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Nanoparticles NPs stresses as *In vitro* plant innovative tool

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Abstract

The use of nanoparticles is one of the important applications that can affect the global economy, industry and people positively, with the global development taking place in both agricultural production and food industries, and for this the importance of developing innovative means to advance agricultural production has become evident and the food industry by diagnosing and treating diseases, and improving the ability of plants to absorb elements and resist infections by various pests and pathogenic microorganisms. To ascertain the nature of the positive change in the growth of plant organs grown in the presence of Nano catalysts, it has become imperative for researchers to understand the negative dimensions of these stimuli, as is the case with the positives envisaged by their use. Most of the concerns that were raised on this subject came with new proofs that opened the door for botanical researchers to test these small-sized nano-chemical elements and compounds to be a new generation of mutagenic substances that can cause changes at the DNA level.

Keywords: Nano Particles; Tissue Culture; Abiotic Stress; Agriculture; Plant Biotechnology.

1. Introduction

Agriculture is the backbone of developing countries, as more than 60% of the world's population depends on it for their livelihood, and nanotechnology has the potential to revolutionize the agricultural and food industry with new tools for molecular management of diseases, rapid detection of diseases, and enhancing the ability of plants to absorb nutrients As well as developing systems to monitor environmental conditions and the need for pesticides, New materials to detect pathogens and protect the environment are important examples of nanotechnology for the science and engineering of agricultural and food systems. Nanotechnology works at the same level with viruses or germs that cause diseases and thus has the ability to detect and eliminate them initially. Long before symptoms appear on plants, nanotechnology will indirectly protect the environment through the use of alternative (renewable) energy supplies, filters or catalysts to reduce pollution and get rid of pollutants in soil and water (Wheeler, 2008).

2. Nanotechnology in agriculture

Population growth and climate change have an impact on food security and agricultural production as well as biotic and abiotic stress that poses a threat to the global environment. Therefore, botanists are interested in promoting environmentally friendly methods using nanoparticles (NPs) due to their unique physical and biochemical properties in addition to their role in various scientific applications (Kumari et al. 2022). Since the advent of nanotechnology, this science has focused on its applications in agriculture as an emerging field of research. In the past decade, new methods of manufacturing nanomaterials from plants have been explored to reduce their toxic and chemical environmental impact. The biosynthesis method has been proposed and its performance has been tracked on physiology, absorption, mechanisms of action, and plant stress responses, Despite the good applications of nanoparticles and biosynthetic as a new agricultural practice to overcome any difficulty in maintaining grain production worldwide and enhancing essential

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nutrients and reducing toxic elements in the edible parts of grains for human health. However, the side or harmful effects of these substances on crop physiology, their effect on grains, and their mechanisms of action are still under study, as metal nanoparticles (silver, gold, and iron) and metal oxides (titanium dioxide, zinc oxide, and iron oxides) were reviewed (Ovando et al. 2022). In the rice plant (*Oryza sativa* L.), the bioavailability of nutrients such as carbohydrates and proteins increased when certain changes were made in the germination process. After adding levels of nanoparticles, some bioactive compounds such as kappa-aminobutyric acid (GABA) and antioxidants increased, as well as increased levels of fiber (Nascimento et al. 2022).The plant scientific community followed with interest the capabilities offered by nanotechnology in various fields of life until this technology entered several applications inside the plant because of its effects on the level of living cells, as its ability to enter the parts of the cell because of its size and physical and chemical properties that improve growth In some cases, this technology has made this technology a new path for plant research, especially its use as stress catalysts. This technology was included in the research aimed at increasing the production of secondary metabolite compounds in a number of plants. The research indicated the positive effect to a certain extent in the production of secondary metabolite compounds (for specific compounds) by tissue culture technology in the presence of nano stress factors (nano chemical compounds or elements).

3. Nano technology

Pena et al. (2020) refer to the use of nanotechnology to control bacteria that attack stored and canned food, and then preserve food for a longer period. Nanotechnology has shown its ability to genetically modify crop plants, as the desired DNA is delivered to plants through the use of nanomaterials through nano capsules (Kim et al., 2017). Nanomaterials also helped improve vegetable crops through the use of nano fertilizers (elements loaded on nanoparticles). It should also be noted that there is a danger to these nanomaterials, Where they are able to penetrate the cell wall and cell membranes of plants, and can even be transmitted to soft tissues and from there also to other organs inside the cell (DeRosa et al. 2010).

