Chapter 15
Future Roadmap for Plant Nanotechnology

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Abstract Nanotechnology has started to play a promising role in agriculture and plant biology in the last few years. The experimental base for "nanoagriculture" is still limited. Several research groups demonstrated that nano-sized materials can be useful for the delivery of nucleic acid, pesticides and fertilizers to plants, activation of seed germination and plant growth, suppression of plant diseases caused by pathogens, and sensing of critical plant molecules with a high level of sensitivity. Success in the development of efficient "nano-agro-technologies" will require the creation of reliable and accurate methods of detection of nanomaterials inside plant cell or tissue, the understanding of the biological mechanisms of effects of nanoparticles in plant systems, and the clarification of properties of nanomaterials that can be associated with observed biological effects. Involvement of nanotechnology in agriculture will eventually enhance the flow of nanomaterials into the food chain. Thus, the risk assessment of agricultural plant products contaminated with different nanoparticles intentionally or nonintentionally is the most important task for future plant nanotechnology.

Keywords Nanodelivery · Nucleic acids · Pesticides · Fertilizers · Growth regulators · Suppression · Nanosensors · Risk assessment

The study of nanoparticle–plant interaction is a new, emerging area of modern nanobiotechnology. However, the number of publications associated with the effects of nanomaterials on plant organisms is dramatically lower compared with articles focused on effects of nanoparticles on animals/humans or animal cells (Fig. 15.1).

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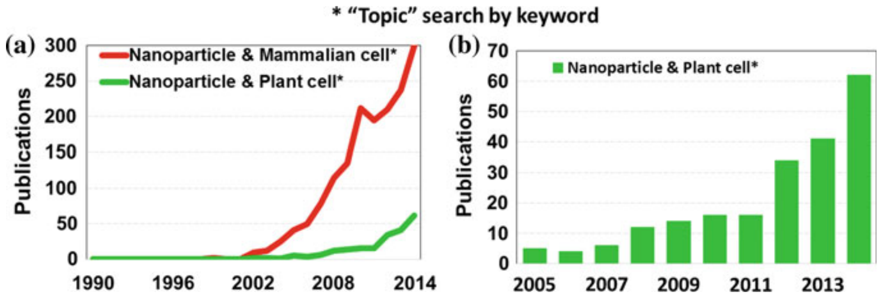

Fig. 15.1 Comparative analysis of some manuscripts published in the area of nanoparticle–plant and nanoparticle–animal interactions during 1990–2014 (**a**). The increase of publications associated with effects of nanoparticles on plants through 2005–2013 years (**b**). The analysis was performed using available data of the Web of Science (WoS) database. Keywords indicated on graphs were used for this search

The interest of research groups to understand how different nanomaterials can affect plant physiology and development significantly increased after 2011 and continues to be elevated (Fig. 15.1b). Such phenomena can be explained by the recent discoveries of the benefits of nanomaterials for fundamental plant biology and applied plant science. During the early years of nanotechnology, investigators mostly focused their efforts on understanding the toxicity of carbon-based (CBNs) and metal-based (MBNs) nanomaterials to different plant species and plant cells. To achieve visual symptoms of phytotoxicity, researchers mostly worked at a range of very high doses (1000–2000 μg/ml) of tested nano-sized materials (Lin and Xing 2007; Stampoulis et al. 2009). As a result, authors noticed no toxicity or visible toxicity of full range nano-sized materials applied at high doses to different plants (Lin and Xing 2007; Stampoulis et al. 2009). However, it was later demonstrated that a significant decrease of working nanomaterial concentrations can change the response of treated plants dramatically. Particularly, it was shown that carbon-based tubular nanomaterials (carbon nanotubes, nanohorns) in concentrations between 10 and 100 μg/ml were sufficient to activate seed germination and plant growth (Khodakovskaya et al. 2011, 2012; Villagarcia et al. 2012; Lahiani et al. 2013; Khodakovskaya et al. 2013; Lahiani et al. 2015).

