Navjot Gill *et al*, International Journal of Advances in Agricultural Science & Technology,
Vol.9 Issue.5, May-2022, pg. 50-65

ISSN: 2348-1358
Impact Factor: 6.901
NAAS Rating: 3.77

Seed Treatment Improved Heat Stress Tolerance During Seed Germination

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DOI: 10.47856/ijaast.2022.v09i05.006

ABSTRACT
Heat stress is a major problem raised in agriculture sector due to increasing temperature in temperature. Due to Increase in temperature plant en counter physiological and biochemical changes which directly affect plant growth and production. There are multiples method of seed treatment for improving heat stress tolerance during seed germination for better growth and production. There are different biotic and abiotic stresses throughout plant ontogeny. To overcome these stresses different approaches have been made and practiced, there are few methods that are still in research in different research institute. The negative effects of heat stress can be reduced by developing plant extracts through the development of thermo tolerance using a variety of genetic methods. For this purpose, however, a complete understanding of plant life responses to high temperatures, heat tolerance methods and possible techniques for improving plant heat tolerance is essential. Some treatment can be particularly time consuming, while other treatment are denied by countries in the world.

OBJECTIVES
- Protect against soil and seed born diseases or insects
- Enhancement of seed germination
- improved methods of seed treatment
- different types of biostimulants and their effects

Keywords: Seed treatment, Heat Stress Tolerance, Bio stimulant, biotic – abiotic stress, seed coating technique.

INTRODUCTION

Plants do encounter various abiotic stresses during their life cycle and these stresses may have a significant impact on plant growth and productivity. Different approaches have been used to enhance plant stress tolerance. Some treatments can be particularly time-consuming (e.g., conventional breeding), while others treatments are not accepted by all countries in the

ISSN: 2348-1358
Impact Factor: 6.901
NAAS Rating: 3.77

world (e.g., plant genetic modification- which involves adding a specific stretch of DNA into a plant's genome like golden rice). Seed priming could be an alternative way to prepare plants to counteract more successfully abiotic stress conditions [Filippou et al., 2013]. Recent studies say that different molecules have the potential to act as a biostimulant against different abiotic stresses. The application of amino acids, hormones, reactive oxygen-nitrogen–sulfur species, or just water can also be effective in enhancing plant tolerance to different abiotic stresses [Savvides et al.,2016]. For both the academic and seed industry the search for a new substance able to act as a biostimulant has become an important target. Biostimulants could play a key role as a seed-treatment agent [Masondo et al., 2018]. modified biostimulants could be complex mixtures derived from raw materials of greatly diverse origin, including extra waste from food and paper industries. It's considered safe for the environment and possesses a broad spectrum of biological activities [Bulgari et al., 2019].

BIOSTIMULANTS

Biostimulants could be a natural or synthetic materials that can be applied to seeds, plants, and soil. These materials cause changes in vital and structural processes to influence plant growth through improving tolerance to abiotic stresses and increasing seed or grain yield and quality. biostimulants reduce the need for fertilizers. In the past 25 years, plant biostimulants have received considerable attention since these innovative products offer a potentially novel approach for the modulation of physiological processes in plants to stimulate growth, enhance stress tolerance, and increase yield [Du Jardin p. 2015]. For all these reasons, has found an important application in modern crop production [Yakhin et al., 2017]. Biostimulants could be classified depending on the mode of action and the origin of the active ingredient; while Ref [Bulgari, et al., 2015]. suggested biostimulants have been classified based on their activity on the physiological plant responses rather than on their composition. In addition, has emphasized the importance of the final impact on plant productivity which suggests that any definition of biostimulants should focus on the agricultural functions of biostimulants, either on the behavior of their constituents or on their modes of actions. In general, temperature changes significantly affect seed germination through the inhibition of radicle emergence and post-germination growth in seedlings. For these reasons, the use of biostimulants to overcome heat stress became an important method to preserve the final crop production and yield [bulgari et al., 2019].

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A Biostimulant Seed Treatment Improved Heat Stress Tolerance During Cucumber Seed Germination.

