Seed Bio-priming for Biotic and Abiotic Stress Management

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Abstract

In modern agriculture, advance technologies are being deployed for breaking yield barriers and enhancing crop productivity. Devising varied seed enhancement technologies is an important domain assuring uniform field emergence, better crop stand and realisation of higher yield in different crops. Integration of diverse plant extracts, microbial products and biotic agents through bio-priming for managing seed crop targeting against biotic and abiotic stresses has been considered as a unique approach, as it requires lesser amounts of chemicals, enhances efficacy of the seeds, reduces the cost of management and eliminates pollution hazards while causing minimum interference with biological equilibrium. Seed bio-priming is one of the vital seed enhancement tool in management of biotic as well as abiotic stresses and guarantees uniform stand establishment under stress conditions. Therefore, research programmes encompassing identification and genetic manipulations of novel biocontrol agents (fungal and bacterial strains) along with its commercial application needs to be devised.

Keywords

Bio-priming • Rhizosphere • Induced systemic resistance (ISR) • Systemic acquired resistance (SAR)

12.1 Introduction

Seed is a growth driver of agriculture and efficacy of all other agricultural inputs, viz. irrigation, fertilisers and plant protectants, and human labour revolves around the use of quality seed. Seed is a tool for delivery of improved technolo-

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gies and is a mirror for portrayal of inherent genetic potential of a variety/hybrid. Seed offers to integrate production, protection and quality enhancement technologies through a single entity, in a cost-effective way. Seed can play a pivotal role in achieving higher productivity; the use of quality seeds alone could increase productivity by 15–20 % which highlights the important role of seed in agriculture.

In modern agriculture, advance technologies are being deployed for breaking yield barriers and enhancing crop productivity. Devising varied seed enhancement technologies is an important domain assuring uniform field emergence, better crop stand and realisation of higher yield in various crops. The quality of seed can be enhanced by different methods, viz. physical, mechanical, chemical and physiological seed treatments. Seed enhancements may be defined as "postharvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at the time of sowing". Seed priming is a technique of controlled hydration (soaking in water) and drying that result in more rapid germination when the seeds are reimbibed. There are different methods of priming like hydropriming, halopriming, thermopriming, bio-priming, etc. Numerous invigoration protocols as well as seed coating and pelleting technologies are used for enhancing planting value and storability of high value and poor storer seeds. Seed quality enhancement through second-generation drying, packing and quality enhancement technologies, viz. intelligent coating molecules, time and target-oriented seed additives, electron treatment, magnetic treatment, plasma coating and its commercial application holds the promise to deliver seeds with high vigour and better adaptability to biotic and abiotic stress. Use of third-generation seed quality augmentation strategies viz., nanotechnology for external as well as internal designing has unlocked new avenues in precision agriculture. Different types of seed enhancement technologies are being developed and deployed for seed invigoration and biotic as well as abiotic stress management.

12.2 Seed Enhancement Technologies

Any postharvest treatment that improves germination/seedling emergence or facilitates the development of more number of normal, rapid, uniform and healthy seedlings in the field condition is termed as seed enhancement. Various environmental factors can be circumvented by using seed enhancement techniques, viz. seed invigoration (priming), coating and pelleting.

12.2.1 Seed Invigoration or Priming

Seed invigoration or priming is a treatment, in which seeds are soaked in an osmotic solution/ other solutions containing different active ingredients, that allows water imbibitions and permits early stages of germination but does not permit radical protrusion through the seed coat.

12.2.2 Osmopriming

Soaking the seed in osmotic solutions is osmopriming. Water is either made freely available to the seed (as in steeping or soaking) or restricted to a pre determined moisture contents, typically using water potential between – 0.5 Mpa and −2.0 Mpa. Several osmotica like inorganic salts such as potassium nitrate, potassium phosphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulphate, magnesium chloride, calcium chloride, sodium chloride, sodium nitrate, sodium polypropionate, sodium sulphate, chemically inert compounds such as PEG 6000, PEG 8000 and mannitol are used. Details of different osmoticum used for priming in vegetable seeds are given in Table 12.1.

12.2.3 Solid Matrix Priming

Pre-sowing hydration in a solid-based medium is called solid-based matrix priming, and it is used for increasing the efficiency of fungicide/insecticide to control the seed-borne infection and soil

Table 12.1 Effect of osmotic seed priming in different crop species

Crops	Osmoticum	Results	References
Cabbage	PEG 305 g/kg seed 15 °C for 14 days	Accelerated emergence in heat-damaged seed	Ralph (1978)
Carrot	PEG 273 g/kg seed 15 °C for 14 days	Accelerated germination, field emergence and increased plant fresh weight	Broklehurst and Dearman (1983)

Table 12.2 Polymer film coating with reference to storage potential of seed

Crop	Finding	Reference
Turnip, carrot and cabbage	Coating seed with polyvinyl resin didn't decrease germination consistently after 18 months from storage	Sauve and Shiel (1980)
Tomato	Seed treated with Vitavax Power at 2 g + polymer coating at 20 ml per kg of seeds enhanced seed quality attributes and storability	Harish et al. (2014)

insects. In solid matrix priming seed slowly imbibes to reach an equilibrium hydration level, determined by the reduced matrix potential of the water adsorbed on the particle surfaces.

12.2.4 Seed Hardening

It is a process of soaking seeds in water for a precise period followed by drying, re-soaking and re-drying. This process of alternate hydration and dehydration cycles with water and later drying to original moisture is called seed hardening.

12.2.5 Seed Coating

Seed coating in broad sense includes seed film coating, seed colouring and seed pelleting. Details of the use of different chemicals for seed coating are given in Table 12.2.

