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A review on biopriming in vegetable crops for yield and quality traits

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Abstract

The use of micro-organisms to improve yield and quality parameters in crop plants is the basis of biopriming treatment in different crop plants. Inoculation of microbes to hydrated seeds initiates metabolic activities without actual germination. The micro-organisms multiplying on the seed stimulate the release of growth hormones and volatile compounds by plants, improve the availability of nutrients in the soil and initiate defence signals in stress conditions. Ultimately, better quality and higher yield is obtained by increased nutrient use efficiency, enhanced tolerance of plants against various biotic and abiotic stresses and improved physiological and morphological traits in different vegetable crops. Among various methods of microbe application, priming treatment was found to be a promising technique. It also reduces the use of chemicals and their harmful effects. Therefore, biopriming can be effectively used as a simple and inexpensive substitute to other seed treatment methods for crop improvement. Here in this review, different microbes and their potential use as growth and development stimulators through priming treatment in the vegetable are discussed.

Keywords: Biopriming, nutrient, stress, quality and yield

Introduction

Food security is of great concern in developing countries like India where the population is ever increasing (Sreekanth et al. 2015). This growth in population, by many activities caused changes in environmental condition (Tian et al. 2016) creating demand for quality food. In this context production of sufficient food with higher nutrient content is of more importance (Bahadur et al. 2018). Different compositions of nutrients are found in vegetables which can protect the population from malnutrition (Iyaka et al. 2014). Vegetables are composed of antioxidants (Baiano and Del-Nobile 2016) like phenolic compounds, flavonoids, vitamins, tocopherols, pigments and sulphur containing compounds (Doleman et al. 2017) which possess health benefitting properties (Padayachee et al. 2017). The awareness about the importance of nutritional food have changed lifestyle and food habits of people. Realising the opportunity, for greater advantage farmers are moving towards commercial vegetables cultivation (Schreinemachers et al. 2018). With a view to increase production many modern techniques are practiced continuously. These methods like use of chemicals have severe negative impacts (Sharma et al. 2019) creating a need for environmentally friendly methods to improve overall crop production of different vegetables.

Seeds are the primary input used for transfer of novel technologies (Wimalasekera 2015). Non availability of sufficient quantity of seeds, infected seeds, seeds with lower viability and vigour and poor germination of seeds lead to poor crop stand. Therefore, it is of great importance to use vigorous seeds to get quality produce. Seed treatment with microbes was found to be better than seedling or soil treatment as it improves the rhizosphere by increasing the microbial population (Mehjabeen et al. 2020). Biopriming is a novel approach of seed treatment where the seeds are treated with different beneficial micro-organisms along with hydration of seeds in controlled condition (Sukanya et al. 2018). It is recently used as an alternative method for controlling many seed and soil borne pathogens (Mona et al. 2017) and also to improve the economics in crop production (Devika 2019).

The major objectives of concern while biopriming are the different positive impacts of the treatment that includes increase in nutrient use efficiency,

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improved water use efficiency, uniform emergence, growth and development of plants, better quantity and quality of yield production with reduced impact of abiotic and biotic stress (Rakshit 2019).

Types of Seed Priming and its procedure:

There are numbers of priming approaches being expedient in numbers of vegetable crops.

