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Nano-elicitation: An efficient method for enhancement of secondary metabolite production in plant cultures

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Abstract

The positive effect of bioactive compounds present in plants has been exploited in nutraceutical and pharmaceutical industries. However, over-exploitation of germplasm and poor extraction methods cause difficulty in obtaining these compounds directly from the plants. Hence, elicitation has emerged as an effective strategy for *in vitro* plant cultures to enhance the metabolite production. Use of metal oxide nanoparticles as novel elicitors is an appropriate choice for secondary metabolite production due to their non toxic and stable nature. Thus in the present review, the mechanism and knowledge gaps in the field of nano-elicitiation for secondary metabolite production has been discussed.

Plants are rich source of secondary metabolites that provides protection to plant against various abiotic and biotic stresses[23]. These bioactive metabolites have a positive effect on human health. Despite their *in vitro* production, there are some limitations that includes their lower productivity, inconsistent yield and poor quality at an industrially competitive level. To counter this, different strategies have been devised. Among them, elicitation has emerged as an effective yield increasing strategy, by activating the plant defense system. Hence, nanoparticles have evolved as novel elicitors. These nanoparticles have size that ranges from1-100 nm[18]. Also, they possess unique physical and chemical properties that differ from the bulk material[22]. They are potential tools with wide range of applications in diverse branches of medicine, agriculture, biotechnology, electronics, food and cosmetics[14,26,45]. Different types of nanoparticles includes ferromagnetic, ferroelectric and superconducting[11]. They can also be classified from the bulk material as nano allotropes of carbon including fullerenes, nanotubes and grapheme, metallic and oxide molecular building blocksthat includes Ag, Au, Fe, ZnO, CeO_2, nano-silicates, ceramics, dendrimers, polystyrene, CdSe, CdTe etc. and biological molecular building blocks including cyclodextrins, liposomes and monoclonal antibodies[8]. Further, they can be classified into one, two and three dimensions in the regime of nanoscale[9].

Size of nanoparticles is one of the most essential characteristic as it exerts impact on their properties. The uptake of small sized nanoparticles by cells through the membrane pores means they are not recognized as foreign materials[37]. Different enzymatic and non-enzymatic defense systems can activate the nanoparticles uptake which in turn stimulates the metabolite production[36].Nanoparticles also exhibit different morphological shapes such as rod, triangle, round and octahedral. Besides, they have various porous shapes including porous spheres, nanorod, distorted cubes etc[1]. These characteristics influence the internalization process, distribution and compliance time via the cells[25,43].

In plants, bioactive compounds such as alkaloids, terpenoids, flavonoids and phenolic acids are essential for the adaptation of plant to an adverse environment conditions along with their commercially importance in pharmaceutical, agriculture, cosmetic, food industry and nanotechnology[17,21]. Due to their abundant applications, diverse strategies have been developed including precursor feeding, immobilization, metabolic engineering, bioreactor cultures, elicitation and synthetic biology[40,42,45]. Among them, elicitation is one of the most effective strategy for enhanced production of bioactive compounds[33]. Thus, nanoparticles have the potential to be used as an effective abiotic elicitors by activating the genes implicated in the production of secondary metabolites in plant cell and tissue cultures[47].

Previous studies have reported that nanoparticles trigger the accumulation of reactive oxygen species (ROS) which in turn induces oxidative stress that affect the primary and secondary metabolism in the plant cells[28]. The oxidative stress activation and inactivation depends on various factors such as plant species, type and age of tissues, type of stressor and tolerance[19,41,44].

Nanoparticles uptake and translocation in plants :

The uptake of nanoparticles in plants takes place through three main ways, viz foliar spray, soil and by growing the tissues or an explant on artificially prepared nutrient media. Due to their unique physicochemical characteristics, nanoparticles can interact with plant cells and tissues (Fig. 1). The first step involves crossing of nanoparticles across the plant cell wall. Hence, nanoparticles ranging in size between 5 to 20 nm can simply make the entry into the plant cells because of small dimensions as compared to cell wall pore diameter[46]. But few studies reported the entry of large size nanoparticles either by producing new cell wall pores which are larger in diameter or by changing the pre-existing size of cell wall pores[5,29,36]. This is followed by diffusion of nanoparticles towards the cell membrane. From the plant cell membrane, the subsequent movement of nanoparticles with regard to cytosol and other organelles happen either by specific membrane bound transporter proteins such as aquaporins (water channels), endocytosis, plasmodesmata’s and via production of new pores with the use of ion carrier substances[5,13]. The transportation of nanoparticles from one cell to other cell occurs through apoplastic and symplastic pathways. Once inside the plant cell, the interaction of nanoparticles with intracellular molecules and organelles results

