	90.18 ± 1.0^{cd}	93.28 ± 0.94^{b}	103.44±2.35a	94.36±0.8c	97.83 ± 1.9^{b}
T3	74.51 ± 2.14^{bc}	70.30 ± 0.79^{d}	71.24 ± 1.8^{cd}	93.66 ± 1.5^{b}	81.20 ± 1.9^{f}	87.83 ± 2.5^{cd}	100.85±1.85a	90.18±1.7d	95.47 ± 1.5^{bc}
T4	69.17 ± 2.0^{de}	63.0 ± 1.9^{g}	66.72 ± 1.98^{ef}	87.67 ± 2.5^{cd}	76.47 ± 1.5^{gh}	80.28 ± 1.0^{f}	95.19±1.4bc	83.98±0.4fg	94.31 ± 0.7^{c}
T5	64.96 ± 2.90^{fg}	52.81 ± 1.7^{ij}	56.33 ± 0.9^{h}	85.19 ± 1.9^{de}	73.57 ± 1.6^{i}	77.52 ± 1.0^{g}	91.13±1.9d	81.65±0.8gh	88.37 ± 1.9^{de}

Table 16.3 Effect of soil types, seedling bio-priming, and graded N dose application on chlorophyll content

Treatments	Alluvial soil	Red soil	Black soil
T1	1.43 ± 0.1^{a}	0.66 ± 0.1^{a}	1.06 ± 0.1^{a}
T2	3.46 ± 0.2^{d}	2.83 ± 0.1^{d}	3.03 ± 0.1^{d}
T3	3.26 ± 1.0^{d}	2.43 ± 0.8^{c}	2.83 ± 0.9^{c}
T4	2.83 ± 0.1^{c}	2.16 ± 0.1^{c}	2.53 ± 0.1^{c}
T5	2.23 ± 0.1^{b}	1.5 ± 0.8^{b}	1.7 ± 0.1^{b}

$P \leq 0.05$

potential with graded N fertilizer application and seed bio-priming by *T. harzianum*. In present study, 100% RDF-treated plants showed maximum chlorophyll content followed by seedling treated with *T. harzianum* + 3/4th N and RDF of P and K fertilizers in comparison to control.

16.5 Nitrogen Uptake

Application of 100% RDF in pots resulted in significantly higher N uptake in plants at all growth stages in rice crop as compared to control. The N uptake in rice plants was gradually increased and reaches maximum at 90 DAS in all soil type under T2 followed by T3>T4>T5>T1. Meena et al. (2016) has reported that the N accumulation in wheat plants was increased with N fertilizer doses and was recorded maximum at 100% RDF. Significantly higher N uptake was noticed at 60 DAS under following treatment T2 >T3> T4 >T5 >T1.

At 90 DAS increased N uptake was recorded and ranged from 6.4 to 61.86 and 3.79–24.93 and 4.47–50.78 mg plant^{-1} under alluvial, red, and black soil, respectively. Higher N uptake by plants may be attributed to the ability of *Trichoderma* to facilitate the plant growth, nutrient availability, and plant health (Meena et al. 2016; Adesemoye et al. 2009; Singh and Singh 2011).

16.5.1 Grain Nitrogen Content

Significantly higher grain nitrogen content was observed in treatment T2 under alluvial, black, and red soil, respectively, in comparison to control (Fig. 16.1). Nitrogen content in grain of rice was also influenced by different soil types. Meena et al. (2016) have reported maximum grain N content in wheat plant under alluvial soil treated with 100% RDF. In present study T3 seedling treated with *T. harzianum* + 3/4th N:P:K @ 90:60:60 showed significant increase in grain N content in comparison to control.

Table 16.4 Effect of soil types, seedling bio-priming, and graded N dose application on nitrogen uptake by plant (mg plant^{-1}) at different growth stages of rice

	30 days			60 days			90 days		
	Alluvial soil	Red soil	Black soil	Alluvial soil	Red soil	Black soil	Alluvial soil	Red soil	Black soil
T1	14.0 ± 1.2^{a}	8.6 ± 0.6^{a}	10.9 ± 0.06^{a}	39.2 ± 1.0^{a}	25.30 ± 0.7^{a}	34.58 ± 1.1^{a}	6.47 ± 0.5^{a}	3.79 ± 0.8^{a}	4.47 ± 0.6^{a}
T2	100.4 ± 1.7^{e}	68.4 ± 0.7^{e}	85.4 ± 1.1^{e}	150.3 ± 1.3^{e}	95.32 ± 0.7^{e}	136.61 ± 1.1^{e}	61.86 ± 1.2^{e}	24.93 ± 1.3^{d}	37.38 ± 0.8^{c}
T3	90.7 ± 1.0^{d}	61.2 ± 1.3^{d}	70.18 ± 1.3^{d}	119.5 ± 0.9^{d}	75.65 ± 1.1^{d}	102.05 ± 1.2^{d}	27.43 ± 0.6^{d}	21.78 ± 1.3^{c}	26.59 ± 1.1^{b}
T4	66.3 ± 0.7^{c}	49.8 ± 0.8^{c}	56.1 ± 1.2^{c}	70.8 ± 0.9^{c}	52.23 ± 0.8^{c}	82.05 ± 1.3^{c}	20.73 ± 1.3^{c}	11.6 ± 0.7^{b}	50.78 ± 0.9^{d}
T5	37.5 ± 1.7^{b}	26.4 ± 0.9^{b}	33.9 ± 1.1^{b}	58.1 ± 0.8^{b}	46.33 ± 0.8^{b}	50.45 ± 0.9^{b}	11.59 ± 1.2^{b}	9.49 ± 0.7^{b}	27.93 ± 1.3^{b}

