Mirza Hasanuzzaman
Vasileios Fotopoulos *Editors*

Priming and Pretreatment of Seeds and Seedlings

Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants

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Preface

Climate change-related environmental conditions, such as drought, salinity, and heat, are among the most devastating abiotic stresses for crop yield. Their impacts are further exacerbated due to anthropogenic contribution, thus generating a major threat to global food security. Simultaneously, agriculture is one of the top water-consuming sectors globally (69% of the water withdrawal), and agricultural intensification is expected to increase the demand for water worldwide. Drought, salt, and heat tolerance improvement in major crops is a valuable tool to reduce crop yield losses, to increase agricultural water use efficiency, and to cultivate areas that were unable to be cultivated due to low precipitation and/or salt accumulation.

Different methodologies have been employed for enhancing climate change-related stress tolerance in plants and therefore increase crop water use efficiency; some are particularly time-consuming (e.g., conventional breeding), and others are currently unacceptable in many countries around the world (e.g., plant genetic modification). Priming is a rapidly emerging field in plant stress physiology and crop stress management. Plants treated with certain natural or synthetic compounds (i.e., chemical agents) and/or biological agents (such as PGPB and AMF) prior stress events show enhanced tolerance when exposed to sub-optimal abiotic conditions. There are different kinds of priming, like hydropriming, halopriming, magnetopriming, and chemical priming, which have become very popular in the last couple of decades. Recently, seedling pretreatments with a low dose of antioxidants, trace elements, amino acids, phytohormones, and other signaling molecules were found to provide enhance stress tolerance under stressful conditions. Stress impacts on plant growth and yield in primed plants are remarkably reduced in comparison with non-primed plants. However, further research is needed to better understand how plants better adapt to multiple environmental constraints after priming and establish stress management practices based on biological and chemical priming, while very few studies were dedicated on seed priming against combined abiotic stresses to date.

The editors and contributing authors hope that this book will provide an up-to-date view on the exciting field of seed/plant priming and lead to new discussions and efforts to further develop this multifaceted technology. Therefore, the present volume would be a valuable source of scientific information to advanced students, early-stage researchers, faculty, and scientists involved in agriculture, plant sciences, biotechnology, plant breeding, and related areas.

We, the editors, would like to give special thanks to the authors for their outstanding and timely work in producing such fine chapters. We are highly thankful to Ms. Mei Hann Lee, editor, *Life Sciences*, Springer, Japan, for her prompt responses during the acquisition. We are also thankful to Selvakumar Rajendran, project coordinator of this book, and all other editorial staff for their precious help in formatting and incorporating editorial changes in the manuscripts. Special thanks to Dr. Md. Mahabub Alam, Mr. Abdul Awal Chowdhury Masud, and Khursheda Parvin of Sher-e-Bangla Agricultural University, Bangladesh, for their generous help in formatting the manuscripts. The editors and contributing authors hope that this book will include a practical update on our knowledge for the role of plant nutrients in abiotic stress tolerance.

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Vasileios Fotopoulos is associate professor in Structural and Functional Plant Biology and head of the CUT Plant Stress Physiology Group established in 2008. His main scientific research focuses on the study of nitro-oxidative signaling cascades involved in the plant's response to stress factors, while emphasis is being given in the development of chemical treatment technologies towards the amelioration of abiotic stress factors and promotion of plant growth. To date, he is the author of 62 scientific papers published in peer-reviewed journals (h-index=23, Source: Scopus), as well as 7 book chapters. He currently serves as associate editor in *BMC Plant Biology*, *Gene*, and five other journals. He has also been assigned to evaluate competitive research proposals from different countries (France, Belgium, Poland, Chile, Latvia, Greece, Italy, Portugal, Israel, Qatar, Austria, Cyprus, Denmark), EU proposals (EUROSTARS), while he is an active review panel member for COST Actions (EU) in the field of "Natural Sciences." Finally, he has acted as examiner of MSc theses/PhD dissertations from Italy, South Africa, and the Netherlands.

Methods of Seed Priming

Ahmad Sher, Taskeen Sarwar, Ahmad Nawaz, Muhammad Ijaz, Abdul Sattar, and Shakeel Ahmad

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Abstract The food security of world population depends on our limited agricultural land and the reproductive capacity of field crops. Hence, plant scientist are putting their efforts to increase the crop yield by using the existing resources. Seed priming is a very promising, efficient, and low-cost approach to increase the germination, the growth, as well as the productive capability of crops. Water, inorganic salts, sugars, solid medium with water and nutrients, beneficial microbes, micronutrients, hormones, rhizobacteria, and organic sources are used as priming agents for seeds. Seed priming is not only used for improving the plant growth and yield but it also increases the abiotic stress tolerance. This chapter will focus on classification of seed priming by using different priming agents.