4. Green nanotechnology/phyto-nanotechnology

Green nanotechnology plays an important role in facilitating environmentally friendly methods using synthesized green NPs, which has received great attention in the development of resistance to abiotic stress in plants. have the ability to synthesize a wide range of NPs such as Pd, Fe, Pt, Au, Ag, Cu, Zn, and Se into various plant parts such as leaves, stems, roots, peels, bark, flowers, fruits, and seeds. The root acts as an essential part of the plant that maintains the transport of substances, its fixation in the soil, the interactions of roots with microbes and the absorption of nutrients. All these qualities and characteristics of the roots affected their effective role in green nanotechnology found that the reducing ability of oxalic acid, ascorbic acid, phenylpropanoids, zingerone, gingerol, shagaols, and paradol present in Zingiber officinale root extract helped the formation of silver NPs (AgNPs) and gold NPs (AuNPs). Titanium dioxide NPs (TiO2NPs) were also synthesized from *Withania somnifera* root extract, which contains the bioactive metabolites namely withanolides, sitoindosides, amino acids and flavonoids. The leaves are an excellent source for the manufacture of NPs. The synthesized bimetallic (Ag-Cu) NPs were eluted using Vitex Negundo leaf extract, The compound platinum NPs (PtNPs) were obtained using Diospyros kaki papers with a reaction temperature of 95 °C and a concentration of 10%, which played a positive role in controlling the size of the synthesized NPs in the range of 2–12 nm (Kumari et al. 2022). The stem is also a prominent source for the biosynthesis of NPs. Silver NPs were extensively synthesized using stem extract of Miswak plant such as *Salvadora persica*, zinc oxide NPs (ZnO NPs) were synthesized using *Mussaenda frondosa* stem extract inhibiting amylase and glucosidase enzymatic activities, which indicates its role in the treatment of diabetes. Similarly, an extract of the stem of *Swertia chirayita* was used for the synthesis of ZnONPs and was found to be effective in terms of enhanced structural, high photocatalytic and antimicrobial activity (Sa ha et al. 2021).