Cucumber (*Cucumis sativus L.*) is an important vegetable crop, mainly produced in Asia and Europe, also used as a model organism. Along with tomato, onion, and melon, cucumber is the most widely cultivated vegetable species in the world [newstrome et al., 2019]. now, we evaluate the potential effects of KIEM®, an innovative biostimulant based on lignin derivatives (lignosulphonates) and containing plant-derived amino acids and the nutrient molybdenum (Mo) on cucumber seed germination under heat stress conditions. More than 90% of amino acid-based biostimulants employed in agriculture are related to animal-derivative hydrolysates. while those of plant origin are less common, due to their recent introduction into the biostimulant market [Colla et al.,2017]. However, several reports that plant amino acid-based biostimulants have positive effects on seeds in the early stages of germination [Amirkhani, et al.,2017]. Moreover, Mo is also used for seed treatments and is known to be helpful during the germination process. In legumes, Mo can help the formation of root nodules, involved in nitrogen fixation, while in non-legume plants this micronutrient enhances the use of nitrates absorbed from the soil [Farooq, et al., 2012]. Appling Mo directly on seeds looks more effective than applying it on soil and it could also be involved in seed protection against abiotic stress conditions, by increasing the activity of Mo-containing enzymes [Badenko, et al.,2020].

A Biostimulant Seed Treatment Improved Heat Stress Tolerance During Rice Seed Germination.

Rice is an important food crop that is monocotyledonous Rice seed germination could determine its seedling growth and yield to some extent. Plants have evolved different mechanisms to protect and repair the damage for ensuring the seed. Preserve germination capability along with them, seasonal dormancy is a normal selection for environment tolerance [frontiersin, et al 2016]. However, mostly cultivated seeds of rice have no or zero degrees of dormancy. The mature seeds could germinate in panicles called pre-harvest sprouting or vivipary in some cultivars. Encountering suitable climate conditions, which reproduce seed yield and quality. Genes related to ABA synthesis have been proved to be involved in the pre-harvest sprouting of rice seeds [Fang J, et al 2008].

ISSN: 2348-1358
Impact Factor: 6.901
NAAS Rating: 3.77

DISCUSSION

1. Biostimulants and abiotic stresses in plant

An environmental condition that reduces growth and yield below optimum levels is defined as abiotic stress (Cramer GR et., al 2011). Abiotic stress such as cold, drought, and salt largely influence plant development and crop productivity. Abiotic stress has become a major threat to food security due to the constant changes in climate and deterioration of the environment caused by human activity. To cope with abiotic stress, plants can initiate several molecular, cellular, and physiological changes to respond and adapt to such stresses (Huang et., al 2013). Abiotic stresses may be prevented by optimizing plant growth conditions and through the provision of water and nutrients and plant growth regulators (PGRs—auxins, cytokinins, gibberellins, strigolactones, and brassinosteroids). In addition to these traditional approaches, biostimulants have been highlighted as a promoter of optimizing productivity by modifying physiological processes in plants. Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological processes in plants to stimulate growth, mitigate stress-induced limitations, and reduced yield (Yakhin OL et al 2017). The plant hormone auxin is the key regulator of many aspects of plant growth and development, including cell division and stretching, differentiation, tropisms, apical dominance, senescence, abscission, and flowering. The cytokinins are mainly responsible for cell division, besides affecting many other processes, such as vascular development, apical dominance, and nutrient mobilization, especially when interacting with auxins (Teale WD, et al 2006). Gibberellic acid has a marked effect on the seed germination process, activating hydrolytic enzymes, such as α-amylase and protease, which actively act in the unfolding of the reserve substances, facilitating the mobilization of the endosperm. In addition, they promote the breakdown of dormancy, stem elongation and growth, cell division, and, consequently, leaf expansion (Taiz L, et al 2009). According to (Santos CGM, biostimulant is composed of cytokinin, indolebutyric acid, and gibberellic acid, applied in seed, increasing the seedling emergence percentage of *Gossypium hirsutum* L., as well as leaf area, height, and growth of seedlings. The algal extract applied via leaf yielded a higher seed yield of *Glycine max (Santos CGM, 2005)*

2. Biostimulants and temperature stress in plants

Temperature stress in plants is classified into three types depending on the stressor, which may be high, chilling, or freezing temperature. Temperature-stressed plants show low germination rates, growth retardation, reduced photosynthesis, and often die. The development of temperature stress can be induced by a high- or low-temperature, the ad may depend on the duration of the exposure, the rate of temperature changes, and the plant growth stage at which stress exposure occurs. However, plants possess a variety of molecular

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mechanisms involving proteins, antioxidants, metabolites, regulatory factors, other protectants, and membrane lipids to cope with temperature stress (Kai H, Koh I. 2014). The temperature factor can be a relevant obstacle to the germination and early development of many horticultural species. Studies have shown deleterious effects on germination when seeds of various crops are exposed to high temperatures Biostimulants are therefore options for mitigating such effects and, by presenting defensive properties against abiotic stresses, such as drought, salinity, and high variation of temperatures; they can alleviate the plant defense system of such stressors [Du Jardin p. 2015].