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Keywords Seed treatment · Hydropriming · Halopriming · Pre-treatment · Seedling vigor

1 Introduction

Seed priming is the control hydration of seeds in water or a solution of low osmotic potential to initiate the germination metabolism without radical protrusion. Many studies have reported that seed priming improves the stand establishment and productivity of field crops (Farooq et al. 2005, 2006a, b, c, d, 2007, 2008a, b, 2010). There are several types of seed priming including (i) hydropriming, (ii) halopriming, (iii) osmopriming, (iv) solid matrix priming, (v) biopriming, (vi) nutripriming, and (vii) seed priming with hormones, plant growth regulators, and other organic sources. Each of this seed priming technique has been discussed in detail below.

2 Hydropriming

In hydropriming, the seeds are simply soaked in water prior to sowing for a defined period of time depending upon the radical protrusion time of each plant species. Aeration may or may not be provided to the seeds. It is a very simple, low-cost technology as simple water is used in this priming technique. Hydropriming is followed by surface drying or redrying of seeds to their original weight. Many studies have reported that hydropriming may improve the stand establishment, seedling vigor, and productivity of field crops under optimal and suboptimal conditions. For example, Roy and Srivastava (1999) found that hydropriming of wheat seeds improved the seed germination in a saline soil. In another study, seed emergence and plant growth at vegetative and reproductive stages were improved in maize due to hydropriming (Nagar et al. 1998). Many studies have reported that hydropriming of seeds improved the seed germination and seedling emergence of rice (Basra et al. 2005; Farooq et al. 2006d), maize (Mohammadi et al. 2008; Dezfuli et al. 2008), wheat (Harris et al. 2001; Afzal et al. 2007; Nawaz et al. 2017), safflower (Bastia et al. 1999), chickpea (Harris et al. 1999; Kaur et al. 2002; Kamithi et al. 2016), lentil (Ghassemi et al. 2008), safflower (Ashrafi and Razmjoo 2010), mountain rye (Ansari and Zadeh 2012), and pearl millet (Kumar et al. 2002). In conclusion, hydropriming improves seed germination, seedling emergence, and productivity of field crops.

3 Halopriming

Halopriming involves the soaking of seeds in the aerated solutions of inorganic salts (potassium nitrate, sodium chloride, calcium sulfate, and calcium chloride) of variable concentration. Various studies have reported that halopriming improved the stand establishment, seedling growth, and productivity of diverse crop species under optimal and suboptimal conditions. For example, Khan et al. (2009) found a significant improvement in the stand establishment and seedling growth of hot pepper due to seed priming with sodium chloride under saline soil conditions. In another study, the priming of wheat seeds with sodium chloride and potassium chloride was useful for improvement in its performance in saline soil (Iqbal et al. 2006). Under salt stress, priming of sorghum seeds with potassium nitrate or calcium chloride improved the activity of amylase and proteases (Kadiri and Hussaini 1999) and increased the soluble sugars, free amino acids, and proteins in pigeon pea (Jyotsna and Srivastava 1998). In another study, Sedghi et al. (2010) found that seed priming with sodium chloride or potassium nitrate improved the stand establishment and seedling growth of sunflower. Improvement in α-amylase and dehydrogenase activities due to seed priming with potassium nitrate solution in muskmelon has also been reported (Singh et al. 1999). The priming of wheat seeds with calcium chloride improved the performance of wheat under saline stress (Roy and Srivastava 1999). Likewise, seed priming of millet and sorghum with calcium chloride and potassium nitrate improved the activity of α-amylase and proteases under salt stress (Kadiriand Hussaini 1999). Chang-Zheng et al. (2002) also reported that seed priming with mixed salt solution improved the seed germination and activities of α- and p-amylase and root dehydrogenase in rice under salt stress. In conclusion, halopriming improves the stand establishment, germination metabolism, and productivity of many crops under optimal and suboptimal conditions.