1. **Biopriming:** Seed imbibition coupled with biocontrol agent or plant growth promoting rhizobacteria (PGPR) inoculation of seed (Callan et al. 1990).
2. **Hydro priming:** Prior to sowing, seeds are soaked in pure water and re-dried to their original moisture content. Because no additional chemical compounds are used as a priming ingredient, this process is both inexpensive and ecofriendly (Taylor et al. 1998).
3. **Osmopriming:** Osmopriming is the process of soaking seeds in a saline solution with a low water potential rather than pure water. Water enters seed slowly due to the low water potential of saline solutions, allowing constant seed imbibition and activation of early stages of germination but limiting radicle protrusion (Di Girolamo and Barbanti 2012).
4. **Solid matrix priming:** This method involves combining and incubating seeds with a moist solid water carrier for a set amount of time. Following that, the seeds are separated from the matrix, cleaned, and back-dried. The use of solid medium allows seeds to progressively hydrate and stimulates the natural imbibition process that occurs in the soil (McDonald 2000).
5. **Hormopriming:** Hormopriming occurs when seeds imbibe in the presence of plant growth regulators, which can have a direct impact on seed metabolic processes. Abscisic acid, auxins, gibberellins, kinetin, ethylene, polyamines, and salicylic acid (SA) are common growth regulators utilized in hormopriming. On heavy metal polluted soil, gibberellic acid (GA_3) and PEG priming improved photosynthetic characteristics, antioxidant system, seedling emergence, and growth of white clover (Galhaut et al. 2014).
6. **Chemo-priming:** Chemo-priming refers to seed treatment using various chemical solutions as priming agents. Priming with a wide range of natural and synthetic substances such as antioxidants (ascorbic acid, glutathione, tocopherol, melatonin, and proline), hydrogen peroxide, sodium nitroprusside, urea, thiourea, mannose, selenium, chitosan, fungicide, and so on is part of this procedure. Several studies have pointed to the positive effects of chemo-priming with various priming agents in a variety of environmental conditions (Patade et al. 2012).
7. **Nutri-priming:** Nutri-priming is the process of soaking seeds in solutions containing the limiting nutrient rather than pure water. The idea behind this strategy is to get nutritional influence along with biochemical priming effects in order to improve seed quality, germination characteristics, and seedling establishment (Farooq et al. 2012).

Role of biopriming in nutrient use efficiency

Nutrient use efficiency is the unit output of the crop per unit input of nutrient (Meena et al. 2017). Use of microorganisms in seed priming treatment enhances the availability and utilisation of nutrients of varius vegetable crops (Table 1). By increased enzymatic activity bioagents increase the nutrient contents in soil and hence can be used as a substitute to inorganic fertilizers (Ji et al. Rakshit 2021). Similarly, Pal and Singh (2018) reported that *Trichoderma harzianum* NBRI 1055 increased N, P, K, Fe, Zn, Cu and Mn content in okra. *Glomus sp*. improved content of N, P, K, Ca, *fluorescens* and *Bacillus subtilis* increased N, P, K, Cu, Fe and Zn in red cabbage (Sarkar and Fe, 2020). When *Burkholderia gladioli*, *Pseudomonas sp.* and *Bacillus subtilis* were used as a consortium in biopriming of tomato and fenugreek, maximum

Table 1: Nutrient use efficiency of different vegetable crop as influenced by the bio-priming intervention

S. No.	Crop	Bioagent	Nutrient use efficiency			References
			Primary nutrients	Secondary nutrients	Micro nutrients	
1	Tomato	*Trichoderma harzianum* T22	K (9.7%); P (38%); N (2.5%)	Ca (22%); Mg (20%)	Fe (46%); Zn (27%)	Molla et al., (2012)
		BioF/liquid (broth of spores	K (15.3%); P	Ca (18.2%);	Fe (64.6 %);	

		suspension of *Trichodermaharzianum* T22)	(24.7%)	Mg (24.4%)	Zn (45%)	
		Trichoderma harzianum T969	P (65.85%); K (324.35%)			Azarmi et al., (2011)
		Trichoderma harzianum T447	P (359.53%); K (782.97%)	Ca (528.63%); Mg (220.86%)		
		Trichoderma harzianum T969	P (42.98%); K (162.82%)	Ca (31.46%); Mg (38.98%)		
2	Broccoli	AM fungi	N (102.08%); P (53.33%)			Tanwar et al., (2013)
		Pseudomonas fluorescens	N (235.42%); P (163.33%)			
		Trichoderma harzianum	N (735%); P (210%)			
3	Melon	*Trichoderma harzianum*	N (27.03%), P (137.8%); K (27.96%)			Martınez Medina et al., (2009)
		Trichoderma harzianum under conventional fertilization dosage	N (20.6%); K (30%)			
4	Cucumber	*Trichoderma asperellum* strain T 34			Cu (25%); Zn (11.4%); Zn (29.5%); Mn (58.6%); Cu (10.5%); Fe (85.7%)	Santiago et al., (2012)
		Trichoderma harzianum	N (13%); P (12%); K (11.7%)	Ca (13.5%); Mg (3.7%)	Fe (9%); Mn (8.2%); Cu (35%); Zn (5.7%)	Moharam and Negim, (2012)
		Trichoderma viride Tv2	N (5.9%); P (1.2%); Ca (5.3%)		Fe (7.5%); Mn (1.1%); Cu (13.8%); Zn (1.4%)	
		Trichoderma harzianum	P (30%)		Zn (25%); Mn (70%)	Yedidia et al., (2001)
5	Cabbage	*Bacillus megaterium* TV-91C	N (17.95%); P (10.28%); K (5.01%)		Fe (14.69%); Mn (14.99%)	Turan et al., (2018)
		Bacillus subtilis TV-17C	N (10.26%); P (6.26%); K (4.59%)		Fe (27.97%); Mn (10.85%)	
6	Chilli	*Pseudomonas stutzeri* + *Azospirillumbrasilense* + *Agrobacterium tumefaciens*	P (4–29%)			Abbasi et al., (2015)
7	Okra	*Trichoderma harzianum* NBRI 1055	N (49.18%); P (39.56%); K (38.89%)		Fe (69.04%)	Pal and Singh, (2018)
8	Red cabbage	*Pseudomonas fluorescens* + *Bacillus subtilis*	P (0.37%); K (2.84%)		Fe (160.12 mg kg^{-1}); Zn (34.18 mg kg^{-1})	Sarkar and Rakshit, (2021)