Increasing doses of Stimulate® biostimulant (0, 4, 8, and 12 mL L−1) as a thermal stress reliever (temperatures 25 and 40°C) on germination and initial growth of melon favoured the germination rate by the increase of the doses of biostimulant at both temperatures (vendruscolo EP, et al 2016). Thus, the biostimulant can be used to improve the germination of the melon in high-temperature conditions and to improve the initial development of the melon in regions that present high temperatures. The research was conducted to determine the effects of two biostimulants (humic acid and biozyme) or three different salts (NaCl) concentrations on parsley, leek, celery, tomato, onion, lettuce, basil, radish, and garden cress seed germination at 10, 15, 20, and 25°C. It resulted that two applications of both biostimulants increased seed germination of parsley, celery, and leek at all temperature treatments. In addition, interaction among biostimulants and temperatures was significant
in all of the vegetable species (Yildirim E, et al 2002). The effectiveness of a product obtained from the enzymatic hydrolysis of porcine hemoglobin (PHH) as a biostimulant that lessens the effect of thermal stress in plants, was observed by two experiments carried out in which lettuce plants were subjected to short-term episodes of intense cold and heat, with different doses of PHH. The results showed that at the highest tested doses, the PHH product ameliorated the negative effects on lettuce growth caused by the increase in temperature and lessened the harmful effects of the cold, i.e., promoted a reaction that lessened the harmful effects caused by the intense cold and heat treatments (Polo J, et al 2006). Evaluating PHH, specifically porcine blood, on strawberry plants in the initial growing stages after being transplanted and subject to conditions of intense cold, an experiment was carried out to compare two doses of PHH with a commercial biostimulant (CB) and a control treatment (C). The results showed that the highest dose of PHH produced more biomass of newly formed roots, that both doses of PHH produced early flowering, and that both doses of PHH led to a significant increase in the early production of fruit compared with the C treatment. None of the biostimulant treatments improved the survival ratio of the strawberry plants compared with the control treatment. (Marfa O, et al 2009).

Plant thermal acclimation mechanisms include the accumulation of compatible N-rich solutes, such as amino acids, that confer stress tolerance. Thus, to assess the effect of exogenous amino acids treatments, several experiments with plants (lettuce and ryegrass), subjected to three different types of cold stress, were conducted by applying an amino acid product obtained by Enzymatic Hydrolysis (Terra-Sorb® Foliar). Results showed that treated lettuce plants have a higher fresh weight than control plants, exhibiting a higher stomatal

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conductance, which implies productive improvements. In addition, at a high temperature (36°C), ryegrass treated with Terra-Sorb® Foliar showed a superior photosynthetic efficiency (Fv/Fm) and maintains higher levels of chlorophylls and carotenoids. These findings suggest that Terra-Sorb® Foliar has a similar effect to natural plant amino acids and promotes a better prompter crop recovery from temperature stress. (Botta A. 2009).

A major concern in turfgrass management is the summer decline in turf quality and the growth of cool-season grass species (Xu Y, Huang B. 2010). Based on this, these researchers investigated whether foliar application of tranexamic-ethyl (TE) and two biostimulants (TurfVigor and CPR) containing seaweed extracts would alleviate the decline in creeping bentgrass (*Agrostis stolonifera* L.) growth during the summer months and examined the effects of TE and the biostimulants on leaf senescence and root growth. Foliar application of TE resulted in significant improvement in turf quality, density, and chlorophyll content compared with the control. Both TurfVigor and CPR significantly improved visual quality by promoting both shoot and root growth. This study suggests that the proper application of TE and selected biostimulants could be effective to improve the summer performance of creeping bentgrass. Perennial ryegrass plants treated with a product-based protein and exposed to prolonged high air temperature stress exhibited both an improved photochemical efficiency and membrane thermos ability than untreated plants (Kramer PJ. 1980). These results provided consistent and interesting results and showed that foliar applications of protein hydrolysates can positively affect plant tolerance to heat stress (Kauffman GL, et al 2007). The stress protection of bacterial biostimulants to rained field crops can be of particular relevance under increasing temperatures foreseen by most prediction models of climate change. Wheat inoculated with the thermotolerant *Pseudomonas putida* strain AKMP7 significantly increased heat tolerance. Inoculated plants had increased biomass, shoot and root length, and seed size (Shaik ZA,2011). Bioactive compounds present in the seaweed extracts enhance the performance of plants under abiotic stresses. Spray applications of extracts have been shown to improve plant tolerance to freezing temperature stress. Moreover, commercial *A. nodosum* extract was also reported to promote the performance of lettuce seedlings under high-temperature stress. In addition, seed germination of lettuce was influenced by priming with *A. nodosum* extract in that germination improved under high-temperature conditions (Battacharyya D, 2015).