4 Osmopriming

In osmopriming, the seeds are soaked in aerated solution of sugars (sorbitol, mannitol, etc.) or polyethylene glycol (PEG), followed by surface drying or redrying to their original weight. It is also known as osmotic priming or osmo-conditioning. Many studies have reported that osmopriming improves the stand establishment and seedling/crop growth under optimal and suboptimal conditions. For example, osmopriming (20% PEG-8000) of ryegrass and sorghum seeds improved the germination rate of both crops under suboptimal conditions (Hur 1991). Salehzade et al. (2009) also found that osmopriming (with PEG-8000) of wheat seeds improved the stand establishment. In another study, the osmopriming (with PEG-As) enhanced the ATPase activity in peanut with substantial improvement in RNA syntheses and activity of acid phosphatase in the cotyledon and embryonic axis (Nawaz et al. 2013). In tomato, osmopriming (with PEG 6000) improved the seedling emergence, seedling vigor, stay green, and leaf growth as compared to unprimed seeds (Pradhan et al. 2015). In rice under salt stress, seed priming with mannitol (1, 2, and 3%) improved the seedling dry weight, the membrane stability, and stay green with a simultaneous reduction in the Na^{+}/K^{+} (Theerakulpisut et al. 2017). Improvement in stand establishment of sorghum and peanut owing to mannitol seed priming has also been reported (Safiatou 2012). Many other studies have reported that seed priming with PEG improved germination in bell pepper (Thakur et al. 1997), soybean (Gongping et al. 2000; Sadeghi et al. 2011), tomato (Nagarajan and Pandita 2001), muskmelon (Nascimento 2003), bitter gourd (Pandita and Nagarajan 2004; Thirusenduraselvi and Jerlin 2009), cauliflower (Pallavi et al. 2006), safflower (Nabizadeh et al. 2012), barley (Shukla et al. 2016), and wheat (Azadi et al. 2013). In conclusion, osmopriming improves the germination metabolism in field and horticultural crops under a diverse array of environmental conditions.

5 Solid Matrix Priming

Matrix priming, also known as the solid matric conditioning, is accomplished with the controlled and limited hydration, as in hydropriming and osmopriming. However, the matrix priming utilizes the solid medium (matrix which delivers water and nutrients to seed prior to emergence of radical including vermiculite and diatomaceous and water-absorbent polymer) for seed priming purpose (Taylor and Harman 1990). These solid matrix materials have low bulk density and low osmotic potential and high water-holding capacity. Khan (1992) recommended the use of matrix priming due to the good water-holding capacity of the solid matrix and its ease of removal from the seed. The matrix priming has the ability to provide a good amount of oxygen to the seed during the process of hydration. In a study, the germination metabolism was improved by the matrix priming in *Helichrysum bracteatum* L. (Grzesik and Nowak 1998). Ilyas et al. (2002) found that matrix conditioning of hot pepper seeds improved the protein level and fruit quality. In another study on pepper and tomato by Kubik et al. (1988), it was found that solid matrix priming was much useful for improvement in germination. In conclusion, solid matrix priming might be a good option to boost up the seed germination in different crop species, especially the horticultural crops.

6 Biopriming

It is a newly emerged technique of seed treatment which integrates the physiological (seed hydration) and biological (seed inoculation with the beneficial organisms) mechanisms (Reddy 2012; Rakshit et al. 2015). Biopriming has been recently used as an alternate method of managing the soil- and seed-borne pathogens. Callan et al. (1990) first introduced the procedure of biopriming. Like other seed priming techniques, radical protrusion in not allowed in the biopriming. The exudates released from the seed may serve as source of energy and nutrients to the biocontrol agents during biopriming (Wright et al. 2003), thus facilitating the proliferation and the colonization of these biocontrol agents over the surface of seeds which facilitate the nutrient/water uptake. According to Rakshit et al. (2015), the biopriming with beneficial microbes offers an innovative crop protection toll by improving the seed quality, seedling vigor, and plant ability to withstand the suboptimal growth conditions, thus ensuring the sustainable crop production. Mahmood et al. (2016) reviewed that biopriming speeds up germination, ensures the uniformity of seedling emergence, and thus improves the crop yields and quality. Recently, the nutrient-solubilizing bacteria have been used as seed priming agent along with the nutrient media to enhance the nutrient uptake in crops. For example, Rehman et al. (2018a, b) found that *Pseudomonas*-aided zinc application or Zn seed priming with endophytic bacteria improved the productivity and biofortification of bread wheat. In conclusion, biopriming with beneficial microbes may be useful to combat the disease problem and improve the bioavailability of the micronutrients to the crops.

