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Seed Bio-Priming : Plant Growth Promoting Microorganisms in Enhancing Crop Productivity and Stress Tolerance - A Review

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ABSTRACT

Beneficial microbes are considered to be a natural alternative path to ease the pressure on the environment resulting from conventional farming. Proper application of beneficial microbes to the seed or soil is essential for their efficacy and efficiency. Among the several methods of inoculation *viz*., soil application, seed inoculation, root dipping, foliar application and seed coating or covering; seed inoculation is found to be the potential method for ensuring the survival and colonization of microorganisms. 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. Biopriming with *Trichoderma* spp. has shown better survival and colonization on/in the seeds with plant growth promoting and stress tolerance activities in many crops. A diversity of biopriming methods have been used for biocontrol of pathogens, *Pseudomonas, Trichoderma, Serratia* spp. These microbes can help plants to maintain or increase productivity while reducing the input of agrochemicals, restoring soil fertility and or overcoming problems caused by abiotic and biotic stresses. This review article shed some light on beneficial microbes, comparison of methods of inoculation, biopriming and its potentiality in agriculture.

Keywords: Plant growth-promoting microorganisms, Seed biopriming, Crop productivity, Stress tolerance

THE agriculture sector contributes 7.8 per cent to Indian economy which is much higher than the world average *i.e.,* 6.1 per cent (Anonymous, 2017). Increasing population and awareness of health leads to huge pressure for quality adequate foods at right time and that should be free from an unacceptable level of chemicals. To accomplish this goal, many countries switched over to organic farming or sustainable farming. Seed is a vital source for sustained growth in agriculture since ninety per cent of the food crops are grown from seed. Good quality seeds with rapid germination, producing synchronized vigorous seedlings, higher yield potential and productivity are the vital factors in agriculture. Use of quality seeds alone could increase productivity by 15–20 per cent which highlights the important role of seed in agriculture. 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 and hence there is a need to search for sustainable production technology to meet the requirements. Pre-sowing techniques comes as an opportunity to enhance the crop stand establishment inclusive of enabling the plant to cope up with the stress without compromising on yield, coupled with no or minute deleterious effect on the surroundings.

The priming technique has gained importance in crop production for its role in activation of physiological processes without the emergence of the radicle of seed. 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). Different seed priming methods are available which are depending on the substances used named as hydro priming, halo priming, osmopriming, hormonal priming and bio-priming (Farooq *et al.,*2017 and Jisha *et al.,* 2013).

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. The incubation duration allows the beneficial microbes to colonize the seed surface which enters the seeds where needed.

The potential of agriculturally important microorganisms to reduce or replace the agrochemicals has been so far evaluated. Beneficial microorganisms including biological control agents (BCAs), plant growth promoting rhizobacteria (PGPRs), fungi (PGPFs) and endophytes play a crucial role in sustainable crop production. It improves seed viability, germination, vigour indices, plant growth, subsequent protection against diseases and finally enhances crop yield. PGPB (Bacteria) 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 such as, 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 (Meena *et al.,* 2015 and Santoyo *et al.,* 2016). Similarly, PGPF (Fungi) 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.

Augmentation of PGPR into plants can be achieved by different methodologies including direct soil application, root dipping method, seed coat pelleting and seed priming. On the other hand, seed inoculation can be a cost-effective way to deliver microbes in large-scale field applications (John *et al.,* 2010 and O'Callaghan, 2016). All the application methods have different merits and demerits (Mahmood *et al.,* 2016). But, there is a need for more effective application method, which supports 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).

Considering all these issues, this review focuses on biopriming technique, method of inoculation and potential of this technique in enhancing plant growth and productivity and its role in ameliorating biotic and abiotic stresses.