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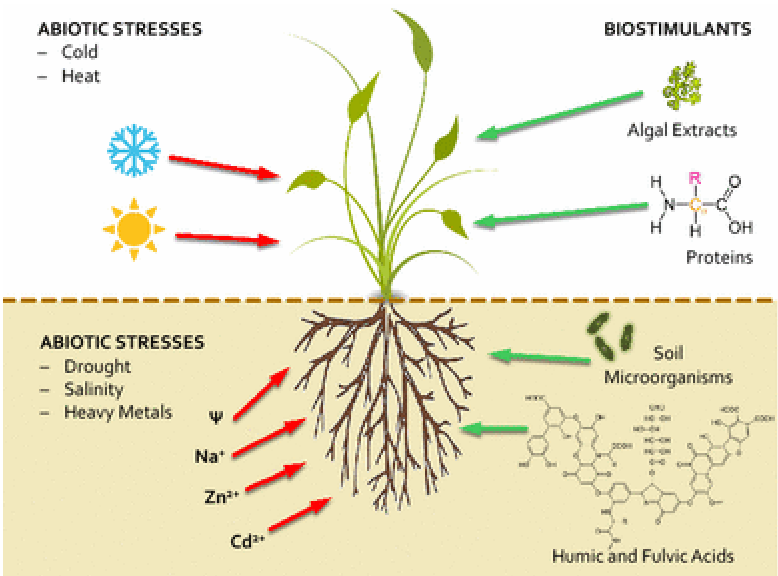

Fig.1: Role of biostimulants to avoid negative effect of abiotic stress in plant

Seed treatment on germination under different abiotic stresses Arabidopsis and Rice.

To expose seeds to heat stress, a slightly modified version of the basal heat stress method used by (Silva- Correia 2014) was applied: treated and untreated *Arabidopsis* seeds were washed and kept at 50°C in a heat block (ALB-120, Iwaki Asah Techno Glass, Japan) for 65 min and then transferred to 23°C conditions. Photographs were taken every 12 h. Maximum germination was measured at 96 h after transfer to the chamber. Treated and untreated *Arabidopsis* seeds were germinated on agar in the presence of 120 mM and 150 mM NaCl and kept vertically in a growth chamber. Photographs were taken every 12 h after transfer to the growth chamber. For rice seeds, treated or untreated seeds were germinated in water containing different concentrations of NaCl ranging from 25 to 200 mm.

Seed Treatment and Germination Parameters in cucumber.

Cucumber seeds were treated by following the protocol provided by Embrapa in 2005 (Henning, 2005) and are currently used in Brazil for seed treatment with different products, including phytochemicals and biostimulants (Dos Santos et al., 2018). This application method allowed the use of the minimum amount of product still ensuring its homogeneous distribution on the cucumber seed surface. Moreover, due to the low dosage of the product, this application method is estimated to be one of the most efficient and safe for both seeds and the environment (Braccini et al., 2015; de Andrade et al., 2018; dos Santos et al., 2018). Briefly, 2 mL of KIEMR solution was diluted in distilled water to reach the final volume of

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8mL. The KIEMR (Kannad institute of engineering and management) –the diluted solution was then added drop by drop to 2.5 g of dried seeds kept in continuous shaking until the complete and visible distribution of the product on the seed surface was obtained. Following the treatment, seeds were dried at room temperature and then placed in glass Petri dishes (20 cm Ø) containing two filter papers saturated with 15 mL of distilled water. Seeds are treated with the same protocol, but with distilled water instead of the biostimulant as controls. For both treatment and control, three replicates were used. Each replicate was composed bof100 see and ds, and placed in five different Petri dishes (20 seeds _ 5 Petri Dishes). Finally, the Petri dishes were incubated in the dark at standard (28_C) or heat stress (35_C) conditions for 24 or 48 h. At 48 h, germination percentage and fresh biomass were measured to evaluate differences between KIEMR -treated and untreated seeds. Before performing the following experiments, teguments were removed from seeds, and cotyledons were dry-blotted on filter paper.

