Research Article

Nanoelicitors Application Promote Antioxidant Capacity of *Asparagus officinalis* (In Vitro)

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ABSTRACT

Nanoparticles recently play remarkable roles in modern agriculture and biotechnology due to specific exclusively physicochemical and biological characteristics of the particles. In recent years, nanoparticles are been using as fertilizers and elicitors to improve crops. Nutritionists are constantly seeking natural antioxidants without side effects to using for healthcare and treatment. *Asparagus officinalis* L. as medicinal plant treated by iron (0, 10, 50 and 100 mg/L) and selenium (0, 0.5, and 1 mg/L) nanoparticles as nano elicitors. Then the antioxidant capacity of *A. officinalis* L. was detected and measured by α , α -diphenyl- β -picrylhydrazyl (DPPH) assay, for assessment of the antioxidant activity. The iron nanoparticles concentration significantly increases the antioxidant activity of both male and female asparagus stem, as well as selenium nanoparticles. When combined iron and selenium used as nano elicitors then cause the antioxidant activity significantly decreases. But the integration of two nano elicitors (iron and selenium) decreased antioxidant capacity while the use of nano-selenium could enhance antioxidant capacity. The application of nano elicitor increased antioxidant capacity in the female stem than male.

Keywords: Nanoelictor, Iron nanoparticles, Selenium nanoparticles, Antioxidant capacity, *Asparagus officinalis*, Plant tissue culture

Introduction

Nanoparticles are a complex of many defined atoms of specific element. Generally, the most application of nanoparticles, in submicron range (less than 20 nm), belongs to Nano biotechnology. Nanoparticles of gold, silver, zinc oxide, titanium oxide, iron (Fe) oxide and selenium (Se) are widely employed in nanobiotechnology. The particles have been dramatically applied in biotechnology [1] and agriculture [2]. High contact surface of nanoparticles is biochemically and physicochemically effective [3]. So they are susceptible to be absorbed by cells then have function [4].

Nowadays, nanoelicitors application has been one of the most popular majors in agricultural

biotechnology, particularly plant tissue culture as a beneficial recommended approach to produce secondary metabolites of medical plants [5]. Elicitors could stimulate expression of the genes to produce enzymes to promote pathway of secondary metabolites [6]. Defensive responses can induce a network of signal transduction in plant. Acceptors molecules recognize and bind to the elicitors to promote this process. Regulatory hormones such as jasmonate, ethylene and salicylic acid are the most prominent precursor of promoting defensive responses. Biosynthesis of these hormones is caused by the effects of the elicitors, too [7].

According to the current studies, silver nano

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particles utilized to stimulate secondary metabolites in *Trigonella foenum-graecum* L. was significantly effective to enhance phytochemical compounds production [8]. As well, aloein concentration was enhanced by silver and titanium dioxide nanoparticles [9]. Also, it has also been showed nanoelicitor application in cell suspension of *Oryza sativa* L. makes some changes in hydrolyzates concentration [10]. It has also reported that nano titanium dioxide effects on Nitrogen metabolism by enhancing nitrate reductase and glutamate dehydrogenase activity to increase photosynthesis and growth [11]. Nano Chitosan is used to improve phenylethanoid glycosides biosynthesis in cell suspension culture of *Cistanche deserticola* Y.C.Ma [12].

Iron (Fe) nanoparticles can be formed in the form of iron oxide. They have been known as low-toxic and high eco-friendly nanoparticles [13]. It is reported that 0.75 g/L of iron nanoparticles positively influence on pod and leaf weight and dry weight of soybean. The highest yield (48%) was observed by 0.5 g/L iron nanoparticles [14]. Also the best yield of carrot was observed by 50 mg/kg nano-iron oxide particles [15].

Selenium is adsorbed from soil by the roots of plants [16]. Role of the element in plants is a serious debate between plant scientists; nevertheless low concentration of selenium is effective for herbal cells metabolism [17]. Regarded to scientific evidence, selenium can increase growth and yield of plants. Some plants are susceptible to accumulate selenium into their tissues [18]. Many studies confirm that the element is the most remarkable part of antioxidant enzymes structure. Selenium is generally as an essential element of 30 selenoenzyme and selenoprotein which can protect cell against free radicals. Selenium can be integrated with proteins of cells' membrane and tissues to protect them against oxidative stress [19]. There is a study to indicate that low concentration of selenium nanoparticles can enhance growth, physiological characteristics and chlorophyll content of Onion (*Allium cepa* L.) [20].

