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SEED PRIMING AND FORTIFICATION OF SEEDS USING NANOTECHNOLOGY: A REVIEW

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Abstract

A new agricultural revolution is required to improve crop yield while also ensuring food quality and safety in a sustainable manner. Nano-priming affects biochemical pathways as well as the equilibrium of reactive oxygen compounds and plants growth hormones. This enhances stress and disease tolerance, resulting in a decrease in fertilizers and pesticides. Nano-priming alters biochemical systems and the balance of reactive oxygen compounds and PGR, causing stress and disease resistance and a reduction in fertilizers and pesticides. The current study gives an overview of achievements in the sector, highlighting the obstacles and opportunities for using nanotechnology in seed nano-priming to contribute to sustainable agriculture practices. Nano priming can be treated to seeds to protect them during storage, promote germination, germination synchronization, and plant development, and boost crop tolerance to biotic or abiotic stress conditions, which can assist to minimize the amount of pesticides and fertilizers needed.

Keywords: *Nano-priming, Seed germination, priming, nanoparticle, ROS, nanotechnology, nanocarriers.*

1. INTRODUCTION:

Nanotechnology, a new and exciting subject of research, is currently useful in a variety of fields. It has a wide range of applications in agriculture. Nanotechnology is defined as "the science and art of manipulating matter on a nanoscale." Nanotechnology is concerned with the manipulation of particles with at least one dimension ranging in size between 1 to 100 nanometres. [1](Kah, 2015). Pre-sowing treatment known as "seed priming" alters the physiological makeup of the seed to speed up germination. Nano-priming is a cutting-edge seed priming method that improves germinating seeds, seed growth, and yield by offering plant tolerance to diverse challenges. [2](Bruce et al., 2007). When compared with all other seed treatment procedures, nano-priming is far more effective. The main characteristics of nanoparticle in seed priming are electron exchange and increased surface reaction capabilities connected with distinct plant cell and tissue components.

2. History:

Science and technology is the result of human creativity. Since the advent of civilization and agriculture, people have come to understand that some seeds are difficult to germinate. Theophrastus (circa 372-287 BCE) established that pre-soaking *Cucumis sativus* L. seeds in water improved germination. As a result, Oliver de Serres (1539–1619) described prehydration techniques for various species before seed sowing to improve germination in ancient times. Numerous biologists have discussed the value of seed treatment for enhancing germination and physiological characteristics in the 19th century [3](Evenari, 1984). Nanotechnology is the one of the creativity of human mind which has tackled all the fields in science. Richard Zsigmondy, who coined the term nanometer and won a chemistry noble prize in 1925. He used microscope and determined the size of gold colloids, he introduced the idea of manipulation of matter at atomic level [4](Home | National Nanotechnology Initiative, n.d.). New ways of thinking were introduced thanks to this creative strategy, and Feynman's theories were later confirmed to be accurate. He is regarded as the founding father of contemporary nanotechnology for all of these reasons. Nearly 15 years after Feynman's lecture, the Japanese scientist Norio Taniguchi coined the term "nanotechnology" to describe semiconductor operations on the order of a nanometer. He advocated the notion that materials could be altered, isolated, stabilised, and deformed using just one atom or one molecule [5](Taniguchi, N. (1974) *On the Basic Concept of Nanotechnology. Proceedings of the International Conference on Production Engineering, Tokyo, 18-23. - References - Scientific Research Publishing, n.d.*).

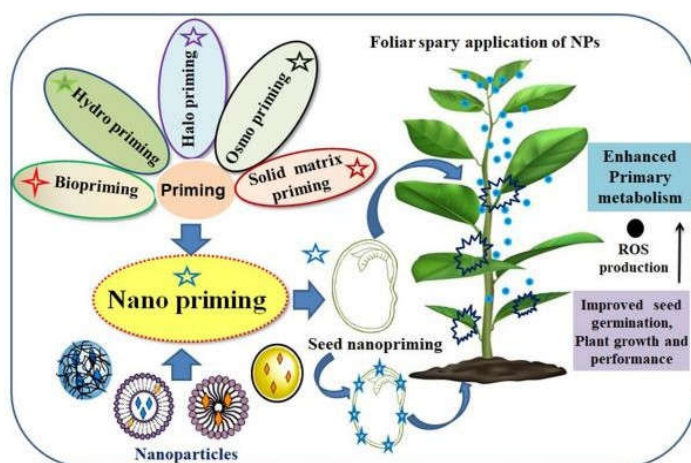


Fig1. Types and applications of seed priming [13](Nile et al., 2022).