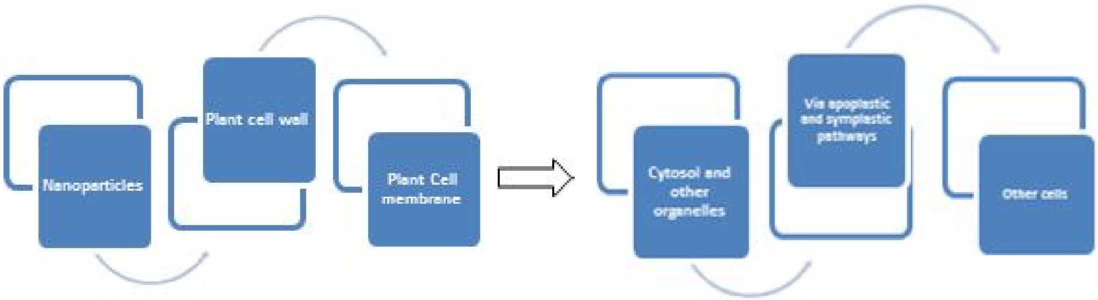

Figure 1. Entry and translocation of nanoparticles into plant cells and tissues

in abrupt disruption of primary as well as secondary metabolism by producing oxidative stress[27]. Till date, few reports indicate the use of nanoparticles in the plant cell cultures.

Mechanism of elicitation by Nanoparticles:

The mechanism procured by nanoparticles as elicitors for elicitation is complicated and distinctive process. In the cell membrane, various elicitor-binding sites have been recognized for several elicitors. Trans-membrane receptor-like protein kinases have been identified as one of the well-known receptors with the knowledge of diverse types of stimuli[49]. There are several factors which affect the production of bioactive compounds in plants including the morphology of an elicitor, concentration of an elicitor, type of *in vitro* culture, exposure time, nutrient composition, plant species and growth regulators[4]. Few nanoparticles possess the ability of binding the receptors such as chitosan nanoparticles, while some enhance the production of endogenous messenger that may attach with the receptor[7]. After binding, the initial exchange of ions *i.e.* Ca^{2+} influx and Cl^- and K^+ efflux takes place across the plasma membrane. Furthermore, cytoplasm acidification which is important for signal transduction, oxidative burst and synthesis of secondary metabolites in plants, occurs with regards to H+-ATPase inactivation and enhancement in extracellular pH. It also involves the activation of protein kinases and mitogen-activated protein kinases. During elicitation, G-proteins show their response by inducing phospholipases, which leads to the downstream production of secondary metabolites in the plants[3,6,35].

However, second calcium influx takes place via PLC/IP3-DAG/PKC cascade[30] followed by activation of NADPH oxidase due to increase in intracellular Ca^{2+} level and subsequently there is generation of reactive oxygen species[7]. This further triggers the various defense genes, biosynthetic genes and signaling molecules including ethylene, salicylic acid and jasmonic acid. These signaling molecules play key role in production of bioactive compounds via various pathways in plant cells[28].

Effect of Nanoparticles on metabolite induction :

Several previous studies demonstrated that the nanoparticles possess the ability to