Natural Antioxidants extracted as prominent medical secondary metabolites have been being considered by nutritionists to alter chemical synthetic antioxidants [21]. There can be found many plant tissue culture approaches employed to enhance biosynthesis of the compounds in medical plants [22] such as *Asparagus officinalis*

L. This plant belongs to genus *Asparagus* that is used to cure several diseases [23]. Furthermore, *A. officinalis* L. is monocot and belongs to family Asparagaceae. The plants of Asparagaceae are mainly herbaceous and perennial or biennial and rarely annual, due to possession of tuber, bulb or rhizome [24]. The needle like derivative stems known as "Cladodes" contain chlorophyll tissue as same as leaf [25]. Inflorescences are axial and, in some cases, have single-stranded and dual-base flowers; flowers with six tepals partially fused at base; female flower with three carpels, each ovary with four seeds. [23]. *A. officinalis* L. has been employed as an important medical plant to remedy of several disorders and diseases for ages. Rhizome and spear of the plant is used for this purpose. The rhizome of asparagus can increase the volume of urine [27]. According to the effect, combination of the rhizome and roots of *A. officinalis* L. causes positive physical consequences such as elimination of thirst, jaundice and disorders of heart, bladder and breast. At the contrary, asparagus should not be used for patients with acute rheumatism and kidney stones [28]. There can be found asparagine in all parts of the plant which grow in whether darkness or lighting conditions. In addition, *A. officinalis* L. contains resin compounds such as conifeirine, inosit, tannin and garlic acid. The rhizome consists sucrose, asparagose, choline, arabinose, pentosan, galacton and a kind of heteroside [27]. The spears also contains throzine, conifeirine and succenique acid. Seed oil, about 15 percent of seed weight, can be extracted that it is reddish yellow and dried [29]. In the present study, iron and selenium nanoparticles were utilized as nanoelicitors to evaluate their effects on antioxidants capacity of *A. officinalis* L. evaluated *in vitro* culture.

Material and Methods

Preparing and culturing explants

Plant samples, both female and male spears of *A. officinalis* L. with the accession number 1322, were separately picked 2-3 cm above the soil surface and collected from organic research farm of Imam Khomeini Higher Education Center (Karaj, Alborz, Iran) to laboratory. Both female and male stems of *A. officinalis* L. were used in the experiment followed by three repeats per group. The explants of *A. officinalis* L. were provided by cutting shoots diagonally from

slightly lower than internodes. The culture medium dish containing cultured explants in MS medium [30] were transferred to growth chamber in condition including 23°C, 16 hours of light and 8 hours of darkness. After 30 days, calli and regenerated explants were subcultured in fresh media. After one month, the explants and calli were again subcultured in fresh media to be treated by nanoelicitors.

Nanoelicitors treatment

Selenium and iron nanoparticles were bought and provided from Central Nano Research and Technology Company (Biozar), Khomain, Markazi, Iran. Selenium nanoparticles (nano selenate; 0, 0.5 and 1 mg/l) and iron nanoparticles (nano Ferric. EDTA; 0, 10, 50 and 100 mg/l) were utilized as nanoelicitor treatments.

Extraction of the Essential oil of *Asparagus*

Essential oil was extracted from the explant to measure antioxidant capacity. At the first, 2 g of the sample and 20 ml distilled water were poured in a crucible and squashed completely. Then the sample was shaken by shaker in 140 rpm and for 48 hours. After that, this sample was centrifuged in 3600 rpm, 20°C and for 15 minutes [31]. The supernatant was concentrated by evaporation in darkness and open air for 24 hours, to remove aquatic solvent. The concentrated extraction was utilized to measure antioxidant capacity.

Assessment of the Antioxidant activity of *Asparagus*

DPPH (diphenyl-1-picrylhydrazyl free radical) [32] was employed to measure antioxidant capacity of treated explants of *A. officinalis* L. At the first, 400 g concentrated extraction was solved in 25 ml methanol. At the next stage, 4 mg concentration of diphenyl-1 picrylhydrazyl free radical was solved in 100 ml methanol (0.1 mMol). Then five concentrations of each extraction, solved in methanol, were provided 12.5 - 500 µg/ml. Next, 2 ml the prepared extraction and 2 ml DPPH were combined in a tube. So, there were 5 tubes contain same concentration of DPPH and different concentrations of each extraction. Then, the tubes stayed in darkness for 15 minutes. Finally, antioxidant capacity of the samples was analyzed by spectrophotometer, wavelength 570 nm.

