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Bibliographic review

Potentialities of chitosan nanoparticles in rice cultivation (*Oryza sativa* L.)

Potencialidades de las nanopartículas de quitosano en el cultivo del arroz (*Oryza sativa* L.)

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ABSTRACT: Chitosan nanoparticles (CSNP) are compounds that have great potential in modern agriculture due to the challenges they face such as climate change, the severity of diseases, and the limited availability of important nutrients for plants. Therefore, this article presents a review of the literature on CSNP, their different uses in agriculture, their methods of obtaining them, applications in rice cultivation as a biostimulant, antifungal and resistance inducer against *Pyricularia oryzae*.

Key words: germination, nanotechnology, biocomposite.

RESUMEN: Las nanopartículas de quitosano (NPQ) son compuestos que tienen un gran potencial en la agricultura moderna debido a los desafíos que esta enfrenta como el cambio climático, la severidad de las enfermedades y la limitada disponibilidad de importantes nutrientes para las plantas. Por lo que, este artículo presenta una revisión de la literatura sobre las NPQ, sus diferentes usos en la agricultura, métodos de obtención, las aplicaciones en el cultivo del arroz como bioestimulante, antifúngico e inductor de resistencia contra *Pyricularia oryzae*.

Palabras clave: germinación, nanotecnología, biocompuestos.

INTRODUCTION

The growing world population demands food and other inputs, so the challenge facing agricultural researchers in the 21st century is to innovate and generate technologies to produce sufficient quantity and quality of food to feed the world's growing population, but without degrading soil health and agroecosystems (1). It has been estimated that world food production must increase by 70-100 % by 2050 to meet the ever-increasing demand of the world's growing population (2). However, agricultural production continues to be affected by a large number of insect pests, diseases, and weeds (3).

In recent decades, the use of agrochemicals (substances such as fungicides, insecticides, herbicides, rodenticides, fertilizers, plant growth stimulants, etc.) has increased in different crops, with China, the United States of America and Argentina being the main consumers of these products (4).

Rice is one of the most demanded crops in the world and its consumption has increased in the last decades, with the consequent increase in the application of herbicides, insecticides and fungicides, during different phases of cultivation to increase its production.

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In Cuba, rice is the staple food of the Cuban diet and the population's consumption is more than 75 kg per capita. It is highly dependent on chemical products for its production, which are highly expensive and toxic for man and the environment. For this reason, research is being carried out in the search for new natural, more economical, biodegradable and non-toxic products.

Among the compounds of natural origin and with a wide range of applications directly related to agriculture is chitin and, especially, its best known derivative: chitosan. This active principle has been used in the fungal protection of seeds and seedlings, as a biostimulant of growth and inducer of defense mechanisms in plants, in the post-harvest protection of flowers and fruits, in the manufacture of films for packaging agricultural products (5). In rice, this compound has demonstrated to have antimicrobial activity on pathogens of economic importance, to induce resistance and has stimulated seed germination, growth variables and yield components (6-9).

Therefore, the application of nanoparticles contributes to better plant nutrient uptake, resistance to damage, as well as improved yield and crop quality. There are different methods of synthesis of nanomaterials, these can be physical, chemical or where nanoparticles are deposited on supports such as: ionic adsorption, precipitation, colloid and photochemical (10).

Due to the knowledge derived from the usefulness of this biocomposite, the way to optimize its applications in the agricultural area is being sought through nanotechnology as an integral strategy towards sustainability in productivity, in obtaining high yields and plant protection. The objective of this review is to document the potential of this new technology in economically important crops such as rice.

DEVELOPMENT

Nanotechnology and its applications in agriculture

The so-called nanosciences and nanotechnologies have become the main areas of scientific technological development in the last twenty years (11). Nanotechnology is a new science that involves the manipulation and use of materials smaller than a micrometer.

The word nano is a prefix whose meaning is dwarf, an adjective applied to indicate smaller than average size, usually of a person. When used as a prefix to a unit of measurement, it means one billionth of a unit of measurement (1 nano= $1x10^{-9}$) (12). According to the definition of the International Organization for Standardization (ISO), nanoparticles (NPs) are considered to be those portions of matter whose three external dimensions fall within the nanoscale range (between 1-100 nm) (13). The strength of nanotechnology lies basically in making more efficient, multifunctional and raw material-saving products. Within the global nanotechnology market, interest in NPs and nanocomposites is increasing. In recent years, the study of nanomaterials (NMs) and nanostructured materials, whose main characteristic is the size of the phases involved, which is in the order of nanometers (14), has become more widespread.