solubilisation of nutrients was observed (Kumar et al. 2020). Biopriming with *Trichoderma harzianum*, *Pseudomonas* Mn, Zn, and Cu in pepper (Pereira et al. 2016). Arbuscular mycorrhiza also increased nutrient content in tomato (Bowles et al. 2016).

PGPB was found to increase availability of N, P and K in soil and their uptake by plants in amaranth on biopriming (Negi et al. 2019b) Similar results were obtained in okra on biopriming with *Glomus fasiculatum* and *Gigaspora sp*. (Dhawal 2017) and in pea plants with *Glomus mosseae* treatment (Yadav et al. 2018). (Kumar et al. 2017) observed increased uptake of N, P, K, Ca, B and Mo in okra and pea on *Glomus mosseae* biopriming.

Use of biopriming for biotic stress management

Use of chemicals for disease control is a common practice which is neither safe for environment nor for human health. But microbes can be used as a substitute to chemicals in seed treatment against many seed and soil borne diseases (Reddy 2012). It is simple and inexpensive method helping plants to adapt under stressed condition (Rakshit et al. 2013). Biopriming of tomato using *Trichoderma erinaceum* resulted in defence signalling by WRKY gene against *Fusarium* leading to production of antioxidative enzymes, defence compounds and lignification of the cells (Aamir et al. 2019). Biopriming of pepper seeds with *Streptomyces rochei* IT20 and *S. vinaceusdrappus* SS14 inhibited mycelial germination of *Phytophthora capsici* by inducing systemic resistance through ACCO and SUS gene (Abbasi et al. 2020). Up-regulation of salicylic acid, ethylene and jasmonic acid signalling immunity induction against *Spodoptera litura* was observed when *Bacillus gaemokensis* strain PB69 was used to treat seeds of cucumber and pepper (Song et al. 2017).

The microorganisms along with producing secondary metabolites, stimulate the plants to produce compounds having antifungal, antibacterial and antiviral properties (Shukla et al. 2015). Release of a greater number of volatile compounds like hydroxylamine, dimethoxy dimethyl silane and hexadecanoic acid-15-methyl-methyl ester that is having antimicrobial properties on biopriming with *Bacillus amyloliquefaciens* VB7 in chilli seedlings were observed by (Sathya et al. 2016b). (Singh et al. 2016) observed that under biotic stress conditions, pathogen effect was reduced by enhancing phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase activities with greater accumulation of total phenol content in diverse vegetable crops treated with *Trichoderma asperellum* BHUT8.