Statistical analysis

As mentioned above, three and four different concentrations of nanoparticles, iron and selenium respectively, were used as nanoelicitors affecting on male and female stems of *A. officinalis* L. separately, with three repeats. Statistical analysis was performed using SPSS software and one-way ANOVA with the Duncan Multi-thread comparison test and paired t test. Graphs were also drawn by Microsoft Office Excel.

Results and Discussions

Nanobiotechnology application has widely developed on all majors [1] particularly agriculture [33]. Nanoparticles of silver, zinc oxide, titanium oxide, iron oxide and selenium are applied to produce fungicides, herbicide, pesticide and fertilizer in agriculture [34] due to their high specific and reactive surface [4]. Nanoparticles are employed as common fertilizers because they can be gradually released. So, the nano fertilizers are quickly and completely absorbed by plants [35]. In addition to high efficiency of nutrients absorption, they are susceptible to protect environment [2].

Plant tissue culture is being used as an effective biotechnological technique in agriculture to propagate plants and produce biomass and phytochemical compounds [5]. Elicitors' application is one of the most applicable methods to enhance secondary metabolites production. Biosynthesis of secondary metabolites could be induced by even low concentration of an elicitor [36]. Currently, elicitors are widely used to produce secondary metabolites. Elicitors are classified as two categories; biotic and abiotic [37]. Nanoparticles are considered as an abiotic elicitor which indirectly cause to induce secondary metabolites biosynthesis [38].

There are many studies to employ nanoparticles as nanoelicitor to increased biosynthesis of phytochemical compounds [39]. Nanoparticles, Nano silver and Nano titanium can quickly and positively effects on expression of genes which are involved biosynthesis of sanguinarine in *Papaver somniferum* L. [40], as well as alkaloids in *P. orientale* L. [41]. Biosynthesis of aloin was enhanced by Nano silver and Nano titanium [9]; as well phenylethanoid glycosides biosynthesis was increasingly stimulated by Nano Chitosan [12]. Moreover, it has been reported in some studies that NanoFe application can be increas-

Table 1. Variance analysis of nano selenium and nano iron effects on antioxidant activity

Treatments	F	Mean2	
		Female	Male
Fe	3	11052688 ^{NS}	11560774**
Se	2	9023965 ^{NS}	462961 ^{NS}
Se × Fe	6	137142707**	17016174**
Errors	24	73357524	3631605
Coefficient of variations (%)		9.899	6.536

**($P > 0.01$), *($P > 0.05$), ^{NS}: Not significant

Figure 1. Antioxidant capacity caused by interaction of nanoelicitors in female stem.

ingly effective to growth, yield parameters and physiological features of soybean [14] and carrot [15]. Also, there can be found scientific evidence to demonstrate NanoSe can positively induce growth and yield of plants [18], as well NanoSe low concentration can stimulate Onion (*Allium cepa* L.) growth, physiological characteristics and chlorophyll content [20].

The results show that the effects of several concentrations of iron nanoparticles (NanoFe) were significant on male stem. Also, interaction of both nanoelicitors significantly effects on both stems ($P < 0.01$). In contrast, there was no significant effect of NanoFe on female stem; as well nano selenium (NanoSe) on both stems (Table 1).

As the results of interaction effects of nanoelicitors on female stem, antioxidant capacity of female stems slightly growth though increasing concentrations of NanoSe, in the absence of

NanoFe. Whilst low concentration of Nano Fe can dramatically boost antioxidant capacity, but sharply decrease, then gradually increase in higher concentrations without NanoSe. Overall, higher concentration of both nanoelicitors can lead to increase higher antioxidant capacity. Nevertheless, 10 mg/L NanoFe × 1 mg/L NanoSe can be considered as the most effective treatment to bio-synthesis of highest antioxidant capacity concentration, 11974 µg/ml (Figure 1).