Among the applications that nanotechnology is considering in the agricultural sector is the development of chemicals such as fertilizers, herbicides and growth regulators to increase agricultural production. Other applications in this sector are nanosensors for the detection of plant pathogens and the use of NMs for the stabilization of biopesticides, among others (15,16). As advantages, it allows minimizing nutrient losses in fertilization and improving crop productivity by optimizing the use of water and nutrients (17,18).

It has been demonstrated that the encapsulation of active ingredients in NPs increases the efficacy of their chemical ingredients, since they allow reducing their volatilization, leaching and can reduce the toxicity and contamination of agroecosystems using these nanoproducts (19). NPs used to improve the efficiency of pesticides allow lower doses of the product to be applied in the field (20).

Applications of chitosan nanoparticles in agriculture

In recent years numerous biopolymers such as starch, cellulose, alginate, chitin and chitosan have been used for the development of new materials with environmental sustainability and desirable functionality (21).

As for chitosan, it is a deacetylated form of chitin, which is a linear copolymer of 2-acetamide-e-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose. This polymer is the second most abundant in nature after cellulose and is found in the exoskeleton of crustaceans, cuticle of insects and cell wall of fungi (22). Among its advantageous properties are: abundance, biocompatibility, biodegradability, safety and non-toxicity. In addition to their physical and chemical characteristics, such as size, surface area, cationic nature, active functional groups, greater encapsulation efficiency, ease of mixing with other components (23). Therefore, chitosan nanoparticles (CSNP) can be applied as antifungals (24), antibacterials (25-27), plant growth promoters (28-30) and nano fertilizers (31).

CSNPs have been shown to impact the biophysical characteristics of coffee seedlings by increasing pigment content, photosynthesis rate and nutrient uptake, etc (32).

Although there are many works on the application of chitosan in agriculture, not many have been carried out using CSNPs. Several applications of CSNPs in different crops are shown in the following table (Table 1).

Obtaining chitosan nanoparticles

Chitosan nanoparticles (CSNPs) were first described in 1994 (38). Since then, many methods have been employed for the synthesis of chitosan nanoparticles. Among the various methods are ionotropic gelation, sputtering/ drying, coacervation/precipitation, reverse emulsification, and polyelectrolyte complexation (Table 2).

Table 1. Applications of chitosan nanoparticles in agriculture

Compounds	Crops	Applications	Reference
Chitosan nanoparticles	Strawberry	Postharvest protection	(33)
Chitosan nanoparticles	*Robusta coffee*	Growth stimulation	(28)
Chitosan nanoparticles	Apple	Postharvest protection	(34)
Chitosan nanoparticles -NPK	Wheat	Growth and yield stimulation	(35)
Chitosan nanoparticles	Chili	Antifungal activity	(36)
Chitosan nanoparticles with copper (Cu)	Tomato	Germination, growth and antifungal stimulator	(29)
Chitosan nanoparticles/tripolyphosphate		Herbicide	(37)
Chitosan nanoparticles-Copper (Cu)	Maize	Growth stimulator	(30)

Table 2. Most commonly used methods for obtaining chitosan nanoparticles (CSNP)

Method	Solution Problem	Medium (with)	Nanoparticle forming agent	Separation of nanoparticles
Inotropic Gelation (39)	Solution of Chitosan	Aqueous acid (1mg/mL)	Low molecular weight polyanion Pentasodium Tripolyphosphate (*TTP*), Adenosinetriphosphate (ATP)	Centrifugation
Spraying/ Drying (40)	Solution of Chitosan	Aqueous acid (HAc-0.5 % v/v)	Spraying/ Drying Gas	Filter
Coacervation/ Precipitation (41)	Solution of Chitosan (Use of Surfactants)	Aqueous acid	Sodium Sulfate Solution	Filtration with 400 nm membranes. Centrifugation
Reverse emulsification (42)	Solution of Chitosan	Aqueous	Covalent Crosslinking Agent	Decanting/ Dialyzation/ Lyophilization
Polyelectrolyte Complexation (43)	Solution of Chitosan	Aqueous acid	Polyanion of macromolecular nature	Centrifugation