Biopriming

Bio-priming is a treatment of seed with beneficial microorganism under controlled hydration which enhances the preparatory processes prior to germination without the emergence of the radicle. It is associated with an increase in hydrolytic enzyme activities (Zahra Rezaloo *et al.*, 2018), reactive oxygen species (ROS) detoxifying enzymes activities (Jeevan *et al.,* 2015), alteration in internal plant hormone levels (López- Coria *et al*., 2016) and also a differential expression of genes in plants that contributes the enhanced plant growth and resistance against biotic and abiotic stress. Innovative research studies at biochemical, proteomics and transcriptome levels are necessary to understand the role of biopriming with PGPRs in phyto-stimulation and nutrient enhancement. Biopriming 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), activation of enzymes (Farhad *et al.,* 2011 and 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.

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.

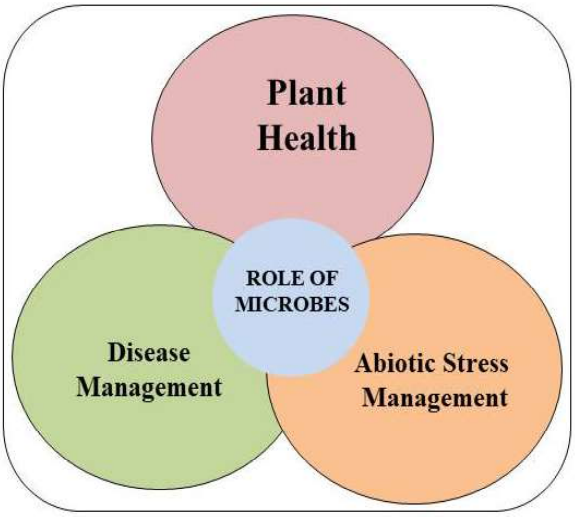

Fig. 1: Diagrammatic representation of plant health, disease and abiotic stress management by microbes

Various species of bacteria including *Pseudomonas, Bacillus, Azospirillum, Serratia, Azotobacter, Enterobacter, Klebsiella, Alcaligenes* and *Burkholderia* have been reported as potential biocontrol agents, biofertilizers and biostimulants (Keswani *et al.,* 2014, 15a), which have been successfully commercialized as the biological control agents. They suppress plant pathogens in soil through production of antibiotics, siderophores and suppress plant diseases through induction of defence response (Bisen *et al.,* 2015; Keswani *et al.,* 2015b and Singh, 2014). It also supports the survival of plants for tolerating biotic and abiotic stress, increase uptake and nutrient availability and improves the soil microflora diversity.

Among various fungal BCAs, *Trichoderm* sp. are mostly studied and are currently being used as biopesticides globally (Singh, 2006; Keswani *et al.,* 2013 and Bisen *et al.,* 2016). *Teleomorph hypocrea,* a saprophyte that commonly reside in root rhizospheric region and *Trichoderma* sp. are the potential plant symbionts reported for antagonistic activity against wide range of soil borne phytopathogenic fungi. More than 60 per cent of the globally marketed biopesticides are based on *Trichoderma* formulation (Keswani *et al.,* 2013 and Singh *et al.*, 2014). About 250 products are commercially available for field applications in India alone (Singh *et al.*, 2012 and Sharma *et al.*, 2014).

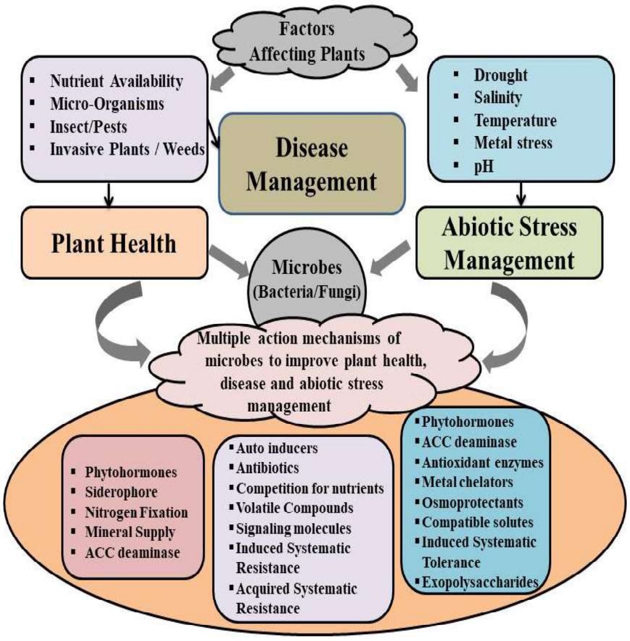

Fig.2: Diagrammatic representation of factors affecting crop productivity and different roles of microbes in stress management