3. Seed Priming:

It is a pre sowing procedure that alters the physiological makeup of the seed to speed up germination. Additionally, it promotes plant resistance to biotic and abiotic stresses, which increases crop activity. Priming is the practise of curing seeds before planting them using age-old techniques like pre-soaking and coating [2][6](Arnott et al., 2021; Bruce et al., 2007). By revealing seeds to solutions containing salt (halo-priming), water (hydro-conditioning), osmotic agent (osmo-priming), phytohormones solutions (hormonal priming), useful microbe solutions (bio-priming), a liquid that contains a solid carrier (matri conditioning), and solutions nano - particle (NPs), seed dormancy can be reduced (nano-priming) [7][8](de La Torre-Roche et al., 2020a; Waqas et al., 2019). For enhancing the performance of the crop's growth, emergence, and yield, seed priming uses a variety of ways. Techniques including hydro-priming, halo-priming, osmo-priming, and hormonal priming are employed.

3.1. Hydro-Priming: Hydro-priming entails immersing the seed in water prior to planting, then air-drying the seeds may or may not come next. Detrimental environmental factors for seed germination and emergence are a major contributor to poor stand establishment and low crop production in many agricultural settings. However, quickly germinating seedlings may emerge and develop deep roots before the topsoil dries out and crusts, which could lead to better crop establishment and a higher crop yield [9](Pill & Necker, 2001). Seed quality includes easily quantifiable characteristics such as suitability, seed lot pureness, health, and structural stress, but it also includes the more ambiguous trait of seed

vigour [10](PERRY, 1980). Some plant species should not be soaked because the rapid staying hydrated may cause the seed to lose vital nutrients, damaging the seed. Numerous strategies have been developed to properly hydrate the seed in order to avoid these potential issues. One approach is seed humidification, a pre-sowing procedure in which seed is treated in an environment with high humidity. Some plant species should not be soaked because the rapid staying hydrated may cause the seed to lose vital nutrients, damaging the seed. Numerous strategies have been developed to properly hydrate the seed in order to avoid these potential issues. One approach is seed humidification, a pre-sowing procedure in which seed is treated in an environment with high humidity [11](Suzuki & Khan, 2000).

3.2.Halo Priming: Halo priming is the practise of immersing seeds in a solution of inorganic salts, such as NaCl, KNO₃, CaCl₂, and CaSO₄. In salt-affected soils, halopriming significantly improved germinating seeds, seedling emergence and introduction, and final crop yield, according to a number of studies. Establishing the seedlings is crucial for the plant's better growth and development, whether it has been primed or not. At every stage of a plant's development, halopriming is crucial for plant growth, seed emergence, and plant growth. Under salt-stress conditions, it was found that paddy seed treated with a mixed salt solution germinated noticeably more quickly than unprimed seed [12](Chang, 2002). Using NaCl and KCl as a primer helped to counteract salts' harmful effects. Under salt-stress conditions, paddy seed allowed to treat with a mixed saline solution germinated more quickly than untreated seed. Halopriming encourages seed germination while also stimulating subsequent growth, increasing crop yield overall [12](Chang, 2002).

3.3.Osmopriming: The term "osmo-conditioning" or "osmotic conditioning" also applies to this. In this method, seeds are air dried before planting after being submerged in sugar, poly(ethylene (PEG), glycerol, sorbitol solutions for a predetermined amount of time. Osmo conditioning of Italian rye grass and sorghum seeds with 20% PEG-8000 for two days at 10°C improved germination percentage, water-stressed, flooded, cold-stress, or saline conditions. Osmo conditioning of Italian rye grass and sorghum bicolor seeds with 20% PEG-8000 for two days at 10°C improved germination rate, water-stressed, waterlogged, or saline [14](Hur, 1991). It was shown that osmotic stimulation of tomato seeds boosted endobeta-mannanase activity in the embryonic cap and decreased its mechanical inhibition of the germination embryo. Lowering the mechanical constraint was shown to be strongly correlated with endo-beta-mannanase activity. Amylases, proteases, and, occasionally, lipases are just a few of the enzymes that are essential to the embryo's early growth and development [15](Maciej Serda et al., 1998). Osmopriming also can increase seed germination by lowering endosperm's mechanical constraint on the growing embryo.