$P \leq 0.05$

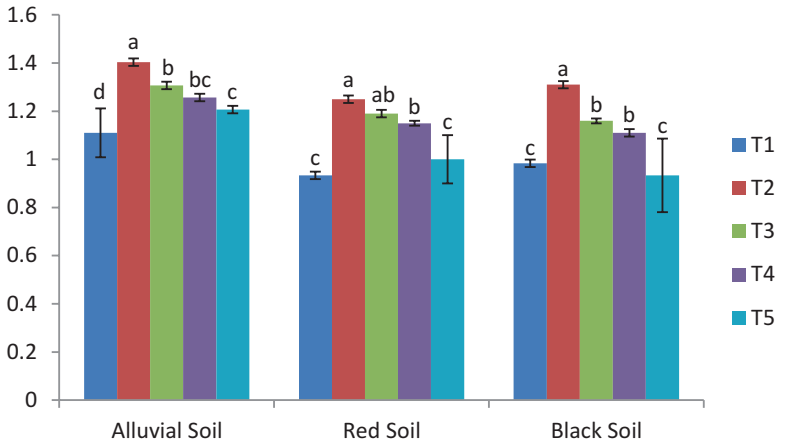

Fig. 16.1 Effect of soil types, seedling bio-priming, and graded N dose application on grain N content

Table 16.5 Effect of soil types, seedling bio-priming, and graded N dose application on agronomic use efficiency and physiological use efficiency

	Agronomic use efficiency			Physiological use efficiency		
	Alluvial soil	Red soil	Black soil	Alluvial soil	Red soil	Black soil
T2	10.61 ± 0.5^{a}	5.57 ± 0.6^{b}	8.54 ± 1.0^{a}	0.37 ± 0.012^{a}	0.25 ± 0.01^{a}	0.33 ± 0.07^{a}
T3	12.73 ± 0.7^{b}	5.62 ± 0.4^{b}	9.87 ± 0.5^{a}	0.56 ± 0.012^{b}	0.31 ± 0.03^{a}	0.45 ± 0.01^{b}
T4	15.20 ± 0.8^{c}	5.45 ± 1.1^{b}	10.48 ± 0.9^{a}	0.65 ± 0.012^{c}	0.42 ± 0.03^{b}	0.50 ± 0.01^{b}
T5	17.99 ± 1.1^{d}	3.75 ± 0.3^{a}	8.62 ± 0.6^{a}	0.71 ± 0.012^{d}	0.41 ± 0.05^{b}	0.51 ± 0.05^{b}

16.6 Agronomic Use Efficiency (AUE)

Present study clearly showed that AUE was significantly influenced by soil type and different level of N fertilizer. Higher AUE was observed in plants grown in alluvial soil followed by black soil and red soil (Table 16.5). Highest AUE in alluvial soil was recorded under T5 (17.99) followed by black soil (15.20) and red soil (12.73), respectively. It may be due to the combined application of graded N fertilizer and *T. harzianum* (Meena et al. 2016; Martinez-Medina et al. 2011; Tripathi et al. 2013).

16.7 Physiological Use Efficiency (PUE)

Pot with alluvial soil showed maximum PUE (0.57 mg g^{-1}) followed by black soil (0.44 mg g^{-1}) and red soil (0.34 mg g^{-1}) of rice plant in comparison to control. PUE of rice plants ranged from 0.37 to 0.71, 0.25–0.41, and 0.33–0.51 in alluvial, red, and black soil, respectively. Data showed in Table 16.5 indicated that in alluvial soil plant under T5 showed maximum PUE (0.71) followed by T4 (0.65) and T5 (0.56). Same pattern was followed in different soil type. Higher PUE under alluvial soil may

be due to the better growth condition provided by soil and proliferation of microbes with different doses of fertilizers (Meena et al. 2016). Higher value of PUE indicates nutrient sufficiency with reference to P followed by optimal plant growth and stress mitigation influenced by various combinations of reccomended dosage of fertilizer and *T. harzianum* (Murphy et al. 2013).

16.8 Conclusions

Present study was conducted to evaluate the effect of different soil type and combined application of graded N fertilizer and seedling bio-priming with *T. harzianum* on nutrient use efficiency and plant growth promotion. Data presented in this study indicated that the application of *T. harzianum* positively influences the growth promotion, nutrient uptake, and nutrient use efficiency in rice plant under different soil conditions. One hundred percent RDF of NPK was the most effective treatment for rice plants in all soil types. Seedling bio-priming with *T. harzianum* + 3/4th N and RDF of P&K treatment was found to be most effective in comparison to control and other combined graded N and *T. harzianum* application.

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