P fluorescens Pf reduced the incidence of *Aspergillus flavus, Fusarium sp*. and non-sporulating fungi in tomato (Mohan and Sundareswaran 2021). (Rahman et al. 2020) reported decrease in soil borne fungi like *Aspergillus*, *Fusarium* and *Penicillium* in cucurbits and okra on biopriming seeds with *Trichoderma harzianum*.Yams when treated with *Trichoderma harzianum* showed lesser incidence of anthracnose disease (Jehani et al. 2019). (Negi et al. 2019a) reported that PGPR-1 and *Rhizobium strain* B1 reduced the degree of rhizoctonia root rot and angular leaf spot effects in french bean. Biopriming with *Trichoderma pseudokoningii* BHUR2 was effective in reducing the occurrence of collar rot in tomato (Rajput et al. 2019). (Avinash and Ravishankar 2017) reported lesser occurrence of fusarium wilt when biopriming with *Bacillus amyloliquefaciens* MIC6 and *Pseudomonas aeruginosa* MTCC2581 was done in cucumber. (Raj et al. 2017) observed that infection by *Aspergillus niger*, *Aspergillus flavus, Fusarium sp*.and *Colletotrichum sp*.was less when chilli seeds were bioprimed with *Pseudomonas fluorescens* Pf 1. Combination of biopriming with *Trichoderma asperellum* BHU P-1 and *Ochrobactrum sp*. BHU PB-1 and ascorbic acid treatment reduced *Fusarium* incidence by higher antioxidant activity, lignin deposition and expressing pathogen resistant gene in tomato (Singh et al. 2020). In conclusion biopriming was found to be better than other seed treatments in controlling diseases in crop plants (Pawar et al. 2019)

Role of biopriming in abiotic stress management

Negative impact seen on plant growth and yield by abiotic stresses can be reduced by biopriming. This could be due to modulation in membrane stability, more of photosynthetic pigments, sugar metabolism and ionic homeostasis which improved germination and growth (Ghezal et al. 2016). Modification of several genes responsible for biotic and abiotic stress tolerance under the influence of plant growth promoting rhizobacteria (PGPR) in vegetable crops have been studied. Upregulation of genes for tolerance, increased chlorophyll and carotenoid content by *Bacillus sp*. caused stress relief in tomato (Yoo et al. 2019).

T harzianum T-78 enhanced the activity of ureases, phosphatase, dehydrogenase and β-glucosidase under saline condition (Mbarki et al. 2017). Under salt stress condition the arbuscular mycorrhizal fungi increased photosynthetic activity, nutrient absorption and antioxidant enzyme activity (Li et al. 2020). Increased radicle and plumule length by PGPR under salinity stress condition in lettuce and radish was observed by (Hussein and Joo 2018). Under nutrient stress condition biopriming with ACC-deaminase containing rhizobacteria improved growth and yield (Hassanet et al. 2015). Better nutrient uptake, antioxidant activity and maintenance of osmotic balance under drought stress condition by *P fluorescens* and *Rhizobium phaseoli* biopriming improved morphological and physiological properties of crop plants (Nawaz et al. 2021). Integration of biopriming with seed coating showed improvement in germination and growth parameters under drought stress condition due to better physiological activities (Piri et al. 2019).

Spinach biopriming with *Ascophyllum nodosum* showed improved antioxidant metabolism and germination percentage, germination speed and seedling vigour under high temperature stress condition (Anjos et al. 2020). (Gowthamy et al. 2018) observed that, under cadmium stress condition biopriming of tomato with *Bacillus amyloliquifaciens* improved seed germination and seedling growth. Biopriming of pepper with *Padina pavonica* and *Jania rubens* regulated sugar metabolism and maintained ionic homeostasis by modulation in membrane stability under salt stress condition (Rinez et al. 2018). *Piriformospora indica* increased tolerance to chilling stress by increasing leaves, leaf dry weight and total dry weight in beans (Forutan et al. 2017). Production of secondary metabolites, synthesis of new proteins, DNA, changes in the number and percentage of bands in the protein profile and ISSR patterns were observed in radish seeds treated with *Codium taylorii* and *Pterocladia capillacea* under salt stress condition (Kasim et al. 2016). In a study conducted by (Bhatt et al. 2015) it was observed that *Enterobacter* strains improved germination and vigour in tomato under osmotic stress. In onion *Citricoccus zhacaiensis* B-4 enhanced synthesis of IAA, GA_3, improved solubilization, zinc solubilization, ACC deaminase activity and ammonia production under osmotic stress (Kumar et al. 2015).