12.2.6 Seed Pelleting

Seed pelleting is the mechanism of applying needed materials in such a way that they influence the seed or soil and the seed-soil interface. Pelleting is defined as the application of a layer of inert material that may obscure the original shape and size of the seed resulting in significant weight increase and improved palatability. These treatments are used to facilitate easy handling, precision placement and incorporation of beneficial microorganism. Seed pelleting is usually practised in seeds which are light in weight and irregular in shape. The largest commercial use of pelleting is for monger sugar beet, carrot, onion, lettuce, tomato and flower spp.

12.3 Bio-priming

Bio-priming is a process of biological seed treatment that refers to a combination of seed hydration and inoculation of the seeds with beneficial microorganisms. It improves seed viability, germination, vigour indices, plant growth and subsequent protection against diseases and finally enhances crop yield. In most of the cases microbial inoculants such as plant growth-promoting rhizo-microorganisms (bacteria or fungi) are used for the purpose of bio-priming of seeds. It is an environmentally sound ecological approach using selected microorganisms which enhance plant growth by producing plant growth-promoting substances or enhancing nutrient uptake or by protecting seedling/plants against soil-/seed-borne plant pathogenic organisms. In present-day agriculture, the biological seed treatment methods using microbial inoculants are providing an alternative to the chemical treatment methods (use of pesticides and/or plant growth-promoting nutrients), being eco-friendly and safer for future agriculture and gaining importance in the seed, plant and soil health improvement programmes.

Crop productivity in India suffers heavy loss due to diseases under field and storage conditions, and a majority of them are seed and soil borne in nature. Chemicals are being used so far to treat the seeds which are not effective under field conditions due to various soil and environmental factors. Moreover, chemicals used for seed treatment mostly act as contact fungicides which are unable to protect the plants from foliar pathogens during the later stages of crop growth. Seed bio-priming is a suitable alternative to seed treatment because the microbes multiply continuously, occupy the growing root surfaces, form a biofilm around the roots and protect the plants from soil-borne plant pathogens throughout the crop-growing stages. Other advantages using microbial bio-priming are the elicitation of systemic resistance in plants that can protect the plants from foliar pathogens during the later stages of their growth and development. This further strengthens the concept of popularising seed bio-priming technique among the farmers which will not only ensure seed and crop health but also help in ensuring ecological sustainability. Alternatively, seed bio-priming can also enhance seed's nutritional and physiological characteristics and result in better germination and adaptation under different soil conditions and when entwined with useful microbial agents associated with plant roots can augment plant productivity and immunity. However, recent work by several groups showed that such microorganisms also elicit so-called induced systemic tolerance (IST) against biotic and abiotic stresses.

Most of these microorganisms increase nutrient uptake from soils, thus reducing the need for fertilisers and preventing the accumulation of nitrates and phosphates in agricultural soils. A reduction in fertiliser use would lessen the effects of water contamination from fertiliser run-off and lead to savings for farmers in addition to impart drought tolerance capacity to plants. The investigators of the present project have been engaged with the isolation, trait characterisation and effective utilisation of several groups of microorganisms that are capable of promoting plant growth and suppressing various seed- and soil-borne diseases as well as foliar disease through induced systemic resistance mechanisms and can withstand high temperature, pH and salt concentrations.

Integration of plant extracts, microbial products and biotic agents along with bio-priming agents for managing plant growth and diseases has been considered as a novel approach as it requires low amounts of chemicals, enhances efficacy of the seeds, reduces the cost of control and eliminates pollution hazards while causing minimum interference with biological equilibrium. The use of bioagents, microbial metabolites or botanicals with priming agents has become an inevitable method of disease control, particularly in the absence of resistant cultivars.

12.3.1 Methodology

The method commonly recommended for bio-priming is to soak the seeds in water for 12 h. Selected formulated product of the microorganism is added to pre-soaked seeds at the rate of 10 g/kg of seed and mixed well. The treated seeds can be taken in polythene bags, heaped and covered with moist jute sack to maintain high humidity and maintained for 48 h at approximately 25–32 °C. During this period, the bioagent adhering to the seed grows on the seed surface to form a protective layer all around the seed coat. These bio-primed seeds can be sown in the nursery bed. Some studies have shown that bio-primed seeds can be safely stored up to 2 months. The microorganisms that have been commonly studied for this purpose include *Bacillus polymyxa*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, *T. viride and Gliocladium* sp. These studies have clearly brought out that bio-primed seeds enhance *percent* germination, seed vigour, plant growth, yield and protection against seed- and soil-borne pathogens in crops like rice, sunflower, rape and several vegetable crops like carrot, radish, etc. Some studies revealed that bio-priming with more than one organism like *Trichoderma harzianum* with *Pseudomonas fluorescens* is more effective in enhancing plant growth compared to bio-priming with single organism. Similarly some workers have brought out that

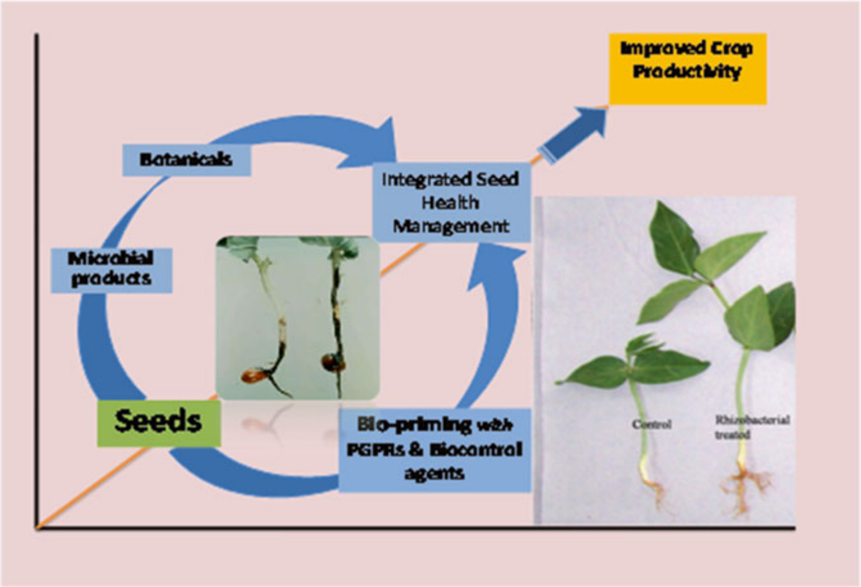

Fig. 12.1 Improving the crop productivity by the application of bio-priming agents