Methods of Microbial Inoculation

Plant Beneficial microorganisms (PBM) are usually added to the soil (direct soil application), the seed (seed-applied inoculant) or the plant (*e.g.,* foliar spray and root dipping) (Adholeya *et al.,* 2005 and Mahmood *et al.,* 2016). Each inoculation method has advantages and disadvantages, depending on the amount of inoculants, availability of equipment, type of seed (*e.g.,* size, shape, and fragility), the presence of inhibiting compounds in the seed (*e.g.,* fungicides, micronutrients, and PBM) and cost (Deaker *et al.,* 2004 and Bashan *et al.,* 2014) (Table 1). In general, direct soil inoculation is used to introduce a large amount of microbial inoculant into the soil, avoiding damage of fragile seeds or protecting the inoculant from inhibiting compounds applied or produced by the seed (*e.g.,* fungicides and antimicrobial compounds). It can be done either using

TABLE 1

Application methods of microbial inoculants

Method	Technique	Advantages	Disadvantages	References
Direct soil inoculation	Granular /powder, liquid inoculation; immobilized microbial cells	Avoids damaging fragile seeds and cotyledons; overcomes the adverse effect of pesticides and fungicides applied to seed; small seed can receive higher dose of inoculants	Requires specialized equipment for application and larger quantities of inoculants; Requires micro storage area and transport; Expensive method	Van Elsas & Haijnen, 1990; Smith, 1992; Deaker *et al.,* 2004; Adholoya *et al.,* 2005 and Bashan *et al.,* 2014
Plant inoculation	Foliar spray; root dipping	Direct application; application of microbial inoculants with high concentration	Expensive; requires large amount on inoculants; laborious and time consuming	Adholeya *et al.,* 2005 and Mahmood *et al.*, 2016
Seed inoculation	Seed soaking; seed coating; bio-priming	Practical and ready to use product; fast, cheap and accurate; require low amount of inoculants; confers other beneficial characteristics to the seed	Poor survival of the inoculants; insufficient amount of microbial inoculants for small seeds; incompatibility of seeds treatments	Kaufman, 1991; Smith, 1992; Adholeya *et al.*, 2006; Doaker *et al.,* 2012; Bashan *et al*., 2014 and Mahmood *et al*., 2016

solid, liquid, or encapsulated formulations at the time of seeding (Malusá *et al.,* 2012 and Bashan *et al.,* 2014). However, direct soil inoculation is not economically feasible in large-scale applications due to the high amount of microbial inoculum required (Deaker *et al.,* 2004; Adholeya *et al.,* 2005 and Vosátka *et al.,* 2012).

Although inoculation of plants through root dipping and foliar is currently being used, these techniques demand large amounts of inoculants and in the case of root dipping; plant nursery preparation is also required. Seed inoculation delivers PBM to the rhizosphere of the target crop, where an intimate plant-microbe contact is established since germination (Philippot *et al.,* 2013).

Biopriming is a method of seed inoculation and it 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 per cent. Almost all the later methods are employed for 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 and 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). Similarly, the wheat seeds were surface sterilized for 1 min. in 70 per cent ethanol and then treated in 5 per cent 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 surface sterilized maize seeds were coated with 20 per cent arabic gum and then suspended in bacterial perlite mixture until the seeds were uniformly coated (Firuzsalari *et al.,* 2012; Sharifi & Khavazi 2011 and Gholami *et al.* (2009). Similarly, selected formulated product of the microorganism is added to pre-soaked (water for 12 h) 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 (Prasad *et al.,* 2016). This method is mainly characterized to be used in biocontrol. 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).

