FRONTIERS IN BIOLOGICAL SCIENCES

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BIO-PRIMING: A REVOLUTIONARY THOUGHT AGAINST CHEMICALS-BASED CROP IMPROVEMENT

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Abstract- The ideal methodology that can be used for rendering resistance to plants against various stressful situations is priming. In priming, physiological state of seeds can be changed by treating with various priming agents like water, chemical and biological organisms. Various microorganisms including plant growth promoting bacteria and fungi are successfully used for bio-priming in plants. Because of the eco-friendly nature of bio-priming, it becomes an apt method that can be practiced for the future for stress alleviation in crops. It imparts both abiotic and biotic stress tolerance in plants. Agriculture sector has been dramatically depending on the chemical pesticides and fungicides for eradicate pathogenic attacks. Bio-priming is a promising strategy for imparting various biotic/abiotic stresses thus paving way for the removal of chemicals from crop protection.

Introduction - The incipient object of an agricultural sector is the better establishment of crop in a cost-effective method. For many years researches have been investigating to improve the crop production for achieving sufficient food for the future generations (Singh et al. 2016.). Abiotic stress remains the greatest constrain to crop production worldwide. It has been projected that more than 50% of yield reduction is the direct result of abiotic stresses. Biotic stress is another struggle to plant, it includes attack of various pathogens such as viruses, bacteria, fungi, nematodes and herbivores. These pathogens directly deprive their nutrients of its hosts, and that can lead to death of plants. Plants are offsetting to this stress by its own defense mechanisms (Yadav et al. 2020). To reduce the pathogenic attack in crops, the farmers always depend pesticides and fungicides over the past decades. So, it is the need of the day to find a biological remedial system against soil borne pathogens, an effective alternative counter to pesticides, through that to increase the crop production without compromising the environmental dynamics (El-Mohamedy and Alla 2013).

Implementing priming to agriculture is the best method to improve our crop productivity. Priming is a pre-sowing technique which will impart physiological changes before germination in seeds (Mahmood and Kataoka, 2018). It enhances the capability of plants to produce different metabolites which are decisive in their stress management to impart immunity to them against a wide spectrum of environmental challenges (Farook et al. 2008; Capanoglu, 2010; Jafar et al. 2012; Jisha and Puthur 2015). Its effects are stronger and faster when compared to other methods (Thiebaut et al. 2019). The different seed priming methods include hydropriming, osmopriming, chemical priming, hormonal priming, biological priming, redox priming, and solid matrix priming etc. Several priming agents can be used for this such as, water, various chemicals, synthetic compounds, radioactive rays and biological organisms (Jisha et al. 2013; Thomas and Puthur 2020). Although, the efficacy of priming agent may vary according to the crop and stress (Jisha et al. 2013; Sen et al. 2021). This article is deals with the efficacy of biopriming on biotic stress management.

Bio-priming

In 1990, Callan coined the term 'bio-priming'. Seed bio-priming is the pre-germination technique in which seeds are undergoing two combined processes, firstly hydration of seeds and secondly inoculation with biological agents (Devika and Rakshit, 2021). It enhances the capability of plants to produce different metabolites which are decisive in their stress management (Capanoglu, 2010). So, the plants will able to put up with the stressful environment (Babu et al. 2014; Cornejo et al. 2014; Meena et al. 2015; Jisha and Puthur, 2016; Meena et al. 2016). Biopriming is recently used as a nature friendly remedial method for eliminate soil borne and seed borne pathogens. (El-Mohamedy et al. 2015). Through bio-priming, plants gain many benefits through changes in the biochemical and physiological processes, leading to induced systemic resistance and help to maintain the soil texture (Berendson et al. 2012; Pathak et al. 2016).

Methods of bio-priming

Fig. 1: Bio-priming procedure

In priming, the seeds were partially hydrated in a controlled environment and then shade dried (Thiebaut et al. 2019). Callan (1990) worked in sweet corn seeds with bacterial suspension in a regulated imbibition up to 35-40%. After the work, almost all bio-priming methods were based on the soaking of seed material in microbial suspension. During bio-priming, the water uptake is determined by the adjustment of the temperature, duration and water quality. These parameters should be in optimized level, through the imbibitional chilling injury can be minimized.