bio-priming along with osmopriming (with NaCl) is more effective in improving seed invigoration and seedling growth. Bio-priming process has potential advantages over simple seed coating with bioagents and results in more rapid and uniform seedling emergence even under adverse soil conditions (Fig. 12.1).

12.3.2 Signal Pathways of Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR)

Biocontrol agents, particularly rhizobacteria, have been shown to be effective in suppressing disease infection by inducing a resistance mechanism called “induced systemic resistance” (ISR) in varied crops (Van Loon et al. 1998). Induced resistance is defined as stimulation of plants with enhanced defensive ability of plants against different pathogens. Van Peer et al. (1991) showed that inoculation of *Pseudomonas fluorescens* strain WCS417r in the stem of carnation resulted in low infection of Fusarium wilt. This low level of Fusarium wilt was attributed to the induced resistance and deposition of phytoalexins in the stem region of carnation plant. Similarly, Wei et al. (1991) demonstrated that seed treatment with PGPR strains in cucumber resulted in reduction of anthracnose disease and further suggested that application of PGPR strains to seeds triggered induced systemic resistance, protecting leaves of cucumber plants against anthracnose disease caused by *Colletotrichum orbiculare*.

Beneduzi et al. (2012) stated that rhizobacteria-regulated induced systemic resistance and plant pathogen-induced systemic acquired resistance are almost regulated through same signal transduction pathway (Fig. 12.2). In case of ISR, jasmonic acid (JA) and ethylene (ET) responsive pathways are involved in defensive response of plants, whereas in case of SAR, salicylic acid pathway is vital to activate defence mechanism against pathogens. Both types of mechanisms are effective in ensuring protection to plants from various plant pathogens *viz*., fungus, bacteria, nematodes and insects.

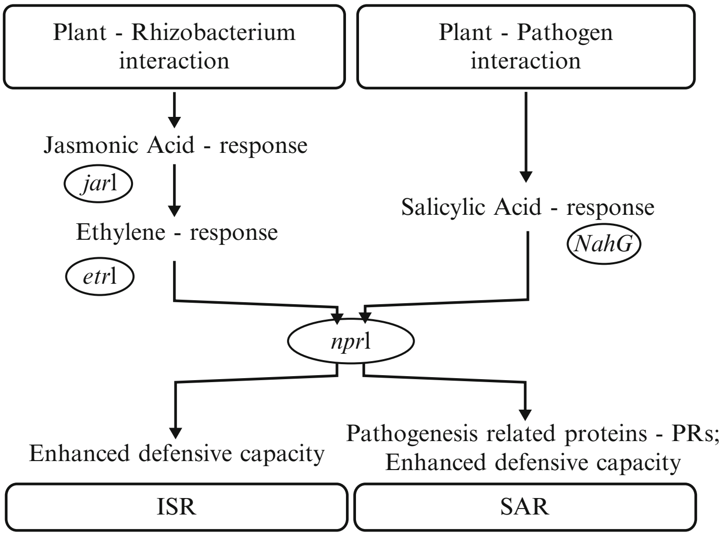

Fig. 12.2 Signal transduction pathways leading to pathogen-induced systemic acquired resistance (SAR) and rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis thaliana* (Source: Beneduzi et al. 2012)

12.4 Seed Invigoration Using Bio-priming

Seed germination, seedling emergence and crop establishment are important aspects of agricultural and horticultural production and are important components of seed/seedling vigour. Seedling vigour is critical when competition for light, nutrients, air and water becomes severe. Seedlings with a vigorous growth pattern can compete successfully under stress, influencing stand establishment and ultimately grain yield. The role of seed vigour comes in light when seeds are sown in adverse conditions, and the vigour of a seed becomes a deciding factor for the crop establishment and yield compared to normal conditions of plant growth.

Infestation by pathogens in seeds could adversely affect the ability of seed to germinate normally, resulting in loss of seed vigour (McDonald and Copeland 1997). Seed germination of Jasmine 85 rice affected by discoloration resulted in decreased number of filled grains/panicle and test weight (Phat et al. 2005). Hamman et al. (2002) concluded that high- and medium-vigour seed lots of soybean always showed higher final emergence (FE) and plant establishment. It was also observed that seedling with low vigour could not withstand stressful conditions, viz. deep planting and pathogen-infested soils during growth, and failed to attain final emergence.

Entesari et al. (2013) investigated efficacy of seed bio-priming treatment with fungal biocontrol agents, viz. *Trichoderma harzianum* (T. AS 19-2, T. bp4, T. BS1-1), *T. virens* (T.As19-1, T.As17-4, T.As10-5), *T. atroviride* (T.As18-5, T.cs5-1, T.Cs2-1) and a bacterium, *Pseudomonas fluorescens* (utpf5) on soybean seed. *Trichoderma harzianum* strain BS1(Th.4) showed positive correlation with soybean growth factors and resulted in enhanced shoot and root length, seedling dry weight and total chlorophyll content as compared to control.