5. Nano applications in plant tissue culture

Nanoparticles have become one of the most important materials on which modern scientific studies are based in various scientific branches. This may be due to the characteristics that these small particles enjoy in terms of size and shape, as well as the method of their distribution. This type of research (which is based on nanomaterials and their applications) is developing. rapidly (Jain et al., 2009). A number of researches were conducted in the field of plant tissue culture, which relied on the use of nanotechnology in areas such as seed germination, plant growth improvement, genetic modification, and crop protection, as well as other fields (Wang et al., 2016, Nedecky et al., 2017, Parzymies, et al., 2019 and Pour et al., 2019). Siddiqui and Al-whaibi (2014) showed that the treatment of tomato seeds with 8 gm. L^{-1}SiO2 nanoparticles increased the percentage of seed germination, germination time, germination coefficient, and seed germination strength coefficient, in addition to the fresh and dry weights. In an experiment conducted to find out the effect of the 24 nm gold nanoelement (10, 80 µg per ml) added to the MS nutrient medium on the germination of rock cress seeds or *Arabidopsis thaliana* L. the improvement of germination of germinated seeds increased threefold compared to the control treatment. In addition to the improvement in plant growth as well as the production of free radicals (Kumar et al., 2013), Kim et al. (2017) mentioned in their study the great success of silica nanomaterials in reducing the incidence of bacterial infection in the cultures of a number of different plants outside the living body, as well as improving the growth of the cultivated plants, as indicated by the presence of genetic variations Somaclonal Variation. In another study, different concentrations of silver nanoparticles (0, 25, 50, 100, and 200) mg were used. L $^{-1}$, which was added to the MS medium when growing *Vanilla planifolia*, where the re-vegetation and growth of the cultivated branches decreased, and the percentage of bacterial contamination in them at concentrations of 50, 100 and 200 mg. L^{-1} While the concentration is 25 mg. L^{-1} was sufficient to cause an increase in the growth of branches resulting from re-sexing (Santoscoy et al., 2017),In the same context, Helaly et al. (2014) found when planting banana branches on the MS nutrient medium that was fortified with both zinc and zinc oxide nanoparticles at concentrations of 50, 100 and 200 mg. L^{-1} Nine types of bacteria and some fungi, which were diagnosed in the control treatment, disappeared after the addition of nanoparticles, compared with plants grown in the control treatment, which died due to bacteria and fungi. It is another result obtained by the researcher in the treatment of 100 mg. L $^{-1}$ of Zn and ZnO had a good re-crossing in addition to the formation of good branches and roots, which led to the success of 98% of the plants in crossing the acclimatization stage. Upon examination of the tested plants after a month of treatment, it was found that they contained larger quantities of chlorophyll dye as well as proline. In another study to find out the effect of silver nanoparticles by interfering with specific combinations of growth regulators, the combination was achieved between 10 mg. l^{-1} Ag NPs and 2.5 mg. L^{-1} BA and 0.1 mg. L^{-1} of IAA added to the MS medium in the cultures of Farfarin or Rohida *Tecomella undulata*, The highest number of branches formed, the highest percentage of plant parts that produced new branches, the highest fresh weight of cotyledons, and the highest number of resulting plants, and this is due to the effect of this combination in reducing the production of ethylene. The researcher also confirms that the nano silver treatment is 60 mg. L^{-1} had clearly reduced the growth of branches, meaning that the stimulating effect was reversed to an inhibitor at high concentrations of silver nano treatment (Aghdaei et al., 2012).In another scientific experiment targeting the copper nanosulphate compound (CuSO4), Genady et al. (2016) tested adding copper nanosulphate to the MS food medium at concentrations of 5, 10 and 15 mg. L^{-1} for the development of shoots of *Verbena pinnatifida*. The results showed the excelled of the 5 mg treatment. L^{-1}, as it achieved an increase in the height of the branches that reached 52% compared to the control treatment, as well as an increase in the length of the roots amounted to 21% compared to the control treatment, and an increase in fresh weight amounted to 39% compared to the control treatment, and in this experiment also the phenolic compounds increased in the plant with increasing concentrations of CuSO4 nanoparticles. In another experiment to study the effect of different concentrations of silver nanoparticles added to the MS diet on the production of blackcurrant callus *Solanum nigrum* L. They are (0, 2, 4 and 8) mg in sequence. L^{-1} Treatments with low concentrations of silver nanoparticles produced a greater amount of callus produced as well as a greater fresh weight, but with increasing concentrations of silver nanoparticles, callus formation decreased (Ewais et al. 2015).Sereda et al. (2016) studied the effect of adding nanoscale copper (0.5) mg. L^{-1} and Nano Cobalt (0.8) mg. L^{-1} to the MS nutrient medium, as the results confirmed a significant improvement in each of the number of branches, their height, and root formation in *Mentha longifolia*. Anwaar et al. (2016) concluded that after adding naturally produced CuONPs from rice plant leaf extract, ex vivo callus formation in rice plants increased significantly up to a concentration of 10 mg. L^{-1} As for concentrations higher than 10 mg. L^{-1} has led to a decrease in callus formation and tissue death. This effect has been due to the great importance of the copper element in various vital activities and plant growth, as it enters as a catalyst in a number of proteins as well as enzymes that regulate a number of these important vital activities. Ebadollahi et al. (2019) concluded that callus produced In vitro under exposure stress to nanomaterials (TiO2) was able to produce essential oil compounds (hypericin and pseudo hypericin) better than callus untreated with nanomaterials in *Hypericum perforatum*. In the same context, Kavianifar et al. (2018) reported that low concentrations of nanocomposites ZnO, SiO_2, and Al_2O_3 were more effective in increasing the fresh weight of callus of *Linum usitatissimum* L. But callus induction required the presence of auxin and cytokinin to obtain good results, in addition to the increase in its medically effective compounds.

6. Nanotechnology and the genetic aspect

Those who follow the technology of tissue culture and its development on the past decades and its effective entry into other technologies such as biotechnology and genetic engineering find that there is room for research in genetic changes at the level of cells, tissues and organs grown on the food medium in tissue culture between the attempts of mutagenesis by different physical and chemical mutagens with the aim of Improvement (Kumar et al., 2016), and between warnings about somaclonal variation in tissue culture, which may lead to a change in the cultivated plant.