3.4.Hormonal priming: The pre-seed application of various hormones, including as salicylic, ascorbate, kinetin, etc., to encourage the development and growth of the seedlings is known as hormonal priming. Wheat seeds were exposed to sodium salicylate (0.6 mM) or vitamin c and thiamin (0.3 mM) while under salinity stress at 120 and 160 mM NaCl. [16](Hamada, 2001). Abscisic acid (ABA), salicylic acid (SA), and ascorbic acid were used to treat the wheat cultivar Auqab-2000 before it was sown under normal and saline conditions (15 dSm⁻¹). Results showed that under saline conditions, these treatments reduced the time for 50% germination, increased germinated seeds count, and significantly increased fresh and dry weight, but ascorbic acid did not exhibit these effects. Hormonal priming has lessened the severity of the effects of salinity, but the amelioration was good due to 50 ppm SA and 50 ppm ascorbic treatments because these demonstrated the best results on seedling growth, both fresh and dried weights under non-saline and salt water conditions, whereas hormonal priming with ABA was ineffective under the current experimental material and conditions [17](Afzal, I., Basara, S.M.A., Faoq, M. and Nawaz, A. (2006) *Alleviation of Salinity Stress in Spring Wheat by Hormonal Priming with ABA, Salicylic Acid and Ascorbic Acid. International Journal of Agriculture and Biology*, 8, 23-28. - References - Scientific Research Publishing, n.d.).

4. Nano particles in seed priming: Nanomaterials, mostly nanoparticles, are used in the novel process known as seed nano-priming. Seed nano-priming and seed priming are fundamentally different from one another since traditional seed priming often use hydro-priming with micronutrients, enzymes, or biopolymers that can adsorption on the seed and produce seed coating. Seeds may or may not absorb the nanoparticles in the suspensions or nano formulations used in seed nano-priming [18][19](*Sci-Hub | Green-Synthesized Nanoparticles Enhanced Seedling Growth, Yield, and Quality of Onion (Allium Cepa L.). ACS Sustainable Chemistry & Engineering | 10.1021/acsuschemeng.9b02180*, n.d.). Different nanomaterials, such as biogenic metallic, polymeric nanoparticles, metallic, also have demonstrated promise for seed nano-priming, which would stimulate plant development and enhance morphological and metabolic features. By altering the transcription of genes that regulate metabolic activities including the generation of phytohormones, this mechanism can encourage rapid root and shoot growth. By raising antioxidant potential and enzyme activity, seed nano-priming modifies the defence system's function, making plants more resilient to pest and other abiotic and biotic challenges in the field. [20,21,22,23,24](Abdel Latef et al., 2016; Duran et al., 2017; Kasote et al., 2019; Li et al., 2019).

4.1.Nanoparticles and Nanocarriers system: Two categories of nanoparticle applications will be considered: 1. active nanoparticles and 2. controlled release nanocarrier systems.

4.1.1.Active nanoparticle: Active nanoparticles are those that have the potential to influence biological processes by acting as stimulants, anti-pathogens, or both. Nanoparticles can be loaded with active ingredients, whether they are

present in the nanoparticle itself or not, and these systems allow for continuous release of the substance throughout time. Metallic nanoparticles are typically less than 100 nm, including those created utilising biogenic processes. As many of these systems are created with metals that are crucial for plant metabolism and biofortification, they have garnered special consideration for seed nano priming [25, 26](Das et al., 2018; de La Torre-Roche et al., 2020b). Additionally, because of their tiny diameters and increased ability to activate secondary metabolites, biogenic metallic and metallic nanoparticles have a stronger effect on embryo development.

4.1.2. Mechanism for Sustained Release Nano-carriers: Potential for seed priming has been demonstrated by nanocarrier technologies for plant growth regulators, offering enhancements from seed germination through production using lignin nanoparticle loaded with gibberellic acid in tomato. Polysaccharides, lipids, and proteins may be combined to create biopolymeric nanoparticles, which are biocompatible and biodegradable and can be engineered to react to a variety of environmental cues. [32][33](Camara et al., 2019; Fraceto et al., 2016). Metal micronutrients can be slowly released into the body by using biopolymeric nanoparticles as carrier systems. Hydrolytic enzymes were activated in maize seeds as a result of priming with zinc-loaded chitosan nanoparticles, promoting germination. Another biopolymer utilised to create nanocarrier systems for agricultural purposes is chitosan. This polymer works in plant metabolism and possesses fungicidal characteristics, which activate protection systems. [34](Fischer et al., 2019).