12.4.1 Alleviation of Biotic Stress through Bio-priming

Different fungi isolated from the rhizosphere of various plants capable as biocontrol agents are given in Table 12.3; however, most of the research work is carried out to test efficacy of *Trichoderma* sp. in controlling plant pathogenic fungi. Major problem for commercial application of this biocontrol is its multiplication, formulation and suitable delivery method at end user. Bio-priming is an effective tool for delivery of biocontrol agents, and priming (bio-priming) is seen as an ideal delivery method for inducing resistance, which amplifies the efficiency of rhizobacteria-induced resistance in plants.

Table 12.3 Fungi isolated from the rhizosphere of various plants capable as biocontrol agents

Alternaria sp.	*Epicocuum* sp.	*Paecilomyces* sp.
Aspergillus sp.	*Fusarium* sp.	*Penicillium* sp.
Cephalosporium sp.	*Gliocladium* sp.	*Rhizopus* sp.
Chaetomium sp.	*Humicola* sp.	Sterile mycelia
Cladosporium sp.	*Mortierella* sp.	*Talaromyces* sp.
Coniothyrium sp.	*Mucor* sp.	*Trichoderma* sp.
Curvularia sp.	Mycorrhizal fungi	*Verticillium* sp.
Cylindrocarpon sp.	*Myrothecium* sp.	

Maize is one of the important cereal crops grown in India, and Fusarium ear rot is one of the most devastating diseases inflicting both pre- and postharvest losses in maize. Further *Fusarium verticillioides* is capable of producing varied mycotoxins, viz. fumonisin, moniliformin, zearalenone and trichothecene, damaging approximately 20 % of grains in storage. Chandra Nayaka et al. (2008) studied the effect of bio-priming with potential *Trichoderma harzianum* on maize to control Fusarium ear rot disease and fumonisin accumulation in different maize cultivars grown in India. They concluded that the pure culture of *T. harzianum* was more effective in reducing the *F. verticillioides* and fumonisin incidence followed by talc formulation than the carbendazim. Formulations of *T. harzianum* (1X 108 spore/ml and 10 g/kg of seed) were effective at reducing the *F. verticillioides* and fumonisin infection and also increasing the seed germination, vigour index, field emergence, yield and thousand-seed weight in comparison with the control.

In maize plants, sh2 genes are responsible for high sugar content leading to increase in occurrence of damping off disease caused by *Pythium ultimum*. Callan et al. (1990) used an isolate of *Pseudomonas fluorescens* (AB254) with at least 1×10^7 cfu/seed and allowed to imbibe moisture up to 35–40 % under warm conditions and offered better protection than chemical seed treatments.

Niranjan Raj et al. (2004) studied the effect of bio-priming on pearl millet seeds with different isolates of *Pseudomonas fluorescens* and concluded that among different isolates, UOMSAR14 and UOM SAR 80 showed enhanced germination and seedling vigour in pearl millet plants. Further, bacterial isolate UOM SAR 14 elicited resistance against downy mildew disease under greenhouse as well as field conditions.

Seed bio-priming provides numerous advantages over other delivery methods, and it is reported to alleviate physiological and pathological stresses in plants. Bio-priming of corn seeds with root-colonising *Pseudomonas fluorescens* AB254 resulted in better plant establishment in *Pythium ultimum*-infested soil and was almost equivalent with fungicide seed treatment of metalaxyl (Mathre et al. 1999). Jensen et al. (2004) demonstrated that bio-priming of carrot seeds with fungal isolates of *Clonostachys rosea* (IK726) assured better protection against seed-borne pathogens *Alternaria dauci* and *Alternaria radicina* without any antagonistic effects on plant establishment in carrot (Fig. 12.3).

Mapping of disease-free seed production zones and combinations of integrated disease and pest management practices ensures quality seed production in various crops. Modifying seed and soil interface with addition of beneficial microbes may be proved as an important tool in quality seed production of vegetable crops. Pill et al. (2009) concluded that slurry coating of osmotically primed or non-prime seeds with a combination of *Trichoderma harzianum* and *Trichoderma virens* is at least as effective as mefenoxam coating reducing damping off caused by *Pythium aphanidermatum*-infested seedbed of cucumber. Further, seeds coated with Th, Tv or ThTv can be stored for 4 weeks with the *Trichoderma* viability remaining fairly stable at 4 °C and increasing from 3 to 4 weeks at 21 °C.