Applications of chitosan nanoparticles in rice cultivation

Difficulties in controlling pests, together with the concern about the indiscriminate use of pesticides in agriculture have been the subject of intense debate and discussion. Currently, work is being done to find alternative methods of pest control to reduce dependence on synthetic pesticides (44). This is the case of chitosan nanoparticles (CSNP) that were used as a vehicle for protocatechuic acid (PCA) to induce resistance against *Pyricularia oryzae*, where CSNP transported PCA molecules into fungal cells, exhibiting a strong antimicrobial effect on the fungus. Therefore, tests on rice plants *in vitro* are recommended to reaffirm this possibility (45). Also, other researchers (46) obtained chitosan guar nanoparticles (CSNPG) by the ionic gelation method, applied it to rice seed and observed stimulation in germination and seedling growth. In addition, they demonstrated the inhibition of the growth of two pathogens that cause damage to rice: *Pyricularia grisea* and *Xanthomonas oryzae*, under *in vitro* conditions. These same authors treated 30-day-old rice leaves with a 0.1 % solution of CSNPG, incubated them for 24 h and then inoculated them with 0.5 mL per leaf of a concentration of $1x10^5$ spores/mL of *P. grisea* and, after 14 days, the incidence of the disease was evaluated and no symptoms of the disease were observed.

Other CSNP synthesized also by the ionic gelation method at the concentration of 0.0065 % were applied on transplant rice and then inoculated with *Xanthomonas oryzae* pv. The results showed that the application of CSNP was able to increase the expression of resistance genes with respect to the control; however, it was not able to suppress the development of the infection (47).

Other researchers (48) also prepared a nickel-chitosan nanoparticle (CSNP-Ni) using nickel chloride and evaluated the growth and inhibition of *Pyricularia oryzae*. For this purpose, they applied CSNP-Ni to rice seeds, which showed a significant increase in germination, shoot and root length and number of lateral roots over the control. In addition, treatment with nanoparticles on plants under greenhouse conditions showed a remarkable improvement in plant growth conditions and showed no toxicity. In addition, reduced symptoms of pyriculariosis were exhibited in leaves treated with nanoparticles over the control under greenhouse conditions, while they showed 64 % mycelial inhibition in Petri dishes. All these results suggest that CSNP-Ni could be used as a plant growth promoter and to control rice blast disease.

Growth stimulation of rice seedlings was also appreciated (49), who, first, obtained CSNP and treated rice seeds at different concentrations (10, 50, 100 and 500 ppm) of this compound, after two weeks they found that the highest growth stimulation was achieved with the concentrations of 100, 50 and 10 ppm. However, seedlings obtained from seeds treated at the 500 and 1000 ppm concentrations did not survive. These same seedlings were exposed to brown grassthoppers (*Chorthippus brunneus*) and evaluated for chitinase activity. A moderate increase in the activity of this enzyme was observed in the seedlings that were treated with concentrations of 10, 50 and 100 ppm, and showed resistance to pathogen attack with respect to the control, which consisted of seedlings obtained from seeds treated with water.

Divya and coworkers (50) prepared CSNP using the ionotropic gelation method. Subsequently, they treated rice seeds with different concentrations of CSNP (0.5, 1.0, 1.5, 1.5, 2.0, 2.5 mg mL^{-1}) at different imbibition times (15, 30, 60, 90 and 120 min). The results showed that all CSNP treatments were better than the control (no treatment). The 1mg mL^{-1} treatment with 120 min imbibition achieved the highest germination percentage and higher growth rates (number of leaves, height, mass and plant vigor) 21 days after planting. Applying the same concentration of CSNP (1mg mL^{-1}) to rice seed, soil, foliar and combination, the authors (51) found that the combined treatment of seed, soil and foliar application was the most efficient. The toxicity of CSNP in soil prior to application was also studied and found to be non-toxic. Some authors synthesized CSNP using an ionic gelation method and applied these compounds to rice seeds which were grown in increasing concentrations of NaCl (52). Where they showed a significantly greater effect on germination, seedling vigor, biochemical and antioxidant responses compared to control seeds.

Lanthanum-modified chitosan oligosaccharide nanoparticles were prepared by ionic cross-linking and applied to rice seeds at the concentrations of 6.25, 12.5, 25, 50 and 100 μg mL^{-1} and sown in a hydroponic, after 15 days the height and fresh mass of the area part were determined. The results showed an increase in these variables with the application of the nanocomposite with respect to the control (53).

CONCLUSIONS

Research has shown that CSNPs have a positive effect on different crops, both *in vitro* and under semi-controlled conditions. In the case of rice, they have been shown to have a biostimulant and protective effect against *Pyricularia oryzae*, so these compounds could be used in agriculture, making it more sustainable.

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