Table-1. Nanoparticles as elicitors for secondary metabolite production

Nanoparticles	Size (nm)	Effective concentr-ation	Plant species	Type of plant cultures	Effects	Refer-ence
Ag	40	60 µg/l	*C. tuberculata*	Callus	Enhanced phenolic and flavonoid content	2
Fe_3O_4	-	50 mg/l	*D. kotschyi*	Hairy root	Total flavonoid contentincreased	31
SiO_2	100	-	*D. kotschyi*	Hairy root	Increased 2-fold production of Cirsimaritin, xanthomicrolandis-okaempferide	32
Fe	<50	135 µg/l	*S. rebaudiana*	Micropropa gation	Total flavonoid content increased	24
SiO_2	10-100	50 mg/l	*H. reticulatus* and *H. pusillus*	Hairy root	Highest flavonoid content after 24 h exposure	20
ZnO	35	1-500 µg/l	*L. usitatissimum*	Cell suspension	Enhanced flavonoid content (123.83 mg/l)	48
SWCNTs	110-170	0.002g/l	*S. chinensis*	Plant tissue	Increase in Flavonoid content	16
MWCNTs	-	0-1000 mg/l	*S. verticillata*	Seedlings	Secondary metabolism enhanced	34
MWCNTs	50 µm	0-2000 µg/ml	*T. daenensis*	Seedlings	Increased TFC, antioxidant and PAL activity	39
Chitosan	40-180	0.01	*C. sinensis*	Foliar spray on leaves.	Enhanced phenolic and flavonoid content.	10
CuO	25-55	3 mg/l	*G. sylvestre*	Cell suspension	9-fold increase in gymnemic acid and 2- fold increase in phenolics and flavonoids	15
Au+Ag	-	3:1 ratio 30µg/l	*P. vulgaris L.*	Cell suspension	1.8 fold increase in TPC and TFC	12

increase the production of bioactive compounds in various *in vitro* cell cultures (Table-1). Ali *et al.*, reported the elicitation of *in vitro* callus cultures in *Caralluma tuberculata* with Ag nanoparticles. They observed an increase in the fresh weight and in the production of total flavonoid and phenolic content, enhanced antioxidant activity and phenylalanine ammonia lyase activity but decrease in total biomass[2]. Chung *et al.*, observed nine fold increase in the production of gymnemic acid and two fold enhancement in the production of phenolic and flavonoid content after elicitation of cell suspension cultures of *Gymnema sylvestre* with CuO nanoparticles[12]. Furthermore, Nourozi *et al.*, recorded secondary metabolite gene expression in the hairy root cultures of *Dracocephalum kotschyi* with the application of Fe_3O_4 nanoparticles[31]. Similarly, enhancement in cirsimaritin, xanthomicrol and isokaempferide in the hairy root cultures of *Dracocephalum* *kotschyi*and enhanced flavonoid content in *in vitro* grown *Stevia rebaudiana* and *in vitro* hairy root cultures of *Hyoscyamus reticulatus* and *Hyoscyamus pusillus* was observed with SiO_2 nanoparticles[20,24,32]. ZnO nanoparticles elicited *in vitro* flavonoid production in cell suspension culture of *Linum usitatissimum*[48].

Apart from the metal based nano-elicitation, several studies reported the induction of bioactive compounds with the application of carbon nanomaterials. The commonly employed carbon nanoparticles are fullerenes, chitosan nanoparticles, single-walled carbon nanotubes and multi-walled carbon nanotubes. Gaafar *et al.,* reported that the application of single-walled carbon nanotubes (SWCNTs) enhanced the production of flavonoids in plantlets of *Simmondsia chinensis* as compare to the control[16]. A group of researchers studied the effect of multi-walled carbon nanotubes (MWCNTs) in seedlings of *Salvia verticillata* and *Thymus daenensis,* that resulted in enhancement in the production of total phenolic content, total flavonoid content, antioxidants, rosmarinic acid and phenylalanine ammonia lyase activity[34,39]. In shoot culture of *Camellia sinensis,* the application of chitosan nanoparticles by foliar spray on leaves, enhanced the production of flavonoids and phenolics by 24% and 20% respectively[10]. Effect of gold and silver nanoparticles (in ratio 1:3) along with naphthalene acetic acid was studied in cultures of *Prunella vulgaris* that resulted in increase in total phenolic content, total flavonoid content and antioxidant activity[15].

As discussed, nanoparticles have the potential to enhance the secondary metabolite production in various plant species. These nanoparticles act as potent tools to increase the primary metabolism in plants. Apart, nano-elicitation has unlocked a new era of research that plays a significant role in pharmaceutical, food and herbal industries. Also, in order to understand the mechanism of nano-elicitation further standardization of plant cultures with specific nanoparticles is necessary. However, nano-elicitationomics based studies are required in near future so as to study the potential relationship between stress caused by nanoparticles and its effect on metabolite production.

Author's contributions :

Authors Tania Sagar, Nisha Kapoor and Ritu Mahajan contributed to the conception of the study, preparation and design of this review article. The literature survey was done by the authors Tania Sagar and Ritu Mahajan. Tania Sagar wrote the first draft of the manuscript and all the authors made their comments on it. All authors read, revised and approved the final manuscript.

Conflicts of interest :

The authors declare no conflict of interest.

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