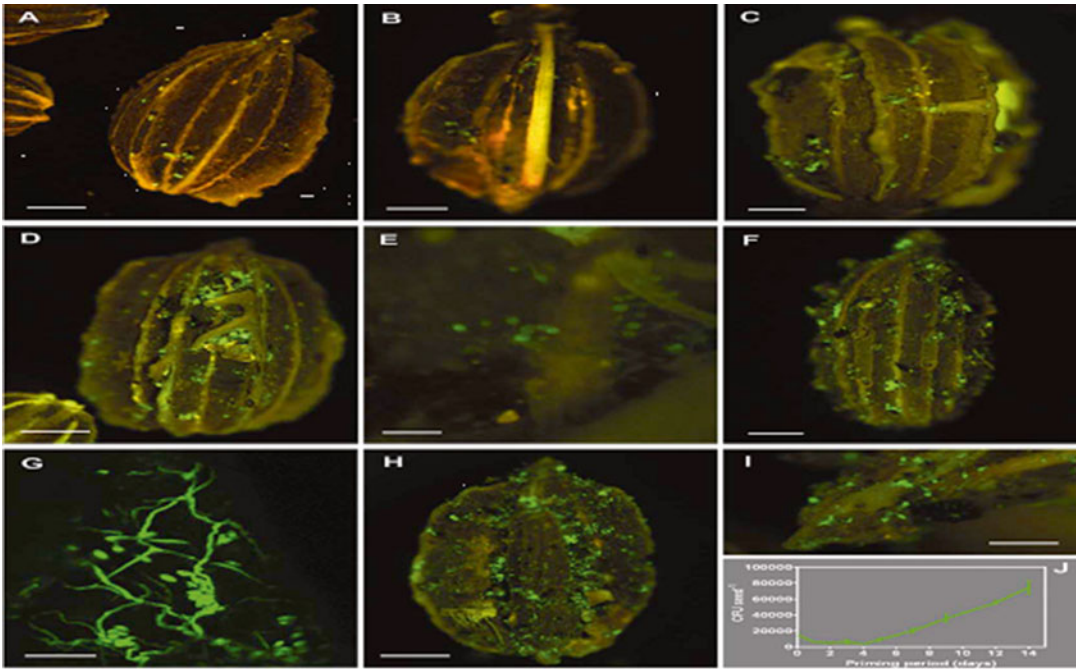

Fig. 12.3 Growth of isolate IK726d11 was visualised with SFM on seeds after a bio-priming period of A, 1 day; B, 2 days; C, 3 days; D, 5 days; F, 7 days; and H, 14 days. E, Sporulation with verticillate conidiophores was observed after 4–5 days. I, Penicillium conidiophores were observed after 6 days. G, With CLSM, a fine web of hyphae was seen on the pericarp at day 7. J, The development in density of *C. rosea* IK726d11 on seeds (CFU/seed) was observed during bio-priming (Source: Jensen et al. 2004)

Today biopesticides with their commercial application are available in India, but biological seed protectant market share is negligible as compared to chemical sales. Progress in biological control with respect to formulation and reliability must be top priority. Commercial formulations available in the market are given in Table 12.4. Key goals of such type of product development invariably should be long shelf life (1–2 years), high density of viable propagules, stability under unfavourable conditions, ease in application and low production cost (Lewis et al. 1991).

12.4.2 Alleviation of Abiotic Stress through Bio-priming

Plants usually face several abiotic stresses that can affect seed quality and yield; these abiotic stresses can decrease germination, vigour and plant stand ultimately affecting the seed yield. In order to optimise the seed crop husbandry, apart from conventional approaches, integration of microbial inoculants in such production systems is gaining importance these days, which is highly efficient and cost-effective. Understanding the complexity of microbial adaptations into stressed rhizosphere and effect of these microorganisms on biological, chemical and physical properties of rhizosphere and plant remains a significant challenge (Yang et al. 2009). At present, significant interest resides in the development and integration of trait-specific microbial inoculants to seed through bio-priming for its enhanced performance in abiotic stress conditions (Nadeem et al. 2007; Yang et al. 2009; Neelam and Meenu 2010). There are satisfactory evidences suggesting that the use of beneficial microbes can enhance plant's resistance to adverse environmental stresses, viz. drought, salt, nutrient deficiency, heavy metal contamination and climate change-induced stresses (Glick et al. 2007).

Table 12.4 Commercial formulations of biocontrol agents available in India

Product	Bioagent	Use
Antagaon-TV	*T. viride*	As seed and soil treatment for control of *Rhizoctonia solani* and *M. phaseolina* in pulses and vegetables
Biocon	*T. viride*	Available in broth and dust used for control of root and stem disease in tea
Bioderma	*T. viride* + *T. harzianum*	Seed treatment against the fungal pathogens in vegetables and pulses
BioGuard	*T. viride*	As seed and soil treatment of seed-borne diseases in vegetables and pulses
Bioshield	*Pseudomonas fluorescens*	As seed, soil and seedling dip against fungal pathogens of cereals and pulses
Biotak	*Bacillus subtilis*	Available in broth formulation and used for the control of black rot disease of tea
Defence-SF	*T. viride*	As seed and soil treatment for control for different diseases in crops

Source: Bhattacharjee and Dey (2014)

12.4.3 Manifestation of Abiotic Stresses in Seed Crop

Stress manifests itself in reduced plant-microbe interaction, water balance and nutrient availability and increased disease incidence and heavy metal toxicity in plant system (Mayak et al. 2004; Egamberdieva and Kucharova 2009). Among the microorganisms many fungal and bacterial strains augmented to seed through bio-priming were found with immense ability to alleviate abiotic stresses by means of various mechanisms thereby enhancing plant growth (Paul and Nair. 2008). Abiotic stresses lead to a series of morphological, physiological, biochemical and molecular changes adversely affecting plant growth and yield (Wang et al. 2001). Various abiotic stress factors like drought, salinity, extreme temperatures and oxidative changes are well connected resulting in cell damage (Wang et al. 2003). High temperature stress results in extensive protein denaturation and aggregation leading to cell death, and low temperature stress weakens metabolic processes by altering the membrane system (Heino and Palva 2003). Heavy metals like Pb, Cu, Hg, etc., were taken up by plant cell and subsequently target enzymes vis-à-vis Cu/Zn-SOD and ethylene receptors and further reduce molecular oxygen leading to formation of reactive oxygen species (ROS) causing extensive cellular damage (Polle and Schützendübel 2003).

12.4.4 Microbial Inoculants for Bio-priming for Alleviation of Abiotic Stresses

The application of beneficial microbes in agricultural production systems started about 60 years ago (Kloepper et al. 1980), and the effect of these microbes was amply addressed in a variety of crops especially in cereals, legumes and oilseeds. Integration of beneficial microorganism into seed crop husbandry through bio-priming for management of biotic and abiotic stresses is gaining enormous importance.

