BIOCELL
2022 46(3): 651-654

Tech Science Press

Impact of nanomaterials on plants: What other implications do they have?

ANTONIO JUÁREZ-MALDONADO*

Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro, Saltillo, 25315, México

Key words: Nanotechnology, Antioxidant defense system, Secondary metabolites, Ecosystems and the environment

Abstract: The use of nanomaterials has spread and has been applied in different industries, including agriculture. Here, the possibilities presented by NMs are very varied, from the biostimulation of favorable responses, or the control of pests and diseases, to the monitoring of characteristics of interest with the use of nanosensors. Particularly the biostimulation of agricultural crops with the use of nanomaterials is very relevant, since from this process stress tolerance, higher content of biocompounds, etc., can be induced. Although the positive impacts on crops are clear, there is not enough information to determine the long-term impacts, both on ecosystems and on human health.

Nowadays, research has intensified on the use of nanomaterials (NMs) in various areas of agriculture, from their application as nano-fertilizers to their application as nanosensors (Moulick *et al.*, 2020; Singh *et al.*, 2021). This has been thanks to the new characteristics that NMs present, mainly due to the surface/volume ratio, but they also have other properties that directly impact on the interaction that occurs with plant cells, among these are surface charge, surface energy, size, shape, roughness, porosity, hydrophobicity, and hydrophilicity (Das *et al.*, 2019; Juárez-Maldonado *et al.*, 2019). Derived from all these characteristics, a range of different responses can be obtained in plants exposed to NMs (Juárez-Maldonado *et al.*, 2021). One of the main approaches that have been given to the use of NMs in agriculture is their application in order to modify the metabolism and physiology of plants. This results in the increase of multiple characteristics of interest such as crop yield, greater tolerance to some type of stress, greater accumulation of bioactive compounds or secondary metabolites, among others (Awasthi *et al.*, 2020; Kumar *et al.*, 2019; Rizwan *et al.*, 2017).

Due to this, it has been proposed to consider NMs as biostimulants in plants (Juárez-Maldonado *et al.*, 2021, 2019), but also their function as elicitors of secondary metabolites has been proposed (Lala, 2021; Rivero-Montejo *et al.*, 2021). In both cases there is sufficient evidence in the literature to support these claims. However, perhaps the most relevant thing is to understand the mechanism of action of NMs that induces the responses observed in different plant species.

It is clear that NMs can enter plants through different routes. However, the route of application can directly influence the final bioaccumulation that can be observed in the plant because NMs, depending on their nature, can be transformed by chemical, physical, biological and macromolecular interactions. The result of these interactions can be agglomeration (NMs that interact with the environment), dissolution (NMs based on metals) or chemical transformation that can be by oxidation or reduction (NMs that interact with the environment or by bioaccumulation) (Petersen *et al.*, 2019). Possibly, once NMs enter plants and interact with cells, organelles and other cellular components, a chemical transformation takes place.

By foliar route they can enter through the stomata or through the cuticle, while through the root they can enter through existing channels, aquaporins, endocytosis, or induce the formation of new pores and afterwards move through the symplast or apoplast until reaching to vascular bundles (Corredor *et al.*, 2009; Pérez-de-Luque, 2017; Tripathi *et al.*, 2017). Once the NMs are in the vascular bundles they can move to the rest of the plant structures (He *et al.*, 2021; Jordan *et al.*, 2018). From the first contact of the NMs with the plant cells, there is an interaction at the physical-chemical level and therefore a series of responses are generated (Juárez-Maldonado *et al.*, 2019). After entering the interior of the cell, there is a new interaction between the NMs and the different cellular organelles, including the nucleus (Ahmed *et al.*, 2021). It is here where a series of changes in cellular metabolism are

*Address correspondence to: Antonio Juárez-Maldonado, antonio.juarez@uaaan.edu.mx
Received: 04 May 2021; Accepted: 16 June 2021

Doi: 10.32604/biocell.2022.017350

www.techscience.com/journal/biocell

actually induced, among which is the modification of the antioxidant defense system (Khodakovskaya *et al.*, 2012; Yan *et al.*, 2013). The interaction of NMs with membranes or other cellular organelles can trigger the production of reactive oxygen species (ROS), which results in oxidative stress (Tripathi *et al.*, 2017). However, ROS induction is dependent on the type of NMs applied, and furthermore the degree of oxidative stress is dependent on the concentration (Juárez-Maldonado *et al.*, 2021). This characteristic of NMs is quite relevant, since ROS at this point function as signals that activate the antioxidant defense system, resulting in the production of enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase) and non-enzymatic (ascorbic acid, glutathione, phenols) antioxidant compounds (Mittler, 2017). Furthermore, ROS can also influence the different metabolic pathways, and even directly on the expression of genes related to these metabolic pathways (Marslin *et al.*, 2017; Nazir *et al.*, 2019).