Initial procedure of every priming technique is the surface sterilization of the particular seed material for appropriate time period. For this purpose, scientists used different chemicals such as, 70% ethanol, diluted 3% Clorox1solution (Roslan, 2020), 0.5% (v/v) sodium hypochlorite (Habib 2016), 1% bleach (Guler, 2016; Pehlivan et al. 2017) etc. For bio-priming, different researchers employed different microorganisms. Researchers used okra seeds and soaked it for two hours in spore suspension of *Trichoderma viride* and conidial suspension of *Pseudomonas fluorescence* (Roslan, 2020; Rai et al. 2019). Whereas, Habib (2016) used seeds of the Malaysian okra seeds which were soaked in PGPR suspension (108 cfu/mL) for 24 h. But, Jenson (2004) bio-primed carrot seeds with *Clonostachys rosea* IK726, in which the clay inoculum ofIK726 was applied during imbibition of seeds (2.5 g of clay inoculum per 500 ml of water) or the IK726 inoculum was dusted onto the seeds after drying the seeds (0.01 g of inoculum per g of seed at 40% MC). Similarly, the sunflower seeds were soaked for 10 min in a cell suspension of isolate of *Pseudomonas aeruginosa* PF23 and PF23EPS-(mutants) along with carboxy methyl cellulose and dried overnight in aseptic conditions (Tewari, 2014). Pehlivan et al. (2017) prepared *Trichoderma lixii* ID11D (TXD)

inoculum blended with 1:1 (v/v) of 2% carboxymethyl cellulose and 1% Tween 20 and soaked the maize seeds for one hour. Another report in tomato showed that in order to maintain the uniform coating of bio-priming agent on seed surface, moist vermiculite with the formulation of *P. fluorescens* and *T. harzianum* were effective (Rajaput et al. 2019). Guler (2016) done the inoculation of *Trichoderma atroviride* (TaID20G) on the maize seeds (*Zea mays* L. RX 9292) in the appropriate microbial suspension and incubated for around 1h. *Arabidopsis* seeds inoculated with bacterial suspension *Pseudomonas* PS01 (106 CFU/ml) by Chu et al. (2019). Rai et al. (2019) performed bio-priming with suspension of *T. viride* and conidia suspension of *P. fluorescence* for two hours. The procedure of biopriming s represented in Fig 1.

Bio-priming agents and its encounter to biotic stress

Plant growth-promoting rhizobacteria

Bio-priming is an efficient scheme for disease management since it guards seeds from soil/seed borne pathogens (Debharma and Das 2017). Many bacteria contribute their service to plant growth promotion, so called Plant Growth-Promoting Rhizobacteria (PGPR). These bacteria are found to be efficient in their catabolic versatility, excellent root colonizing ability and can produce a number of metabolites that help the plant to thrive against various abiotic and biotic stresses. *Pseudomonas fluorescens* primed sweet corn seeds showed resistance against in the damping off caused by *Pythium ultimum* (Callan, 1990), and pearl millet showed tolerance against the downy mildew disease caused by the fungus *Sclerospora germinicola*. Similarly in sunflower, priming with *P. aeruginosa* PF23 under saline conditions, showed increased growth and resistance against *Macrophomina phaseolina* the causative agent of charcoal rot disease in sunflower (Tewari, 2014).

Plant growth promoting fungi

The arbuscular mycorrhizal association can change the plant physiology and leads to better mineral nutrition and enhanced resistance to biotic and abiotic stresses in plants (Jung, 2009). These fungi are collectively called as plant growth promoting fungi (PGPF), because they produce certain compounds like siderophore, phosphate dissolving enzymes, phytohormones, etc. which facilitate the host plant growth (Mirshekari, 2012; Doni et al. 2013; Doni et al. 2014). Arbuscular mycorrhizal association can affect a wide variety of organisms below and above the ground (Jung, 2012). *Trichoderma* is a potential biocontrol agent and it can inhibit the pathogenic enzyme that helps them to invade into the host plant. Direct effect of *Trichoderma* on plants is the induction of systemic resistance to the plants. It can control a wide range of pathogens for instance, fungi, oomycetes, bacteria, and virus by induced resistance (Harman, 2006). All of these effects, they compete with the pathogen for nutrition which is necessary for the pathogen growth (Harman, 2006; Haque et al. 2012; Meena et al. 2015). Furthermore, *Trichoderma* can synthesize fungal elicitors (Woo et al. 2006) there by activate ethylene and jasmonate pathways (Djonovic et al. 2007; Shoresh et al. 2005).