12.4.5 Potential Fungal Bio-inoculants for Bio-priming

Wide range of fungal bioagents through its novel interactions with plant has made it beneficial for alleviating biotic and abiotic stresses. *Trichoderma harzianum* is most widely used for bio-priming for its vast range of antagonism against plant pathogens, mainly fungi and nematode (Singh et al. 2004); increased plant growth especially roots particularly under stress (Harman 2000; Shoresh et al. 2010); systemic resistance to abiotic plant stresses including drought, salt and temperature (Mansouri et al. 2010; Shoresh et al. 2010); decomposition of organic matter thereby increasing humic acid in soil; solubilisation and mobilisation of phosphorus; and increased nitrogen use efficiency and nutrient availability per se (Singh et al. 2004). Symbiotic fungi, vesicular-arbuscular mycorrhiza (VAM), viz. *Acaulospora* sp., *Ambispora* sp., *Gigaspora* sp., *Glomus* sp., *Pacispora* sp. and *Paraglomus* sp., have shown significant influence on plant nutrient uptake, growth and colossal capacity to resist abiotic stress, especially drought situations (Oliveira et al. 2006); however, the success of establishing symbiotic interaction was limited through bio-priming, but recent reports suggest that inclusion of some *biostimulants* has made it successful by increasing the occurrence of viable colonies and *percent* infection at early seedling growth stages. Seeds of tomato treated with *T. harzianum* Rifai strain T-22 alleviated abiotic stress factors like osmosis, salinity, chilling and high temperature (Mansouri et al. 2010). Further, many endophytic fungi confer abiotic stress tolerance as detailed in Table 12.5.

Table 12.5 Fungal endophytes inducing abiotic stress tolerance

Fungal strains	Host plant	Responses	Reference
Drought/water stress			
Neotyphodium sp.	*Festuca pratensis*	Induce resistance by osmoregulation and stomatal regulation	Malinowski et al. (1997)
	F. arizonica		
Acremonium sp.	Tall fescue	Osmotic protection through secondary metabolites	White et al. (1992)
Phialophora sp.	*F. pratensis*	Osmotic adjustments	Malinowski et al. (1997)
Colletotrichum magna	*Lens esculentum* and *Capsicum annuum*	Osmotic protection and increased water use efficiency	Redman et al. (2001)
C. orbiculare			
C. musae			
Fusarium culmorum	*Oryza sativa* and *L. esculentum*	Osmotic adjustments and expression of genes	Rodriguez et al. (2008)
Piriformospora indica	*Brassica campestris* and *Arabidopsis* sp.	Involved in expression of diverse stress-related genes	Sun et al. (2010) and Sherameti et al. (2008) respectively
Trichoderma hamatum	*Theobroma cacao*	Induced systemic resistance	Bae et al. (2009)
Salinity stress			
Piriformospora indica	*Hordeum vulgare*	Symbiotic interaction with enhanced nitrate reductase synthesis	Waller et al. (2005)
Fusarium culmorum	*Leymus mollis*, *L. esculentum* and *O. sativa*	Confers salt tolerance symbiotically in coastal habitats through osmotic adjustments	Rodriguez et al. (2008)
Trichoderma harzianum	*Allium cepa*	Osmoregulation through physiological response	Hanci et al. (2014)
Heat stress			
Curvularia sp.	*L. esculentum* and *Dichanthelium lanuginosum*	Symbiotic association found in geothermal soils of Yellow Stone National Park.	Rodriguez and Redman, (2008)
Fusarium sp. and *Alternaria* sp.	*L. esculentum*	Interaction leads to upregulation of stress-related genes	Rodriguez and Redman (2008)

Source: Singh et al. (2011)

The bipartite and tripartite beneficial interactions among various fungi, bacteria and even viruses within the fungi or bacterial cell against abiotic stresses were well demonstrated. Tripartite interaction among *Paenibacillus lentimorbus*, *Piriformospora indica* and *Cicer arietinum* (chickpea) enhanced root nodulations and plant growth which is evident by enhanced N, P, K and S uptake by plants (Nautiyal et al. 2010). Hence, these fungal bio-inoculants when integrated with seed through bio-priming have potential to alleviate the ill effects of abiotic stresses in different crops.

12.4.6 Alleviation Mechanism of Abiotic Stresses in Fungal Bio-inoculants

A variety of mechanism has been projected for microbial stimulated abiotic stress tolerance in plants. Stress tolerance conferred to plants symbiotically involves two mechanisms: (1) activation of host stress response systems soon after exposure to stress, allowing the plants to avoid or mitigate the impacts of the stress (Schulz et al. 1999; Redman et al. 1999), and (2) biosynthesis of antistress biochemicals by endophytes (Miller et al. 2002; Schulz et al. 2002). The manifestation of biosynthesis of antistress compounds results in various mechanisms like *osmotic adjustment* conferring tolerance to abiotic stresses. Osmotic adjustments through enhanced production of osmolytes result in increased retention of water in cells, thereby increasing water use efficiency of plant. Increased osmolyte concentration in plant cell results in increased cell wall elasticity and turgid weight to dry weight ratio (TW/DW) (White et al. 1992). Endophytes are involved in the synthesis of alkaloids like lolines conferring *osmotic protection* by reducing stomatal conductance and alleviating drought stress (Morse et al. 2002); these alkaloids protect macromolecules from denaturation and/or reactive oxygen species (ROS) associated with drought stress (Schardl et al. 2004). Apart from these other potential osmoregulators and protectants are soluble sugars and sugar alcohols, produced by the endophyte, plant or both (Richardson et al. 1992). Symbiotic endophytes increased biomass levels but decreased water consumption and improved recovery after drought period conferring enhanced water use efficiency allowing plants for alleviating drought/heat stress conditions (Rodriguez et al. 2008). Development of mutualistic association of plants and endophytes also confers some systemic properties that enable plant to scavenge ROS burst initiated in plant system as abiotic stress response and thereby reducing the cellular damage (Rodriguez et al. 2008). It is a common acceptance that antioxidant enzymes play an important role in fungal symbiosis conferring abiotic stress tolerance. Further, Rouhier and Jacquot 2008; Rouhier et al. 2008 reported that ROS scavenging compounds include low molecular weight glutathione, ascorbate and tocopherol and enzymes, viz. superoxide dismutases, catalases, ascorbate- or thiol-dependent peroxidases, glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases.