Role of biopriming in Enhancing Crop Productivity

The biology of the rhizosphere could be exploited by manipulating root and microbial interactions to improve the productivity and sustainability of agricultural systems (McNear, 2013). Using Plant Growth Promoting Microorganisms (PGPM) as biofertilizers in place of synthetic NPK (Maheshwari *et al.,* 2012) and also as biological control agents in the form of biopesticides to control plant pests and pathogens (Mishra *et al.,* 2015) have been considered as best practices for sustaining agroecosystems. Soil is supposed to be fertile if it provides physical, chemical, and biological needs for the growth of plants (Abbott and Murphy, 2007). PGPM play a range of different functions that make soil fertile. The direct benefit given to soil by PGPM is their contribution in the formation of soil organic matter (SOM) (Trabelsi and Mhamdi, 2013). SOM also plays a major role in long-term soil conservation, restoration (Sequi, 1989) and soil having low level of SOM inoculated with PGPM gives better yields (Cakmakci *et al.,* 2006).

Plant growth, nutrient uptake and nutrient use efficiency, synchronization in germination and vigorous plant growth, speed of germination and good plant stand under normal and stressed condition have been increased by seed bio-priming (Yadav *et al.,* 2013; Tanwar *et al.,* 2013; Moeinzadeh *et al.,* 2010 and Muruli *et al.,* 2013). Biopriming enhances seed germination, uniformity in emergence of seedlings along with stand establishment, viability, plant vigour, growth and yield (Prasad *et al.,* 2016, Bhatt *et al.,* 2015 and Nakkeeran *et al.,* 2005). Like 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). Biopriming with different bacterial and fungal strains on various crop plants shown positive effects in enhancing the crop growth and productivity (Table 2).

Application of *Pseudomonas aureofaciens* through drum priming system enhanced the stand establishment in tomato (Warren and Bennett, 1997). Biopriming of pea seeds with *Trichoderma* as a biocontrol agent significantly enhanced the plant growth parameters (Altomare *et al.,* 1999 and Singh *et al.,* 2016a). Among other biopriming applications of *Trichoderma*, it increased the growth of wheat, furthermore nitrogen uptake, recovery, agronomic and physiological use efficiency and also performed well even under 75 per cent of the recommended dose of the fertilizer (Meena *et al.,* 2016). Similarly, specific dose of *Trichoderma* spores in six crops *viz*., brinjal, chilli, guar, okra, ridge gourd and tomato has enhanced the plant growth and induced systemic resistance-related enzyme activity (Singh *et al.,* 2016b). Biopriming with *Trichoderma* fungi has increased germination percentage, seedling length and their fresh and dry weight and vigour index in Canola and soybean (Zahra *et al.,* 2018). When chilli seeds were bioprimed with *Trichoderma* and *Pseudomonas* with varying duration; *Pseudomonas* enhanced the seedling growth and vigour better than *Trichoderma*, but both proved to be way better when compared with non-primed seeds (Ananthi *et al.,* 2014). Seed biopriming with *Trichoderma* 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 the growth and yield of barley at different fertilizer levels (Mirshekari *et al.,* 2012).

Soybean seeds bio-primed with fungal biocontrol agents, *viz*., *Trichoderma harzianum, T. virens, T. atroviride, A. bacterium* and *Pseudomonas fluorescens* (utpf5); *Trichoderma harzianum* strain BS1(Th.4) has enhanced the plant growth factors

TABLE 2

Role of seed biopriming with different plant growth-promoting microorganisms in enhancing plant growth parameters

Strain	Crop	Crop response	Experiment	Priming duration	References
Bacillus subtilis and Serratia nematodiphila	Kale, Carrot and Onion	Increased seed germination, emergence and plant growth	Laboratory	20 min	Ruth Murunde *et al.* (2018)
Trichoderma fungus	Canola and Soybeans	Increased germination percen-tage, root and shoot length, vigour index	Laboratory	–	Zahra Rezaloo *et al.* (2018)
Trichoderma viride and Pseudomonas fluorescens	Chilli	Increased plant height, leaf area index, chlorophyll index, fruit set percentage, seed yield and seed weight	Field	9h and 12 h	Ananthi *et al.* (2017)
Trichoderma asperellum	Maize	Increased root length, shoot length, plasma membrane, andH^+-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, and12 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)

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

(Entesari *et al.*, 2013). 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, 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.