Biopriming and improvement in morphological and physiological aspects

Along with growth parameters, biopriming also improved vigour index in chilli with *Pseudomonas fluorescence* and *Trichoderma viride* treatment (Rai and Behera 2019). Similar results were observed in tomato, brinjal, chilli and onion with *Trichoderma harzianum* and *Pseudomonas fluorescens* treatment (Jaiman et al. 2020). A study conducted by (Monalisa et al. 2017) where *Trichoderma harzianum* and *Pseudomonas fluorescence* were used in biopriming of french bean showed higher seedling vigour index I and II. When Capsicum frutescence seeds were bioprimed with *Kappaphycus alvarezii* (K-sap) and *Gracilaria edulis* (G-sap), (Dutta et al. 2019) observed improved germination percentage, mean germination time, vigour index, and seedling weight with increased total phenol content, DPPH activity (2,2-diphenyl-1-picrylhydrazyl) and ABTS assay(2, 2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), while electrolyte leakage was reduced. *Padina pavonica* L. (B3) and *Sargassum johnstonii* improved germination and growth parameters along with better enzymatic activity in onion and cabbage (Patel et al. 2018). Biopriming of seeds of chilli with microbial consortia consisting of *Funneliformis mosseae* and *Bacillus sonorensis* improved plant height, biovolume index, dry weight of root and shoot, germination percentage and vigour index (Balasubramanian et al. 2018). In the biopriming treatment of chilli seeds with *B amyloliquefaciens*, (Sathya et al. 2016a) observed that the speed of germination, germination percentage, root length, shoot length, dry matter production and vigour index was higher (Zameer et al. 2016).

Biopriming by regulating various physiological, biochemical and molecular activities results in overall growth and development of crop plants. The increased activity alpha amylase, protease and lipase by biopriming helps in the process of germination and seedling growth by rapid metabolic activities (Karthika et al. 2016). Positive impact on seedling growth parameters were observed in biopriming lettuce, coriander and fenugreek with Ulva lactuca (Patel et al. 2019). Bokashi leachate improved seed germination and root growth performance of basella (Phooi et al. 2021). Biopriming of cowpea with Rhizobium and PSB improved plant height, number of branches, number of leaves, number of nodules fresh and dry weight of nodules, fresh weight and dry weight (Brunda 2017). Increased shoot and root growth were observed in sweet pepper and tomato with *Ascophyllum nodosum* biopriming (Silva et al. 2021). *T viride* and *Pseudomonas fluorescens* increased pod yield in okra (Rai et al. 2019a).

Improvement of phytochemical quality and yield through biopriming

Vegetables are the source of several biochemical compounds that have positive health benefits. Change in environmental conditions has severe negative effect on nutritional quality of crop plants (Tuomisto et al. 2017). Biopriming can be used as a simple and effective tool to enhance the nutrient component of crop plants. This treatment showed positive effects on several biochemical properties of the plants such as carbohydrates, protein, pigments and enzymes *etc* and is suitable to provide required nutrition to the population.

Seed inoculation of coriander with combination of *Azatobacer*, PSB and *Pseudomonas* improved water content, total phenol, true protein, Indole-3-acetia acid (IAA), total soluble sugar and reducing sugar (Warwate et al. 2017). Biopriming of kidney bean seeds with *Trichoderma harzianum* increases chlorophyll content (Mehjabeen et al. 2020). Improvement in protein and carbohydrate content was seen in okra on biopriming with *Trichoderma viride* (Rai et al. 2019b). In pepper, enhancement in carotenoids, IAA, GA_3 contents and photosynthetic pigments was observed when seeds treated with *Glomus sp*. (Pereira et al. 2016). Along with chlorophyll, protein and carbohydrate content, alpha-amylase activity was also better on biopriming of common bean using *Trichoderma viride* 40% by (Monalisa et al. 2016). Similar results were obtained on *Pseudomonas fluorescence* biopriming treatment in garden pea (Naik 2015). *Trichoderma harzianum* NBRI 1055 improved the content of protein, crude fibre, phenolic and ascorbic acid in okra (Pal and Singh 2018).