12.4.7 Potential Bacterial Bio-inoculants for Bio-priming

Bacteria are the most abundant soil microbes and integral part in nutrient cycling for maintaining soil fertility. Beneficial bacteria in rhizosphere are of two types: (a) bacteria forming symbiotic relationship through specialised structures and (b) free-living bacteria present in the vicinity of plant domain which are often known as plant growth-promoting rhizobacteria (PGPR). PGPR include a wide range of bacteria belonging to genera *Azotobacter*, *Arthrobacter*, *Agrobacterium*, *Azospirillum*, *Enterobacter*, *Streptomyces*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Pseudomonas* and *Serratia* (Gray and Smith 2005; Vessey 2003). Co-application of PGPR to seed via bio-priming improves plant performance under stress environments and consequently enhances yield both directly and indirectly (Dimkpa et al. 2009). Some PGPR may exert a direct stimulation on plant growth and development by providing plants with fixed nutrients and phytohormones that have been sequestered by bacterial siderophores (Hayat et al. 2010; Rodrìguez and Fraga 1999). Strains of *Rhizobium leguminosarum* bv. *viciae* confer tolerance to abiotic stress factors like drought and salinity by maintaining its capacity to nodulate and fix nitrogen in faba bean (Belal et al. 2013). Some of the PGPR capable of alleviating abiotic stresses in different crops are presented in Table 12.6.

Co-inoculation of PGPR through seed bio-priming shows synergistic effects, where one acts as a helper for enhanced performance of other inoculant. In the rhizosphere the synergism between various bacterial genera such as *Bacillus, Pseudomonas* and *Rhizobium* are well demonstrated to promote plant growth and development. Compared to single inoculation, co-inoculation improved the absorption of nitrogen, phosphorus and other mineral nutrients by seed crop (Figueiredo et al. 2011; Yadegari et al. 2010). Presently bio-priming of seeds for alleviating abiotic stress is achieved through only few PGPR; enormous scope exists for inclusion of underutilised biological agents with varied capacity to confer tolerance for various abiotic stresses.

12.4.8 Alleviation Mechanism of Abiotic Stresses in PGPR Bio-inoculants

Bacteria in association with plant are endowed with certain specialised traits to encounter the ill effects of abiotic stress. Under stress conditions, the endogenous ethylene production in plant system is well documented (Jackson 1997), which adversely affects the root growth and consequently the growth of the plant as a whole. Production of enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves ACC, the precursor molecule of ethylene, is well documented in bacteria (Wang et al. 2001; Saleem et al. 2007). Some PGPR are endowed with certain unique abiotic stress alleviation traits by inducing physical and chemical changes in plants known as *induced systemic tolerance* (IST) to alleviate abiotic stresses (Yang et al. 2009). PGPR induce the expression of drought response-related gene in the plant system through production of some specific inducer and also enhance the level of ROS scavenging enzymatic antioxidants by upregulating the gene involved in its synthesis (Kohler et al. 2008). PGPR are involved in the synthesis of some specialised compounds like *exopolysaccharides* (EPS) which are involved in soil aggregation and help in the maintenance of soil structure in the vicinity of root system even in water stress conditions (Konnova et al. 2001). Plant roots along with fungal hyphae fit in the pores between microaggregates and thus stabilise macroaggregates, thereby increasing the root-adhering soil/root tissue (RAS/RT) ratio (Oades and Waters 1991). Further, PGPR enhance the nutrients uptake of plants in soil conditions where limited nutrient is freely available for plant uptake due to fixation (Munns and Tester 2008). In most cases salinity decreased availability of phosphorus, potassium, iron, zinc and copper to plant (Hayat et al. 2010; Rodrìguez and Fraga 1999). These PGPR convert insoluble form of macro- and micronutrients into available form (Richardson et al. 2009; Khan et al. 2009; Rodrìguez and Fraga 1999). PGPR were also involved in the synthesis of phytohormones, viz. indoleacetic acid (IAA) and gibberellins, which enhance root and shoot development in plant, thereby increasing the plant biomass for better alleviation of abiotic stress conditions (Patten and Glick 2002).

12.5 Conclusion

At present, bio-priming of seeds, development of suitable microbial bio-priming agents and their commercial application to facilitate penetration among farming community are very essential. Therefore, microbial identification and characterisation of potential strains for the development of bio-priming agents, development of formulation and microscale production of bio-priming agents and their suitable delivery mode, mass-scale multilocational field trials and generation of bioefficacy data on different crops, popularisation of technologies among the farmers and registration and commercial production by the agro-industries are needs of the hour. Further