Table 1. Nanoparticles used for coating and priming seeds, displaying their properties and the primary impact they have on the species under consideration.

Nanoparticle	Characteristics	Concentration	Effects	Reference
Lignin nanoparticle encapsulated with gibberellic acid	Size is around 200-250 nm and spherical morphology	1mg/ml in tomato 1.5mg/ml in chickpea	Increased in seed vigour	27(Falsini et al., 2019)
Chitosan nanoparticle	Particle with the size of 259nm	1-100 µg/ml in wheat	Auxin activity, plant morphology increased	28(Li et al., 2019)
Biogenic gold nanoparticle synthesized with rhizome extract	Spherical nanoparticle with size of 50 nm	10-15 ppm in maize	Seed and seedling vigour, biochemical activity increased	29(Mahakham et al., 2016)
Biogenic silver nanoparticle with kaffir leaf extract	Spherical nanoparticle with size of 6-26 nm	10-20 mg/ml in rice	Water uptake, enzyme activity, seed vigor increased	30(Falsini et al., 2019)
Manganese nanoparticle	Spherical nanoparticle with size of 10-30 nm	0.5-1 mg/ml in capsicum	Salinity resistance, Antioxidant enzymes increased	29(Mahakham et al., 2016)
Iron oxide nanoparticle	Particle size <50 nm	50-100 mg/L in sorghum	Seed vigor, biomass, water content in leaves increased	31(Maswada et al., 2018)

5. Impacts of Seed Nano Priming on Biotic and Abiotic Stresses on Plant Metabolism:

Reduced output and financial losses result from biotic and abiotic stressors. Environmental elements including heat, cold, salt, drought, floods, and nutrient-poor soils are examples of abiotic stressors. Microbial diseases (bacteria or fungus), pests, or plants that compete for nutrition are the sources of biotic stressors. [33](Camara et al., 2019). Seed nano-priming can enhance seed quality and boost tolerance to stressful situations. Nanoparticles have the ability to directly combat infections and modify seed and plant metabolism, which strengthens the native immune system, changes hormone synthesis, and increases plant resistance to diseases and abiotic stress. [35](Panyuta et al., 2016).

5.1. Biotic Stress: During agricultural techniques, different periods call for varying levels of seed protection. In order to prevent insects, fungus, and bacteria from harming seeds and reducing seedling growth in the field, protective measures must be taken. The natural immune system of the crops was altered in millet seeds containing chitosan nanoparticles, increasing the plants' resistance to infections. The remedy boosted the production of proteins related to the salicylic pathway, which is connected to plant defence mechanisms against biotrophic infections and raised antioxidant enzyme levels (Siddaiah et al., 2018). In order to reduce the growth of germs during grain storage, techniques used may include regulating temperature and humidity. Chemical items may also be used, however customers who prefer natural goods may not be in favour of them.

5.2. Abiotic stress: Salt content and pollution of the soil can significantly reduce crop yield. Because of water and nutrient deficiencies, direct ionic effects on plant metabolism, and salinity, plant development is inhibited (Maswada et al., 2018). In salty circumstances, jalapeno peppers seeds primed with manganese nanoparticles showed better germination and root elongation. They also reduced salt stress by successfully controlling the distribution of sodium among roots and shoots through regulation of the oxidative stress. (Ye et al., 2020) To avoid the negative consequences of the current global climate crisis or manmade and geogenic effects, and to minimise losses in field output, seed nano-priming is a powerful option.

6. Impact of Zinc oxide nanoparticles on wheat seed seedling and germination development: Due to an increase in reports of zinc shortage in both humans and edible plants, zinc, a crucial mineral for biological metabolism, is attracting attention on a global scale. Both people and plants need it for regular growth and development.

6.1 Treatment of seeds and the formation of ZnO nanoparticles: This report is taken from - (Solanki & Laura, 2018) in which they showed the ZnO nanoparticles effects on wheat.