DISCUSSION

The use of biostimulants to counteract the effect of abiotic stress has been documented and their capability to promote plant defenses against adverse environmental conditions was reported (Alzahrani and Rady, 2019). Seed treatment with bio, stimulants are a technology to counteract environmental stress at the time of sowing and improve yield, all starting From seed germination (Rady et al., 2019). This is a faster method in comparison to conventional breeding or plant genetic modification and could be useful for seed treatment in countries, where the high temperature at sowing could be a limiting factor (Savvides et al., 2016). In this work, the potential effects of the biostimulant KIEMR were tested on cucumber seeds germinated under standard (28_C) and heat stress (35_C) conditions by using different methodologies, such as morphological, biochemical, and transcriptional (qPCR) analyses. About biometric data (table 1), our results suggest a potential effect of KIEMR in promoting germination and seedling growth under heat stress. The final germination percentage was higher in KIEMR -treated seeds at 48 h after incubation at 35_C, while 99% of germination percentage was observed at 28_C (table 1) Therefore, KIEMR is not harmful to seeds and has not shown phytotoxicity as biostimulants used as other studies. (Masonda et al., 2018).

TABLE 1: Germination percentage and fresh weight at 48 h after seed incubation.

Seed treatment	Germination(%)	Fresh weight (g)
28C Untreated	100 _ 0.58a	3.11 _ 0.05a
28C Treated	99 _ 0.6a	2.85 _ 0.01b
35C Untreated	92 _ 1.5b	1.74 _ 0.02d
35C Treated	98 _ 0.6a	2.00 _ 0.02c

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Values are expressed as a mean (_SD). Different letters indicate significantly differences among samples (ANOVA, Tukey's post hoc test, p _ 0.05).

In seed physiology, reactive oxygen species (ROS) are usually considered toxic molecules, whose accumulation leads to cell injury with consequent problems in seed germination and development (Jeevan Kumar et al. 2015). However, there is increasing evidence that ROS, at low concentrations, can act as signaling molecules involved in a wide range of responses to various stimuli. (Barba-Espín et al., 2012). Such mechanisms can scavenge potentially toxic ROS, generally produced under stressful conditions, or rather tightly control ROS concentrations to regulate various signaling pathways. Among ROS, hydrogen peroxide plays a key role during the germination process however; high levels of H_2O_2 can be toxic to the seeds (Wojtyla et al. 2016).

Table 2: Few important crop and uses of biostimulant for mentioned improvement

PLANT/ CROPS	**Scientific Name**	**Biostimulants**	**Improvements**
Maize	*Zea mays*	Humic acids - are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal, and microbial residues.	1. Higher crop yield, fertilizers saving, and reduced loss under environmental conditions. 2. Increased root foraging capacity, and enhanced nutrient use efficiency.
Oilseeds	*Brassica napus*	Seaweed extracts - The use of fresh seaweeds as a source of organic matter and as fertilizer in agriculture.	1. Enhanced nutritional value, 'biofortification' of plant tissues, increased contents in S, Fe, Zn, Mg, and Cu. 2. Improved mineral composition of plant tissues.

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Cucumber	*Cucumis sativus*	Protein hydrolysate	1. Increased crop tolerance to abiotic stress. 2. Higher crop yield under stress conditions.
cotton	*Gossypium hirsutum*	Plant growth-promoting – gibberellic acid	1. Increased crop tolerance to abiotic. 2. Higher crop yield under stress conditions.
Wheat	*Triticum aestivum*	Plant growth-promoting - Rizobacteria	1. Increased root foraging capacity, enhanced nutrient use efficiency. 2. Higher crop yield, fertilisers saving and reduce loss under environmental conditions.