Table 12.6 Bacterial strains inducing abiotic stress tolerance

Bacterial strains	Host plant	Responses	References
Drought/water stress			
Achromobacter piechaudii ARV8	*Lycopersicon esculentum* and *Capsicum annuum*	Synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which reduces ethylene production	Mayak et al. (2004)
Ensifer meliloti bv. *mediterranense*	*Phaseolus vulgaris*	Synthesis of ACC deaminase	Mnasri et al. (2007)
Variovorax paradoxus	*Pisum sativum*	Synthesis of ACC deaminase which reduces ethylene production	Dodd et al. (2005)
Pseudomonas putida, *Pseudomonas* sp. and *Bacillus megaterium*	Undescribed plant	Production of phytohormones	Marulanada et al. (2009)
Pseudomonas sp.	*Helianthus annuus*	Increase in biomass and (root-adhering soil/root tissue) RAS/RT of seedlings	Sandhya et al. (2009)
Bacillus sp.	*Lactuca sativa*	Enhanced AM fungi association in roots and incremental photosynthesis	Vivas et al. (2003)
Pseudomonas fluorescens	*Catharanthus roseus*	Improved plant growth	Jaleel et al. (2007)
Paenibacillus polymyxa and *Rhizobium tropici*	Common bean	Altered phytohormone balance and stomatal conductance	Figueiredo et al. (2008)
Pseudomonas mendocina	*Lactuca sativa*	Increased phosphatase activity in roots and proline accumulation in leaves	Kohler et al. (2008)
Salinity stress			
Pseudomonas putida	Canola	Accumulation of proteins and increased availability of nutrients	Cheng et al. (2007)
Pseudomonas fluorescens	*Arachis hypogaea*	Enhanced ACC deaminase activity	Saravanakumar and Samiyappan (2007)
Rhizobium and *Pseudomonas*	*Zea mays*	Decreased electrolyte leakage and increase in proline production in leaves	Bano and Fatima (2009)
Pseudomonas putida	*Gossypium* sp.	Increase the absorption of useful cations and decrease uptake of deleterious Na^{2+}	Yao et al. (2010)
Bacillus subtilis	*Arabidopsis thaliana*	Decreased electrolyte leakage and increase in proline production in leaves	Zhang et al. (2010)
Azospirillum sp.	*Lactuca sativa*	Increase in N metabolism and synthesis of high molecular weight proteins	Hamdia et al. (2004)
Heat and cold temperature stress			
Pseudomonas putida NBR1097	*Cicer arietinum*	Overexpression of stress sigma factor and biofilm formation	Srivastava et al. (2008)
Pseudomonas AKM-P6	*Sorghum bicolor*	Biosynthesis of HSPs and accumulation of proline in leaves	Ali et al. (2009)

(continued)

Table 12.6 (continued)

Bacterial strains	Host plant	Responses	References
Burkholderia phytofirmans PsJN	*Vitis vinifera*	Increase in root and plant biomass and accumulation of starch, proline and phenolics in leaves	Barka et al. (2006)
P. putida UM4	Canola	ACC deaminase synthesis	Cheng et al. (2007)
Burkholderia phytofirmans	*Solanum tuberosum*	Accumulation of proline, antioxidants and phenolics in leaves	Bensalim et al. (1998)
Pseudomonas fluorescens, Pantoea agglomerans, Mycobacterium sp.	*Triticum aestivum*	Upregulation of stress-related genes	Egamberdiyeva and Hoflich (2003)
Waterlogging stress			
Pseudomonas and *Enterobacter*	*Lycopersicon esculentum*	Synthesis of ACC deaminase which reduces ethylene production	Grichko and Glick (2001)
Heavy metal stress			
Kluyvera ascorbata	*Lycopersicon esculentum*	Toxic effects of Ni^{2+}, Pb^{2+} and Zn^{2+} not pronounced on plant	Burd et al. (2000)
Methylobacterium oryzae and *Burkholderia* sp.	*Lycopersicon esculentum*	Reduced uptake and translocation of nickel and cadmium	Madhaiyan et al. (2007)
Pseudomonas brassicacearum Am3, *P. marginalis* Dp1 and *Rhodococcus* sp. Fp2	*Pisum sativum*	Stimulation of root growth and enhanced nutrient uptake	Safronova et al. (2006)
Rhizobium sp.	*Pisum sativum*	Enhanced plant growth	Wani et al. (2008)
Nutrient deficiency stresses			
Pseudomonas fluorescens and *Bacillus megaterium*	*Lycopersicon esculentum*	Enhanced availability of phosphorus and calcium	Lee et al. (2010)
Azospirillum sp. and *Azotobacter* sp.	*Oryza sativa*	Fixation of atmospheric nitrogen	Wada et al. (1978)
Paenibacillus glucanolyticus	*Piper nigrum*	Solubilisation of fixed potassium	Sangeeth et al. (2012)
Frateuria aurantia	Field and vegetable crops	Solubilisation of fixed potassium	Commercial product
Pseudomonas sp. P29	*Zea mays*	Solubilisation of zinc	Goteti et al. (2013)

research on the viability of the introduced microorganisms and its fate, existence and mode of work on bio-primed seeds is one important area which needs immediate attention.

While *Trichoderma* and *Pseudomonas* have been studied by many researchers extensively, hardly any attention has been paid to identification of crop-specific novel strains of various beneficial microbes. Therefore, future research programme needs to be devised for identification and genetic manipulations of novel biocontrol agents with compatibility studies on seed surface and commercial viability. Further, using bio-primed seeds along with bio-priming of nursery beds with obligate symbionts like AM fungi, particularly in marginal soils, should be given special attention. Similarly, a huge number of fungal and PGPR representing diverse genera have been identified and characterised for their capability to augment the alleviation strategies of plant as a response to biotic/abiotic stress factors in agroecosystem. Application of these bio-inoculants for enhancing performance of seed under limiting environmental conditions through bio-priming has proved beyond doubt, but still a large number of microorganisms remain underutilised for this purpose with the capacity to alleviate varied biotic/abiotic stresses. These bio-inoculants in association with plant have much better stimulatory effect on managing pest/diseases, plant growth and nutrient uptake in stressful environmental conditions. Thus integrating these bio-inoculants to seed through bio-priming can successfully alleviate biotic as well as abiotic stress conditions in agricultural system thereby improving the seed quality and yield in limiting environments.

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