Utilizing sodium hydroxide (0.2M) and zinc sulphate (0.1M) in methanol, ZnO nanoparticles were created utilising straightforward chemical techniques. A UV-maxima peak of 350 nm was generated in the form of 40–50 nm nanoparticles. The treated prepared ZnO (40–50 nm) were suspended in water. The CCSHAU Regional Centre in Rohtak was where they purchased the wheat seed of variety WH-711. To reduce seed germination mistake, the healthier seeds were chosen. With 2percentage sodium hypochlorite and two rinses in distilled water, seeds were surface sterilised. Five ZnO NP doses (250, 500, 1000, and 1500 PPM) as well as commonly used fertilisers like ZnSO₄ and ZnO, together with Control, were included in the experimental treatments.

6.2. Seed germination: The petri-dishes (100mm x 15mm) coated with two layers of sterilised filter paper contained 10 seeds of similar size. The petri dishes received 10 ml of the relevant solution or suspension and were then incubated at 26 °C for 12 days. Five days after incubation, the % germination was calculated. The petri dishes were taken out after 12 days, and measurements of the root growth, shoot length, fresh weight, and dried weight were made.

6.3 Result and Discussion: Zinc in Nanoparticles form has shown positive effect on seedling growth over control. NPs of 40-50nm were used along with the Bulk ZnO and ZnSO₄ at different levels of concentration. The NPs at a concentration of 500PPM had highest positive effect in terms of enhanced seedling growth.

6.3.1. Seed germination: ZnO nanoparticles at a rate of 500 PPM produced the highest germination percentage, which was 99%. It was also shown that ZnO and ZnSO₄ Aggregate have a lower performance in increasing seed germination when compared to ZnO NPs. These results agree with previous reports of a percent germination increase with the treatment of ZnO NPs to *Cicer arietinum*.

6.3.2. Seedling growth: The treatment of ZnO NPs at a rate of 500 PPM resulted in the longest shoot length of 13.6 cm. With the exception of ZnO Nanoparticles at 1500 PPM, further increasing the zinc concentration resulted in a decrease in shoot length. The greatest root length was reported with ZnO NPs at 500 PPM concentration.

6.3.3. Chlorophyll content: At a concentration of 500 PPM, ZnO NPs had the greatest total chlorophyll content. In ZnO NPs, the total chlorophyll concentration was found to be 280% greater than the control.

6.3.4. Seed vigour: In general, all of the ZnO solutions were shown to be considerably superior to the no-zinc control. ZnO NPs at 500 PPM had the greatest seedling vigour rating of germinated seeds when compared to other Zn treatments.

7. Current status of nanotechnology: Nano priming could be applied to seeds to protect them during storage, promote germination, germination synchronisation, and plant development, and boost crop tolerance to biotic or abiotic stress conditions, which can assist to minimise the amount of pesticides and fertilizers needed. Although studies have demonstrated encouraging results, the application of nanotechnology for seed priming is a new field of research. Seed nano-priming can also be utilised for seed protection since many nanoparticles have antibacterial properties and can carry antimicrobial agents. Furthermore, nano-priming may be utilised to target biofortification of seeds in order to encourage an improvement in food quality and output. (Pereira et al., 2021)

8. Challenges of seed nanotechnology: The barrier between fundamental research and application. The implementation of nanotechnology is hampered by high costs and risks, as well as a lack of technical understanding. To create nanomaterials in sufficient quantities with good standard and at a reasonable cost. To provide these nanomaterials in a form (such as correct particle diameter, surface chemistry, compatibility, etc.) that allows integration into processes. (Pereira et al., 2021)

9. Future scenario: Because nanotechnology promises improvements in agricultural methods, great care must be taken to ensure that it does not tamper with the environment. The application of Np must be limited to natural thresholds. Newer targets should be established to investigate the depth of the whole germination process regulated by NPs. It is necessary to examine the entire growth spectrum in terms of germination of old seeds. Because there has been few research in this domain, the robustness of technology is determined by how quickly it can be translated to sectors with reproducible findings. (Pereira et al., 2021).

10. Conclusion: Nanotechnology is a potential field for agricultural use, and seed nano priming is one method that may be used to increase sustainability. The use of nano-based advanced technologies for seed treatment has the potential to shift traditional agriculture away from the use of agrochemicals and toward more sustainable farming, once these systems can advertise plant establishment and provide protection against abiotic and biotic stresses, resulting in higher yields

and food quality. All of these elements together could lead to a system that is safer for producers and customers, as well as the environment, by avoiding the ongoing damage caused by traditional agriculture.

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