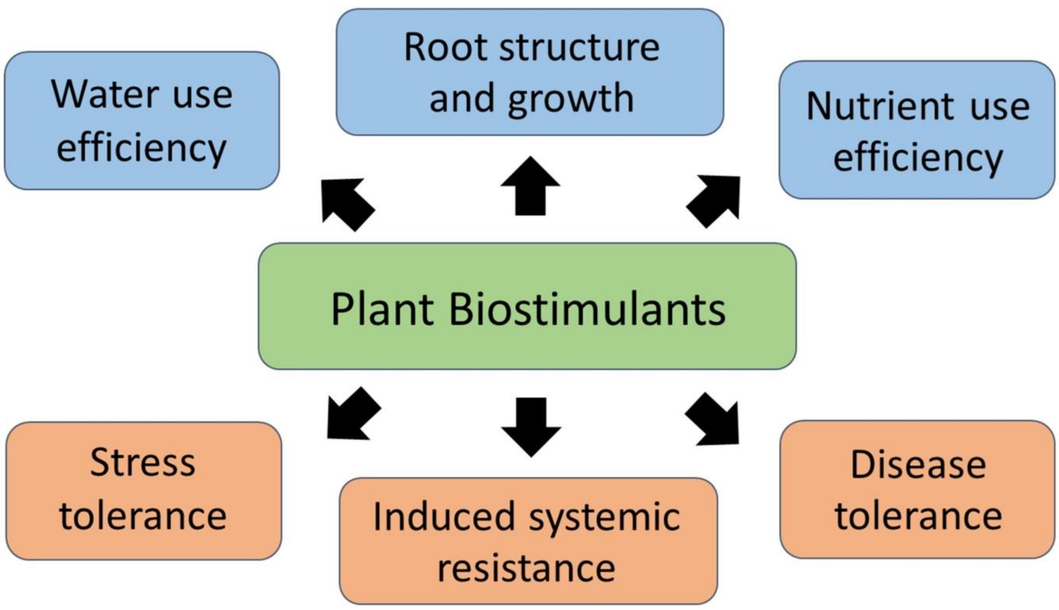

Fig.2 : Affects of biostimulants

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Techniques used for seed treatment

Seed Coating technologies

Applying a film coating on the surface of seeds. The coating may be utilized as a delivery system due to variations in its size, shape, and color. Often, some problems emerge in the discrimination of seeds and the accuracy of planting because of small-size seeds, so they are covered with a coating which is useful for protecting seeds from pests as well as Seeds coating technologies include two methods: pelleting and coating (Taylor et al., 1998).

Seed Coating Technology

A process in which a thin and consistent layer of a special material covers the surface of a seed; by doing so a suitable living environment is provided around the budding area and an advanced movement in critical growing stages will be activated. This technique was applied for the first time in 1977 in Canada. The coating technology is not able to make any changes in the genetic traits of seeds. However, regarding the financial advantages of this process, coated seeds are considered valuable products. This process is worth to be offered to farmers who farm forages and oilseeds (Gustafson, 2006). Coating technologies are the most effective, simplest, and safest methods for seeds improvement.

1) Pre-germination: once the sowing operation is accomplished, it takes several weeks or months for knots to be formed by rhizobia bacteria. However, through seed coating technology the bacteria are close enough to budding areas that lead to an early knot forming.

2) Nutrients: in the seed coating process seeds are provided with a healthy environment in which the growing energy during the initial stages will be increased.

3) Protection against stress, animals, birds, and fertilizers: most rodents and birds cannot identify the coated seed as an edible material (Gustafson, 2006). Using the polymeric seed coating technique increases the percentage of germination through direct fertilizing of the soil (Tilley and John, 2010).

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TABLE 3: POSSITIVE& NEGATIVE EFFECT OF SEED TREATMENT.

Positive effect of seed treatment	**Negative effect of seed treatment**
1) In regions where manual farming is a common method seeds may be placed either too deep or too close to the surface, so the durability of seeds and stability of the plant will be achieved through treatment. 2) In cold areas where the farming period is short, using priming can lead to the early emergence of a place, and hence, it will be provided with a proper opportunity to gain tolerance against cold weather. 3) Seed treatment enhances the tolerance of plants to any unfavorable conditions. 4) Seed treatment can make improvements in the processes of germination and seedling emergence of new plants 5) Enabling adjusting factors of plant growth, makes changes in growing, physiologic processes, enzyme activities, and absorption of nutrients which can resist non-living stresses. (Chastokolenko, 1984).	A significant decline can be observed in the life span of treated seeds compared to untreated ones (Bruggink *et al.*, 1998). proved that the deleterious impacts of seed treatment during storage time weaken the repairing activity of DNA due to advancement in the cellular cycle. An increase in fat peroxidation due to the existing active agent of auxin in absence of protective mechanisms lowers the durability of treated seeds (Bruggink *et al.*, 1998).