7 Nutripriming

Recently, the priming of seeds in different micronutrient soltutions is getting momentum in order to improve the micronutrient availability into plants and their final assimilation in the seed (biofortification) to reduce the malnutrition. Many studies have reported that seed priming with zinc, boron (B), and magnesium at pre-optimized rates improved the performance of different field crops owing to an improvement in seed germination, growth, and yield parameters. For example, Rehman (2012) conducted a field study to evaluate the effect of seed priming with boron in increasing the chickpea productivity. Seeds of two chickpea genotypes Punjab-2008 and T-3009 were soaked in aerated solutions of different B concentrations viz. 0.1, 0.01, 0.001% B. Seed priming in 0.001% B solution improved the stand establishment and uniformity. Seed priming in 0.001% B solution and hydropriming improved the leaf emergence, leaf elongation, plant height, leaf area index, crop and leaf growth rate, reduced time to flowering, caused early maturity, improved grain filling rate and duration, and grain enrichment with boron. In another study, boron nutripriming improved the germination and early seedling growth of rice in Punjab, Pakistan (Farooq et al. 2011). Various other studies have reported an improvement in the stand establishment and productivity of field crops due to nutripriming (Ullah et al. 2017; Iqbal et al. 2017; Rehman et al. 2015, 2018a, b; Farooq et al. 2018). In conclusion, nutripriming improves stand establishment, growth, productivity, and grain biofortification of field crops.

8 Seed Priming with Plant Growth Regulators, Hormones, and Other Organic Sources

Seed priming with hormones, plant growth-promoting rhizobacteria, and other organics sources has been reported to improve the stand establishment, growth, and productivity of field and horticultural crops under optimal and suboptimal conditions. For example, improvement in seed germination in the *Podophyllum hexandrum* (Nautiyal et al. 1987; Nadeem et al. 2000; Thakur et al. 2010), onion (Nalini et al. 2001), okra (Singh et al. 2004), bell pepper (Yogananda et al. 2004), brinjal (Kumar 2005), sunflower (Singh et al. 2006), and sesame (Kyuak et al. 1995) has been reported due to seed priming with gibberellic acid. In a study, Zhang et al. (2007) found that seed priming of lucerne seeds with brassinolide at an optimized concentration increased the seed germination and seedling growth under salt stress.

Seed priming with hormones to improve the crop performance under stressful environments has also attained greater attention in recent past (Sneideris et al. 2015). The hormonal priming generally consists of priming of seeds with different chemicals including hydrochloric acid, hypochlorite and other natural substances. In a study, the seed priming with ascorbic acid and salicylic acid (500 ppm each) improved the seedling growth and seedling dry weight under saline and non-saline conditions (Afzal et al. 2006). In pepper, Khan et al. (2009) found that seed priming with salicylic acid and acetyl salicylic acid improved the uniformity of seedling emergence under saline conditions. In addition to these chemicals, seed priming with ethylene has been reported to improve the seed germination of lettuce under high temperature stress (Nascimento et al. 2004). Afzal et al. (2005, 2006) also reported that hormonal priming in spring wheat with ascorbic acid, salicylic acid, and abscisic acid was useful for alleviation of salinity stress. Basra et al. (2006) found that seed priming in aerated solutions of either, ascorbate or salicylic acid (at concentrations of 10 and 20 ppm for 48 h) improved the stand establishment and seedling dry weight in rice. In conclusion, seed priming with plant growth regulators, hormones, and other organic sources is a viable option to improve the seed germination, growth, and productivity of crops.

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Advances in the Concept and Methods of Seed Priming

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Abstract The critical stages during the growth of crops are the uniform seed germination, early seedling growth, and uniform plant stand. Low crop yield is attributed to uneven seed germination and seedling growth. Therefore, the quality of seed can be improved through priming in addition to the field management techniques for better seed germination. Priming is a physiological technique of seed hydration and drying to enhance the pregerminative metabolic process for rapid germination, seedling growth, and final yield under normal as well as stressed conditions. The primed seeds show faster and uniform seed germination due to different enzyme activation, metabolic activities, biochemical process of cell repair, protein synthesis, and improvement of the antioxidant defense system as compared to unprimed seeds. There are many techniques of seed priming which are broadly divided into conventional methods (hydro-priming, osmo-priming, nutrient priming, chemical priming, bio-priming, and priming with plant growth regulators) and advanced methods (nano-priming and priming with physical agents). However, priming is strongly affected by various factors such as temperature, aeration, light, priming duration, and seed characteristics. This chapter highlights the priming mechanism and the available technologies as a tool for superficial seed germination and crop stand. An experiment with reference to the importance of priming toward vigor seed germination and seedling growth was conducted, and its results have been added in this chapter.

Keywords Seed priming · Germination · Antioxidant defense system · Metabolic activities · Crop growth

1 Introduction

Seed treatment before sowing is the foundation for activation of seed resources that in combination with external ingredients could contribute to the efficient plant growth and high yield. Various physiological and non-physiological techniques are available for enhancing seed performance as well as to combat environmental constraints. The physiological treatments for improving seed germination and stand