The results of the interaction effect of the nanoelicitors on male stem show that the individual presence of NanoSe can modestly increase antioxidant capacity. In the absence of NanoSe antioxidant capacity concentrations can moderately enhanced by NanoFe, then dramatically boosted. Overall, antioxidant capacity can be directly increased by increasing concentration of both Nanoelicitors. It could be considerable that

Figure 2. Antioxidant capacity caused by interaction of nanoelicitors in male stem

100 mg/l NanoFe without NanoSe can cause to extract highest antioxidant capacity concentration, 3361 $\mu\text{g/ml}$ (Figure 2).

Results of this study, individually each nanoelicitor could positively effect on antioxidant capacity of female stem. It can be notable that interaction of higher concentrations of NanoSe and lower concentrations of NanoFe boost to antioxidant capacity in female stem, whilst application of highest concentration of NanoFe without NanoSe is more effective than nanoelicitors interaction to enhance antioxidant capacity in male stem. Overall, application of both nanoelicitors increased antioxidant capacity.

Abiotic stresses such as water logging, intense light, drought, high and low temperature, salinity, environmental contamination and some pathogens as biotic stress can cause oxidative stress in plant tissues. There are many reactive oxygen species (ROS) including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) which play essential roles in oxidative stress. Chloroplast is considered as the main organelle to produce the reactive oxygen species due to imbalance between production and consumption of oxygen caused by stomatal resistance influenced by stress [41]. There is high chemical affinity of reactive oxygen species to react to vital biomolecules. The reactions can damage proteins, amino acids, nucleic acids and lipid membranes peroxidation which disrupt the

metabolism and consequently cause death. Plants are equipped with enzymatic and non-enzymatic antioxidant mechanisms for protection against oxidative stress [42]. There are some prominent enzymes such as superoxide dismutase, catalase and peroxidase which act as effective factors in enzymatic antioxidant mechanisms [43]. Secondary metabolites, as non-enzymatic antioxidant mechanisms, are synthesized by plants to protect themselves against oxidative stress [5]. Antioxidants including ascorbate, glutathione, tocopherols, carotenoids and flavonoids are recognized as the most remarkable secondary metabolites [21]. In a study to evaluate how nanoparticles increase secondary metabolites, it is demonstrated that oxidative stress caused by nanoparticles, commonly stimulates biosynthesis of secondary metabolites [38]. It seems that reactive oxygen species induced by nanoparticles, causes signalling pathway for other messengers such as salicylic acid, jasmonic acid and ethylene, also known as phytohormones, to produce secondary metabolites. It is reported that these phytohormones pathway is commonly affected by nanoparticles to stimulate basic immune response [7]. There cannot be unfortunately found many evidence and reports to understand mechanisms and pathway of effects of nanoparticles on secondary metabolites biosynthesis. It is hypothesized that nanoparticles are recognized Ca^{2+} receptors, channels and $\text{Ca}^{2+}/\text{Na}^+$ ATPases of plasma membrane to induce

oxidative stress. Then signalling molecules are released to activate of transcription factors for transcriptional reprogramming of secondary metabolites. Hence nanoparticles could influence on plant secondary metabolism by stimulating expression of the stress responsive genes involved in secondary metabolites biosynthesis. This ability of nanoparticles can be used in pharmacognosy to produce secondary metabolites [38].

It also seems that female stems of *A. officinalis* L. are more sensitive than male stems against oxidative stress which is induced by the nanoelicitors. Although high concentration NanoFe might be also make toxicity in male stems, NanoSe can probably reduce the negative consequences of high level of NanoFe in female stems, possibly by binding to antioxidant enzymes to stimulate the enzyme which can protect plant against oxidative stress [19]. It could be also considered that low concentration of NanoFe as co-factor can activate antioxidant enzymes including catalase, peroxidase and cytochrome oxidase, in male stem [42]. Moreover, it is reported that the flavone and rutin, as two antioxidant compounds, are produced in male plants more than females [44]. So, it is indicated that there are many functions in male *A. officinalis* L. causing greater longevity than females. Finally, it seems that these functions include enzymatic and non-enzymatic approaches in males [45] to make higher resistance against environmental stresses [43].

Conclusion

It could be concluded that higher concentrations of NanoFe can individually boost antioxidant capacity in both female and male stems. Although individual application of NanoSe can increase antioxidant capacity, it is not significant. In contrast, interaction of the nanoelicitors could be effective to produce antioxidant compounds. Male stem treated by the nanoelicitors contains more antioxidant capacity than female stem. It could be recommended as commercial and economical way that lower concentration of NanoFe can be used in female stem tissue culture to induce antioxidant metabolites production.

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