Trichoderma harzianum is the most potential biological control agent used to eradicate the soil borne, seed borne and foliar diseases in broad range (Ferrigo, 2014). It also synthesizes antifungal compounds (Vinale et al. 2008). Researchers analyzed the systemic resistance of maize plants which were primed with *Trichoderma harzianum T22* against *Fusarium verticilloides* that is the causative organism of ear and kernel rot in maize. They confirmed that strain T22 is a potent biological control agent against fungal pathogen (Ferrigo, 2014). *T. harzianum* plus potassium salt treatment

induces a high decline of root rot disease in green bean (El-Mohamedy et al. 2015). Djonovic (2007) found that the maize plants primed with *T. virens* showed resistance against *Collectotrichum gramicola*. *T. hamatum* can reduce the damping off in peas infected by *Pythium* spp. and it is also reported to be a true biocontrol agent because it attacks and control both *pythium* and *Rhizoctonia solani*, the causative organism of damping off in pea (Chet and Baker 1981). Jenson et al. (2002) tested the biocontrol activity of *Clonostachys rosea* (isolate IK726) spores in barley seeds and found that it had a high biocontrol (>80% disease reduction) efficiency against seedling blight caused by seed-borne *Bipolaris sorokiniana*. Moreover, *C. rosea* also showed very biocontrol efficacy against seed born *Alternaria radicina* and *A. dauci* in carrot seeds (Jensen, 2004). Table 1 represents the various biopriming researches carried out in different plants.

Table 1: Different organisms used for bio-priming and its effect on various pathogens

Sr No	Organisms used for priming	Plant	Disease	Targeted pathogen	Reference
1	*Pseudomonas aeruginosa* PF23	Sun-flower	Charcoal rot	*Macrophomina phaseolina*	Tewari (2014).
2	*Pseudomonas fluorescens*	Pearl millet	Downy mildew	*Sclerospora germinicola*	Niranjan et al. (2003)
3	*Pseudomonas flurescens*	Sweet corn	Damping off	*Pythium ultimum*	Callan (1990)

4	*Trichoderma hamatum*	Pea	Damping off	*Pythium* spp, *Rhizoctonia solani.*	Chet and Baker (1981).
5	*Trichoderma harzianum* T22	Maize	Ear and kernel rot	*Fusarium verticilloides*	Perrigo (2014).
6	Rhizospheric bacteria, Endophytic bacteria	Wheat	Fusarium head blight	*Fusarium culmorum*	Mnasri et al. (2017)
7	*Pseudomonas fluorescens, Trichoderma harzianum*	Tomato	Wilt	*Fusarium oxysporum*	Srivastava et al. (2010)
8	*Trichoderma viride, Trichoderma harzianum, Bacillus subtilis, Pseudomonas fluorescens, Trichoderma hamatum, Bacillus cereus*	Faba bean	root rot	*Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii*	El-Mougy and Abdel-Kader (2008)
9.	*T. harzianum, T. viride*	Green bean	Root rot	*Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina*	El-bab et al. (2013)
10	*T. harzianum*	Green bean	Root rot	*Fusarium solani, Rhizoctonia solani,*	El-Mohamedy et al. (2015)
11	*Clonostachys rosea*	Carrot	Seed born disease	*Alternaria radicina Alternaria dauci*	Jensen (2004)

'Defense priming' travels to subsequent generation

Memory is key source to adaptation and survival amidst of unfavourable circumstances (Baluska et al. 2018). Plants pick up the change in the environment and activate some signal transduction that initiates some modification in the genome for the particular adaptation (Thiebaut et al. 2019). These modifications are called epigenetic modifications and it may transfer in to the next generations (Boyko, 2008). Epigenetic variations are affecting the phenotype of the particular organism, even though it is not associated with the genetic variation (Lämke and Bäurle 2017; Schmid et al. 2018; Thiebaut et al. 2019). The process of formation of new phenotypes as a result of environmental changes can be connected to the epigenetic memory in 'defense priming' (Kinoshita and Sek 2014; Pastor et al. 2013) which regulates the response of plants to a pathogen attack. In defense priming the plant is maintaining its survival chances (Alvarez et al. 2010; Sani, 2010; Slaughter et al. 2012). The period of this memory may only last in range of days to week, but in some cases it may prolong to the offspring and it is called as transgenerational memory (Lämke and Bäurle 2017). Slaughter et al. (2012) reported that offspring of primed *Arabidopsis* showed their parents primed effects without additional treatments. Their results showed that the primed states in plants were transferred their stress tolerance capacity to the next generation, and the progeny possessed the resistance against the pathogen than the unprimed progeny.

Conclusion

Microbial associations are beneficial for plants due to their role in nutrient cycling, nutrient solubilization, production of plant growth hormones, induction of plant defense mechanisms against pathogens etc. Biopriming is the best option that can be exploited for the ecofriendly counter towards plant pathogens. In priming, the seed germination process starts without radicle emergence. So, it triggers many stress responses in plants thus the primed plants show higher immunity than the unprimed ones. Many biological organisms can be used as bio-priming agents to induce tolerance against pathogenic attacks. This could be a green movement against pesticides.

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