In 1981, Larkin and Scowkraft coined a term that describes all the genetic changes that occur in plants at the level of the plant cell, tissue, and organs cultured with the technique of tissue culture by Somaclonal Variation and that this scientific fact may be of great benefit to plant breeders who work to follow specific traits *In vitro* and then try to change them, but on the other hand, it is very harmful to producers of horticultural plants, which are produced by tissue culture technology instead of normal vegetative propagation, as is the case in potatoes. Due to the recent age of nano applications in plants, the focus in research was and still is based on the capabilities offered by this technology (nano stress) in improving plant growth, production of secondary compounds, and genetic engineering (Cunningham et al., 2018). result from the use of such materials (nanomaterials). Atha et al. (2012) was able for the first time to prove the presence of damage at the DNA level in three types of known annual weeds at different levels due to DNA oxidation processes, which causes genetic variation in the resulting plants. They recommended that these researchers intensify efforts to study the genetic variations resulting from the accumulation of nanomaterials, as well as the amount of the appropriate dose to cause this defect in the genome of different organisms, as well as the duration of exposure to these materials, which are newcomers in the field of agriculture. Kumbhakar et al. (2016) also obtained results of the effect of each of nano-copper and nano-cadmium sulfate when they studied *Nigella sativa* L. seeds at concentrations (0.25, 0.5, 1) mg. L^{-1} the seeds were soaked for 3 and 6 hours in order to compare the effect of these two factors with both gamma rays and EMS (ethyl methyl sulfonate) in causing chromosomal aberrations. The study using the Atomic Absorption spectroscopy device demonstrated the deposition of the nanomaterials under study at root apex sites with active cell divisions, and this led to the occurrence of genetic variations. The formation of abnormal shapes among the treatments differed according to the concentration of the materials used, and the results of the researcher showed that each of the EMS, Kama, and NPs treatments caused poisoning of the dividing cells, which reduced the frequency of division and caused abnormalities at the level of cell division compared with the control treatment. The study also showed that the abnormal or deformed cells formed in each of the gamma ray, EMS and CuNPs treatment were higher than the CdNPs treatment, and the shape of the deformed cells was similar in the three treatments with the highest mutation events. Ghosh et al. (2010) when testing the toxicity caused by titanium dioxide in the onion plant, found abnormalities in the DNA and chromosomes, which increase (distortions) with increasing titanium concentrations. The researcher due this effect to the high oxidation of fats or the deterioration of fats due to the attack of free radicals, which causes them to lose electrons and deformities occur at the DNA level. In a study by Naganokar et al. (2015) to investigate the effect of different concentrations of nano copper (CuNPs) on increasing the growth and division of onion *Allium cepa* cells, the results showed that nano copper increased the number of cell divisions to 20 mg. ml^{-1}Then, this index began to decrease with increasing concentration and duration of exposure to copper nanoparticles, causing an increase in the number of dividing cells with abnormal shapes, and these chromosomal abnormalities (mutations) had occurred in the interphase and preliminary phase of division, as well as the presence of empty nuclei. Halder et al. A (2015) induced mutagenicity in a climbing plant of the legume family, *Macrotyloma uniflorum*, by treating the seeds of this plant with different concentrations of CuNPs, EMS, and gamma rays. The results showed that there were eight different mutant individuals from the Cu NPs treatment, whose seeds had little vitality in the third mutant generation (M3), and the researcher stated that this research work is the first of its kind. Ma et al. (2015) and Hossain et al. (2015) when nanoparticles enter the cell, they unite with molecules and components present in the interstitial spaces as well as cell organelles, and the union of these nanoparticles with both protoplasts and mitochondria can be physical or chemically, which causes stress and increases the production of ROS, which in turn affects the structural composition of cell organelles, DNA, proteins, carbohydrates, and fats, as well as secondary metabolites.(Halder et al. B 2015, Ghosh et al. 2017) concluded that nanomaterials are mutagenic in plants compared to known chemical and physical mutagens such as EMS and gamma radiation. In addition, these mutations caused by nanomaterials were the same as mutations created by chemicals and physical mutagens that can be inherited as they were traced back to the M3 generation, and mutations had clear repercussions on the external shape of mutagenic plants. As these nanomaterials resembled the effect of known mutagens on the genetic sites that were affected by them in the plant genome, the mutagen plants were able to produce their seeds. Nanomaterials cause phytotoxic effects at both the structural level of the cell (cell wall, damage at the chromosome level, and disturbance of the biosynthesis of chlorophyll) and physiological level (decreased biomass, inhibition of root elongation) (Hatami 2017 and Hatami et al. 2016). Pramanik et al. (2017) indicated that the incidence of mutagenesis by nanomaterials is higher than known mutagens (gamma radiation and EMS). In a study conducted by Tymoszuk and Kulus, (2020) for the purpose of breeding and improving the phenotypic traits of the Chrysanthemum plant outside the living body, and after adding different concentrations of nano silver , the two researchers were able to obtain genetically heterogeneous plants. As their results showed a change in the shape of the flower and a change in the carotene and anthocyanin pigments in the plant. This study was considered the first in the field of breeding and improving flowering plants using nanotechnology *In vitro*.

7. Conclusion

Nano technology applications rapidly increased in the last decades, this review bring the attention to Nano applications in Agriculture generally, more focus on tissue culture, the innovative use of Nano particles as plant elicitors, clarify of green Nano VS industrial Nano, more info related to genetic parameters impacted by Nano particles.

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