Role of Biopriming in Stress Tolerance

Seed bio-priming alleviate physiological and pathological stresses in plants. The crop plants are prone to seed and soil borne diseases, resulting in heavy yield losses and are usually controlled chemically. However, biopriming being an eco-friendly, economical, and effective technique (Ashraf & Foolad, 2005) is being focused for control of biotic and abiotic stresses. Microorganisms are used as biopriming agents for enhancing seedling growth under stress conditions (Bennett, 1998). 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. Bio-priming also stimulate the production of defense-related enzymes *viz.*, peroxidase, superoxide dismutase, catalase, chitinase, ammonia lyases, etc., which offer fitness benefit to plants against biotic and abiotic stress.

Biopriming : Against Biotic Stress

Almost 10–16 per cent of the global harvest is lost by plant diseases each year costing loss of an estimated US $220 billion. All crop pests (pathogens, arthropods, and weeds) cause preharvest losses of 42 per cent and an additional 10 per cent loss after harvest (Fletcher *et al.,* 2006). The use of pesticides for controlling plant diseases has always been associated with various ecological issues. Biocontrol agents such as PGPR and PGPF offer the advantages of higher selectivity and lower or no toxicity in comparison to conventional chemical pesticides (Mishra *et al.,* 2015). 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). It also helps in initializing the systemic resistance in the plants and 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.

PGPR belonging to the genus *Bacillus* have got recognition for wider biocontrol activity against pests. *Bacillus thuringiensis* (Bt) covers 90 per cent of the biopesticide market in the USA (Chattopadhyay *et al.,* 2004). This bacterium is essentially used for insect pest control and also as 'Bt Genetically Modified (GM) Crops' (Cawoy *et al*., 2011). Besides *Bacillus*, genus *Pseudomonas, Serratia* and *Arthrobacter* have also been reported as BCA (Joseph *et al.,* 2007). *Pseudomonas* strains are known to produce a variety of antibiotics or antifungal metabolites directly involved in the suppression of diseases (Weller 2007; Khare & Arora 2011b and Mishra & Arora, 2012b). Biopriming of *Alternaria radicina* and *Alternaria dauci* infected carrot seeds with antagonistic fungi *Clonostachys rosea* reduced the incidence of these pathogens (Jensen *et al.,* 2001). Similar instances in different bacterial and fungal biopriming agents against several pathogens including *Colletotrichum* (Begum *et al.,* 2010), *Fusarium* (Mnasri *et al.,* 2017; Srivastava *et al.,* 2010), *Rhizoctonia*, *Sclerotium* (El-Mougy and Abdel-Kader 2008) and *Verticillium* (Rybakova *et al.,* 2016) were also observed (Table 3).

At least 750 species of fungi are known to be entomopathogenic (Copping, 2009) and among them, several were used as BCA against phytopathogens. *Metarhizium anisopliae* and *Beauveria bassiana* have

TABLE 3

Efficacy of priming agents against disease causing pathogens in different priming durations