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Seed treatment

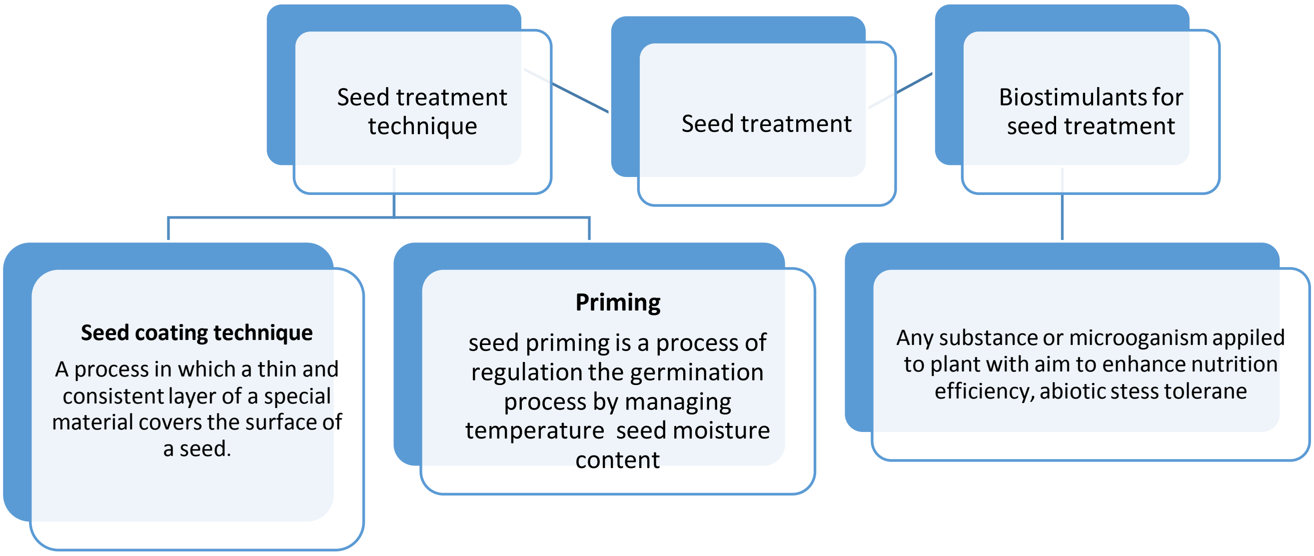

Fig: 3 SEED TREATMENT

Conclusion

There are different biotic and abiotic stress that occurs in a plant life cycle. Abiotic stress such as drought, high soil salinity, cold, and heat stress is the common adverse environmental condition that affects and reduces crop productivity worldwide. To overcome these problems, plant biostimulants are used that include diverse substances and microorganisms that enhance plant growth and resistance to abiotic stresses and also increase crop yield and seed quality. Agriculture biostimulants may contribute to making agriculture more sustainable and resilient, as there are several biostimulants that have a protective effect against abiotic stress. There are other methods of seed treatment used for improving plant growth, yield, and quality, these technique protects the plant against diseases and pest and decreases the use of fertilizers and pesticides. So the farmer can reach a crop with more quality and quantity while expending less time, cost, and effort.

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Figures:

Fig.1:
https://www.google.com/url?sa=i&url=https%3A%2F%2Fchembioagro.springeropen.com%2Farticles%2F10.1186%2Fs40538-017-0089-5&psig=AOvVaw1bZv_KuzPOJNqbfPaUpeSK&ust=1653656777808000&source=images&cd=vfe&ved=0CAwQjRxqFwoTCPDP_cOg_fcCFQAAAAAdAAAAABAD

Fig.2:
https://www.google.com/url?sa=i&url=https%3A%2F%2Fprogressivecrop.com%2F2020%2F07%2Fmaking-sense-of-biostimulants-for-improving-your-soil%2F&psig=AOvVaw1bZv_KuzPOJNqbfPaUpeSK&ust=1653656777808000&source=images&cd=vfe&ved=0CAwQjRxqFwoTCPDP_cOg_fcCFQAAAAAdAAAAABAK

Fig.3: *ibid*