A range of successful experiments identified the most promising directions of nanomaterial applications for plant improvement and agriculture. Thus, the ability of CBNs to improve cell, seed, and plant performance demonstrates a high potential of CBNs as ***regulators of germination and plant growth*** (Khodakovskaya et al. 2011, 2012; Villagarcia et al. 2012; Lahiani et al. 2013; Khodakovskaya et al. 2013; Lahiani et al. 2015). Studies focused on the use of nanoparticles for targeted delivery of pesticides and fertilizers demonstrated good potential in ***disease suppression and crop yield enhancement*** (Perez-de-Luque et al. 2006; Servin et al. 2015). Particularly, this approach has the potential to provide better penetration through plant tissues and allow the slow and constant release of herbicides (Perez-de-Luque et al. 2006). The ability of gold nanorods to stimulate ***delivery of***

phytohormone 2,4-D to plant cells (tobacco cell culture) and activate cell growth was documented recently (Nima et al. 2014).

Silver nanoparticles (Ag NPs) were described as an active nano-sized material for ***prevention of plant diseases*** caused by wide range of pathogens (Lamsal et al. 2011a, b; Kim et al. 2012). It has been demonstrated that they are very useful for the reduction of plant diseases caused by spores producing fungal pathogens (Jo et al. 2009) or reduction of microbial growth for plant cuttings (Liu et al. 2009; Solgi et al. 2009). Recently, nanosilica was recognized as a powerful nanobiopesticide. Practically, nanosilica can be absorbed into the cuticular lipids of insects and cause the death of insects by desiccation (Barik et al. 2008; Rahman et al. 2009).

Plant genetic engineering can benefit from nanotechnology in the area of improvement of plant transformation. Thus, the ***new technology of nucleic acid delivery to plant cells*** using mesoporous silica system (MSNs) has recently become apparent (Galbraith 2007; Torney et al. 2007; Martin-Gullon et al. 2006; Martin-Ortigosa et al. 2012, 2014). Another promising type of nanomaterials for nucleic acid delivery is polymer nanoparticles. Thus, fluorescent conjugated polymer nanoparticles (CPNs) were used to deliver siRNAs and knockdown specific gene target in plant BY-2 protoplasts (Silva et al. 2010). The big advantages of CPNs are very low toxicity of such material for plants.

Creation of new sensors for plants is new and a promising direction of plant nanotechnology. The number of successful studies is still very limited, but nanosensors can be developed in the very near future. A great example is the recent building of single-walled carbon nanotubes (SWCNTs) radiometric sensors (for H_2O_2 and NO) performed by Giraldo et al. (2015) which proved the efficiency of radiometric nanosensing platform for detecting key compounds in plant tissues.

Without any doubts, the range of possible applications of nanomaterials in plant biology is tremendous. However, there are some factors that can limit wide application of nanomaterials *in planta*. First, it is a significant challenge to compare results of independent research groups because investigators are working in different experimental settings. Properties of applied nanomaterials such as size, purity, presence/type of functional groups, doses, the level of agglomeration, and way of delivery are not precisely identical between presented experiments. Thus, reproducibility of successful experiments *in planta* is not at an acceptable level yet. Secondly, the detection of nanoparticles inside plant tissues or cells is a significant challenge. Transmission electron microscopy and methods of spectroscopy including Raman spectroscopy are efficient confirmation of the presence of particular nanoparticles inside plant sample (Khodakovskaya et al. 2011; Lahiani et al. 2013). However, the quantification of the exact amount of absorbed nanomaterials by plant organs is very challenging. For example, the reliable technique for quantitative analysis of carbon nanotubes located inside exposed plants was developed only very recently (Irin et al. 2012; Lahiani et al. 2015). Future progress in the creation and application of new plant-related nanotechnologies will be dependent on accurate quantitative assays of different nano-sized materials inside plant tissues. Thus, new methods of detection and measurement of absorbed nanomaterial have to be suggested and developed. As shown in this book, some positive effects of nanoparticles on plants were documented up-to-date. In the same time, biological mechanisms of observed effects are not clear. The clarification of biological mechanisms of nanoparticle impact in plant systems will require comprehensive transcriptome/proteome investigations of exposed plants in a combination with high sensitive detection of nanomaterials inside plants. Interdisciplinary collaborations between material scientists, plant biologists, chemical engineers, and physicists can help create new platforms for such studies.

To consider the possible use of nanoparticles in plant systems or plant agriculture, the risk assessment of nanomaterial entering into the food chain should be performed in detail. It is critical to understand the effects of short-term and long-term exposure of CBNs and MBNs delivered to humans or animals through exposed plants. However, the methodology of such risk assessment is not yet fully established. Creation of effective, safe, and simple in vitro and in vivo toxicological experimental protocols for each group of nanomaterials is a major step of risk assessment of plants contaminated with nanomaterials.

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