Strain	Crop	Pathogen/Disease Controlled	Crop response	Experiment	Priming duration (in hours)	References
Bacillus gaemokensis	Cucumber and Pepper	*Spodoptera litura*	Higher shoot length, fresh weight and fruit yield	Pot	–	Geun Cheol Song *et al.* (2017) Mnasri *et al.* (2017)
Rhizospheric bacteria, Endophytic bacteria	Wheat	*Fusarium culmorum*	Increased germina-tion and seedling vigor, decreased disease incidence	Pot	24	Rybakova *et al.* (2016) Begum *et al.* (2010)
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	Nayaka *et al.* (2010) Srivastava *et al.* (2010)
Trichoderma harzianum, & *virens*, *Pseudomonas aeruginosa*	Soybean	*Colletotrichum truncatum*	Increased seed germination and seedling establish-ment	Field	12	El-Mougy and Abdel-Kader (2008)
Trichoderma harzianum	Maize	*Fusarium verticillioides*	Increased seed germination, vigor index, field emergence, yield, andthousand seed weight	Pot	24	
Pseudomonas fluorescens, *Trichoderma harzianum*	Tomato	*Fusarium oxysporum*	Increased seed germination, decrea-sed germination time,and disease incidence	Pot and field	24	
Trichoderma viride, *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *BacillusCereus*	Faba bean	*Rhizoctonia solani*, *Fusarium solani*, *Sclerotium rolfsii*	Seed storage experiment	Green house and field	16	

been mainly developed for commercial applications (McCoy 1990). Currently, *Trichoderma harzianum, T. asperellum, T. gamsii, C. minitans, A. flavus* and *C. purpureum* (Auld , 2002) are among the most studied fungal biocontrol agents and their commercialization is increasing day by day (Vinale *et al.,* 2008). The seeds of soybean were bioprimed with *T. harzianum*, *T. virens*, and *P. aeruginosa* separately and in consortium against damping-off causing *C. truncatum*. It was observed that population of bacteria on the seeds increased more than four times after 12 h and reduced the disease incidence up to 97.2 per cent 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, tomato seeds bioprimed with *Trichoderma*, *Pseudomonas* and *Glomus* spp., has significantly decreased tomato wilt up to 74 per cent with decreased seed germination time and enhances seed germination (Srivastava *et al.*, 2010). 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 (Rybakova *et al.,* 2016). Biopriming of cucumber and pepper with *Bacillus gaemokensis* showed control of *Spodoptera litura* pest (Geun Cheol Song *et al.,* 2017). When, faba bean bioprimed against root rot athogens, the seeds can be stored for long durations and disease incidence was completely controlled up to one and three months of storage. However less protection was observed when the seeds were stored for four and six months (El-Mougy and Abdel-Kader, 2008).

Biopriming : Against Abiotic Stress

Abiotic stress can be defined as any factor exerted by the environment on the optimal functioning of a plant (Bohnert, 2007). Abiotic stress affects the productivity of crops as well as the microbial activity in soil (Milosevic *et al.,* 2012). At the global level, crop loss due to abiotic stresses is reported to up to 50 per cent (Rasool *et al.,* 2013 and Rodziewicz *et al.,* 2014). PGPR-mediated tolerance to abiotic stresses has been extensively studied at molecular, physiological and morphological level (Dimkpa *et al.,* 2009 and Lim & Kim, 2013) which strengthens the understanding of enhancing crop productivity under harsh environmental conditions (Yang *et al.,* 2009; Tewari & Arora, 2015 and Tewari & Arora, 2016). The impact of abiotic stresses resulting to anthropogenic activities and climate change such as drought, flooding and salinity has been also studied by several workers (Belimov *et al.,* 2009). Studies suggest that it would be preferable to use PGP microbes for providing protection against such stresses. Further, PGPR help plants to tolerate abiotic stresses by various mechanisms (Yang *et al.,* 2009). Among them, the production of osmoprotectors (K+, glutamate, trehalose, proline, glycine and polysaccharates), stress-induced production of phytohormones (IAA and gibberellins) and stimulation of induced systemic tolerance (IST) are of importance (Yuwono *et al.,* 2005; Saleem *et al.,* 2007; Sziderics *et al.,* 2007 and Barriuso *et al.*, 2008). The most important mechanism reported in several PGPRs under stress conditions is the production of enzyme ACC deaminase. Under stress conditions, this enzyme facilitates the growth of plants by decomposing plant ACC (ethylene precursor in plants) (Saleem *et al.,* 2007). By reducing the level of ethylene, the plant becomes more resistant to stress conditions in the environment (Glick, 2005).