The overproduction of antioxidant compounds has multiple repercussions for the plant, since it is necessary to allocate energy for the synthesis of these compounds without causing irreparable damage to the plant itself. Basically, there must be a balance between the harvesting of energy by photosynthesis and the expenditure on the different metabolic and physiological processes, such as respiration, growth, and secondary metabolism (Juárez-Maldonado *et al.*, 2021).

The biostimulation capacity of NMs in plants can be used for different purposes, such as those previously mentioned. However, considering the antioxidant system and secondary metabolism, the most relevant for the development of crops is the induction of tolerance to a variety of types of both biotic and abiotic stress (Azizi-Lalabadi *et al.*, 2020; Zhou *et al.*, 2021; Zulfiqar and Ashraf, 2021). This is extremely relevant due to the increasingly difficult situation that exists worldwide for the production of agricultural crops, since climate change brings with it repercussions due to the extreme climates that occur. In addition, the lesser availability of soil suitable for the development of crops and the availability of water, due to problems such as erosion, salinity, or contamination by heavy metals (Machado and Serralheiro, 2017; Ruíz-Huerta *et al.*, 2017).

But also, if it is considered that human health depends largely on the intake of foods rich in biocompounds such as vitamin C, lycopene, phenols, carotenoids, due to its high antioxidant activity that reduces the risk of non-communicable diseases such as cancer and cardiovascular problems (Cisternas-Jamet *et al.*, 2020; Cortés-Estrada *et al.*, 2020). So, the use of NMs in agriculture represents perhaps an unprecedented opportunity, which will allow us to improve not only the productivity of agricultural crops, but also the quality of the food we consume. However, one must be very careful with the application of the NMs, Amini *et al.* (2014) suggest adequately studying the exposure routes in industry workers and especially consumers of food products treated with NMs, since a greater and better understanding of the properties of NMs is required to obtain safe food products. Some studies have shown harmful effects in animal species. Amiri *et al.* (2018) conclude that prenatal exposure to Ag NPs (0.26 mg kg^{-1} day^{-1}) can affect the brain and behavior in adult male mice, and suggest further studies to understand the mechanism by which this happens. In contrast, Se NPs can induce beneficial effects because selenium is part of selenoproteins and selenoenzymes resulting in an increase, with the great advantage that Se NPs are less toxic than other organic or inorganic forms of Se, more bioavailable and have the ability to scavenge ROS directly (Amini and Mahabadi, 2018). In addition, secondary metabolites derived from plants are being used in order to synthesize and stabilize NMs, thereby reducing negative impacts on human health and the environment (Amini, 2019). Based on this approach, Au NPs were synthesized using the natural flavonoid apigenin and later these NPs were used to treat cancer in mice; the results obtained indicate that NPs were efficient to eradicate cancer cells, in addition to being more stable in the environment and are much less toxic than other types of Au NPs (Amini *et al.*, 2021). The use of this type of environmentally friendly technologies and with the potential to decrease the negative impact of NMs definitely increases the possibility of being able to apply various NMs in agriculture.

Although all these positive impacts both on the development of agricultural crops and on the increase of bioactive compounds in the plant organs that we use as food are documented (Lala, 2021; Rivero-Montejo *et al.*, 2021; Rizwan *et al.*, 2017), there is a fundamental question that must be answered before commercial application of these NMs. The benefit of using NMs in agriculture is greater than the possible risks that could be observed in the long term, such as the impact on the environment through food chains or even on human health itself? Obviously, to answer this question, it is necessary to deepen the research on the impacts of NMs not only in plant species, but also in other living organisms. In addition, it is necessary to carry out research on the movement that NMs may have through the different trophic levels, since it has been shown that it is possible (Bergami *et al.*, 2017), in order to determine the extent to which complete ecosystems can be impacted, and not only with partial knowledge. Even more so if we take into account that the use of nanomaterials in other industries such as pharmaceuticals, electrical, cosmetic, food, and others, is constantly growing (Ashraf *et al.*, 2021; Wu *et al.*, 2020), which results in a greater production of NMs to satisfy the demand and consequently a greater release of these to the environment (Pereira *et al.*, 2020; Salieri *et al.*, 2018). Fortunately, progress has been made in regulating the use of NMs, and especially in the methods that allow evaluating the potential risks of these materials in the environment (Petersen *et al.*, 2021). This will definitely favor the potential application of NMs in a safe manner. However, and despite the positive evidence of the use of NMs in agriculture, we should consider the possible negative implications that we cannot perceive for now, but that can definitely impact enormously at any given time on food production through agricultural crops and human health, before the extensive use in agriculture.

Author Contribution: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Funding Statement: The author received no specific funding for this study.

Conflicts of Interest: The author declares that he has no conflicts of interest to report regarding the present study.

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