Seed bio-priming is becoming a popular method of inoculation because soil application necessitates a higher proportion of bio-inoculants, which contradicts the economic profitability of agricultural systems. The use of biofertilizers enhanced the benefit-cost ratio (B:C) in cauliflower cultivation from 1.95 to 2.96. (Narayanamma et al. 2005). *Bacillus mucilaginous*, treated as biological potassium fertilizer via seed coating and a full dose of NP fertilizer, produced a higher net return in maize cultivation than full NPK and full NP fertilizer (Jilani et al. 2007). In Himachal Pradesh on-farm experiments, net returns in the cauliflower-cauliflower-pea system were as follows: 100% NPKB + FYM (Rs 462883/ha/year) > 50% NPKB + FYM + Biofertilizers (Rs 213401/ha/year) > 50% NPKB + FYM (Rs 208253/ha/year) (Parmar 2018).The economic verification of a cabbage field trial in Pantnagar (Uttarakhand) revealed a greater B:C ratio and net profit only in fertilization treatments; the minimal contribution of FYM may be attributed to a low breakdown rate during the winter season (Pande and Singh 2016). Fertilization based on target yield (250 q/ha) had a better economic response than broad fertilizer recommendations. Although the gross and net returns in the soybean-potato cropping system were significantly higher in the 100 percent recommended dose of P + biofertilizers (*Pseudomonas striata* + *Glomus fasciculatum*), the net B:C ratio was significantly better in both doses (50 and 100%) of recommended P applied through DAP (Munda et al. 2018). Biofertilizer application in greenhouse tomato cultivation increased total energy input (100.30 GJ/ha), total energy output (119.48 GJ/ha), energy productivity (0.99 kg/MJ), and energy output-input ratio (1.19), compared to control or conventional fertilization (98.45 GJ/ha, 90.52 GJ/ha, 0.77 kg/MJ, and 0.92 kg/MJ, respectively) (Mihov and Tringovska 2010).

Conclusion

Many species of micro-organisms have proved to be beneficial in improving overall crop production. Hence inoculation of these beneficial microbes through priming treatment is advantageous in overcoming problems of poor productivity of vegetables. Biotic and abiotic stress, which are the major problems in crop production can be managed efficiently by biopriming. By increasing the nutrient uptake by crop plants, it not only improves the crop growth and development, but also enhances the nutritional quality of the produce. Many studies conducted on biopriming reveal that, the results of biopriming are better compared to other seed treatment methods. And it can be concluded that it is an economically feasible, environmentally friendly and effective measure to enhance the quality and quantity of yield and yield attributing parameters.

सारांश

सूक्ष्म जीवों का उपयोग करके विभिन्न फसल पौधों में उपज और गुणवत्तायुक्त मानकों के सुधार के लिए बीज बायोप्राइमिंग उपचार मुख्य आधार है। जलयुक्त विधि द्वारा बीजों में रोगाणुओं का टीकाकरण वास्तविक अंकुरण के बिना उपापचय गतिविधियों की शुरुआत करता है। बीजों पर संख्यावृद्धि करने वाले सूक्ष्म जीव पौधों द्वारा वृद्धि हार्मोन और वाष्पशील यौगिकों की क्रियाशीलता को प्रोत्साहित करते हैं, मिट्टी में पोषक तत्वों की उपलब्धता में सुधार करते हैं और पौधों में तनाव की स्थिति में रक्षा संकेतों की शुरुआत करते हैं। अंततः, बेहतर गुणवत्ता और उच्च उपज पोषक तत्वों के उपयोग की दक्षता में वृद्धि, विभिन्न जैविक और अजैविक तनावों के प्रति पौधों की बढ़ी हुई सहनशीलता और विभिन्न सब्जी फसलों में बेहतर शारीरिक और रूपात्मक लक्षणों द्वारा प्राप्त की जा सकती है। सूक्ष्म जीवों के प्रयोग के विभिन्न तरीकों में बीज प्राइमिंग विधि एक आशाजनक तकनीक पाया गया है। यह रसायनों के उपयोग और उनके हानिकारक प्रभावों को भी कम करता है। इसलिए, फसल सुधार के लिए अन्य बीज उपचार विधियों के लिए एक सरल और सस्ते विकल्प के रूप में हमें बीज प्राइमिंग का प्रभावी ढंग से उपयोग करना चाहिए। यहाँ इस समीक्षा लेख में, विभिन्न रोगाणुओं और सब्जियों में प्राइमिंग उपचार के माध्यम से पौधों के वृद्धि और विकास उत्तेजक के रूप में उनके संभावित उपयोग की अनुशंसा है।

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