The use of biopriming against drought, osmotic and salinity stress has been observed in onion, tomato, sunflower, maize, wheat and rice (Table.4).

Among abiotic stress amelioration by biopriming, *Trichoderma* has been used in controlling salinity and drought stresses, where *Trichoderma citrinoviride* and *Trichoderma lixii* when used in maize (Abdullah

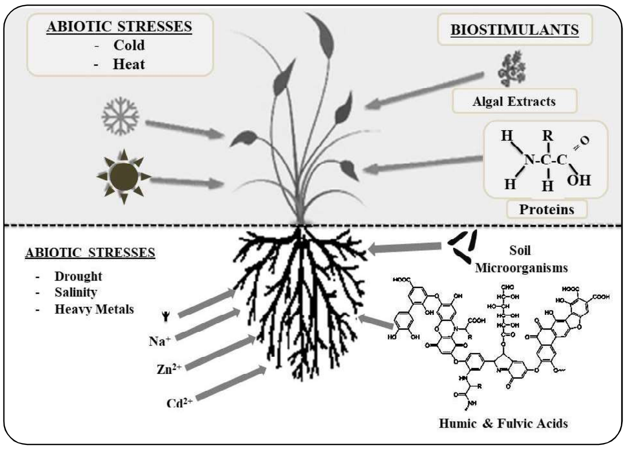

Fig. 3: Key mechanisms of Abiotic factors and biostimulants in plant system

TABLE 4
Efficacy of priming agents against stress condition in different crops

Strain	Crop	Pathogen/Disease Controlled	Crop response	Experiment	Priming duration	References
Pseudomonas geniculate	Maize	Salinity	Increased total chlorophyll content, carotenoids content, total soluble sugar and proline content also enzyme activity	Pot	–	Shailendra Singh et al., (2020)
Trichoderma citrinoviride	Maize	Salinity	Higher photosynthetic pigment, proline, soluble protien, dry weight of root and shoot, relative water Decreased lipid oxidation	Pot	16h	Abdullah et al., (2018)
Trichoderma lixii	Maize	Salinity	Increased length, fresh and dry weights of root/ shoots, relative water content, soluble protein, proline, chlorophyll, and carotenoid content Decreased lipid peroxidation, hydrogen peroxide, and lipid peroxidation	Pot	1 h	Pehlivan et al. (2017)
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 Decreased Na^{+} ion concentration	Pot	30 min	Shah et al. (2017)
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)

Note : Indicates no data given, h stands for hours, and min stands for minutes *et al.,* 2018 and Pehlivan *et al.*, 2017) and *Trichoderma harzianum* in wheat (Rawat *et al.*, 2011) showed better physiological and morphological parameters. Application of *Pseudomonas geniculata* in maize has shown increased plant growth parameters with enzyme activity under saline sodic soil (Shailendra Singh *et al.,* 2020). Use of *Trichoderma harzianum* as biopriming agent against drought stress in wheat crop with 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). The tomato seeds 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 enhanced the seedling vigour in combination with germination (Bhatt *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).

Limitations and Future Prospects

- Enhancing the shelf life of bioprimed seed lots needs to be addressed
- 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
- The microbial identification and characterization 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, popularization of technologies among the farmers and registration and commercial production by the agro-industries are the needs of the hour

Farmers have always tried to improve the chemical and physical conditions of their soils, to make it nutrient rich, to retain moisture and to ease the growth of the plant, but roles played by soil microbes are generally ignored (East, 2013). The use of PGPM (*Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp.) for biofertilization, prevention from deadly diseases, alleviating abiotic and biotic stresses and remediation of contaminated sites can be very useful. 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. 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. This needs better understanding of plant–microbe interactions at biochemical and molecular level. Organic farming process is more eco-friendly than conventional farming. Organic farming keeps soil healthy and maintenance environment integrity there by promoting the health of consumers. Moreover, the organic produce market is now the fastest growing market all over the world including India. To protect seeds from biotic and abiotic stress during organic seed production, seed biopriming can be applied. 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, yield and productivity in controlled environments.

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