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Synthesis of silver nanoparticles of indole acetic acid and its effect on seed germination *of Lablab purpureus* seeds.

Síntesis de nanopartículas de plata de ácido indolacético y su efecto en la germinación de semillas de *Lablab purpureus*

Juhi Anil Naik*, Dr. Pinky Sharma

Madhav University, Pindwara (Sirohi), "Madhav Hills" Opp. Banas River Bridge Toll, N.H.-27, Post office-Bharja, Tehsil-Pindwara, District – Sirohi, Rajasthan – 307028, India.

* Author for correspondence, email: omjuhi29@gmail.com

ABSTRACT

Horticulture is that the branch of agriculture that deals with science and art of the event, sustainable production, marketing, and use of high-value, intensively cultivated food and decorative plants. Horticultural crops are diverse; they include annual and perennial species, delicious fruits and vegetables, and ornamental indoor and landscape plants. It is considered to be the foremost a part of the planet economy. Even though, one among the main challenges, seriously influencing the economic loss of horticulture is rooting of cuttings and root growth inhibiting plant pathogens. To deal this issue through nanobiotechnology, a concept has been built of silver nanoparticles (AgNPs) as "nano-bullets". In this study AgNPs synthesis, using rooting hormones Indole Acetic Acid was done. Further, its effect on Seed Germination of Lablab purpureus seeds was studied with references to three parameters: Seed Germination (%), Root Length (cm) and Fresh Root Weight (gm). As a result, threefold enhacement was observed in seeds treated with AgNPs compared to the control on the sixth day of observation. This study can unravel the relevance, scope and current challenges at horticulture plants root development and disease management for the sustainable agricultural crop production.

Keywords: Horticulture, Nanobiotechnology, Silver Nanoparticles, Indole Acetic Acid, Agriculture

RESUMEN

La horticultura es una rama de la agricultura que se ocupa de la ciencia, el arte del evento, la producción sostenible, la comercialización, la utilización de productos de alto valor, alimentos cultivados intensivamente y plantas decorativas. Los cultivos hortícolas son variados, incluyen: especies anuales y perennes, frutas y vegetales deliciosos y plantas ornamentales de interior y de jardín. Estos cultivos se consideran una de las

partes más importantes de la economía en el mundo, sin embargo, uno de los mayores desafíos, que influye seriamente en las pérdidas económicas de la horticultura, es el enraizamiento de esquejes y el crecimiento de raíces que inhiben los patógenos de las plantas. Para lidiar con este problema mediante la nanobiotecnología, se ha creado un concepto de nanopartículas de plata (AgNPs) como “nano-bullets” (nanobalas). En el presente estudio, se realizó la síntesis de AgNPs utilizando hormonas de enraizamiento de ácido indo lacético. Además, se estudió su efecto sobre la germinación de las semillas de *Lablab purpureus* con relación a tres parámetros: germinación de semillas (%), longitud de la raíz (cm) y peso fresco de la raíz (g). Como resultado, se observó un triple aumento en las semillas tratadas con AgNPs, comparadas con el control del sexto día de observación. Este estudio puede contribuir a la relevancia, el alcance y los retos actuales del desarrollo de las raíces de las plantas hortícolas y la gestión de enfermedades para la producción sostenible de cultivos agrícolas.
Palabras clave: horticultura, nanobiotecnologia, nanoparticulas de plata, ácido indolacético, agricultura

INTRODUCTION

Nano-biotechnology is that the biology-based, application-oriented frontier area of research within the hybrid discipline of Nanoscience and biotechnology with the same contribution. In current nano dynasty, a nanomaterial of organic and inorganic origin ranging from 1 to 100 nm has one- or multi-dimensional application in all fields of science and technology in increasing number. Meanwhile, nanotechnology application to biotechnology also will leave no field untouched by its groundbreaking scientific innovation for human wellness; the agriculture industry is not any exception. Basically, nanomaterials are distinguished counting on the origin: natural, incidental and engineered nanoparticales. Among these, engineered nanoparticales have received wide attention altogether fields of science, including medical, materials and agriculture technology with significant socio-economical growth.
In the agriculture industry, engineered nanoparticles are serving as “nano carrier”, containing herbicides, chemicals, or genes, which target particular plant parts to release their content (Lian et.al., 2012; Zakaria et al.,2015; Wu et al., 2011). Previously nanocapsules containing herbicides are reported to effectively penetrate through cuticles and tissues, allowing the slow and constant release of fertilizers has also become a trend to save lots of fertilizer consumption and to attenuate environmental pollution through precision farming (Jones, 2006). These are only few examples from numerous research works which could open up exciting opportunities for nanobiotechnology application in agriculture. Also, application of this type of engineered nanoparticles to plant should be considered to the extent of amicability, before it's employed in agriculture practices. Based on a thorough literature survey, it was understood that there is only limited authenticity regarding information available to explain the biological consequence of engineered nanoparticles on the treated plants. Certain reports underline the phytotoxicity of various origin of engineered nanoparticles to the plant caused by the subject of concentrations and sizes (Craig and Jason, 2011; Battke et al., 2008; Ghodake et al., 2011; Samardakiewicz and Wozny, 2005; El Sayed, 2004). At an equivalent time, however, an equal

number of studies were reported with a positive out-come of nanoparticles, which facilitates growth promoting nature to treat plants (Nair et al., 2010; Monica ad Cremonini, 2009; Mariya and Mohamed, 2014; Juhel et al., 2011). In particular, compared to other nanoparticles, sliver and gold nanoparticles based application elicited beneficial results on various plant species with less and /or no toxicity (Raqual et al., 2009; Raja Muthuramalingam et al., 2015). Silver nanoparticles (AgNPs) treated leaves of Asparagus showed increased content of ascorbate and chlorophyll (An et al.,2008). Similarly, AhNPs-treated common bean and corn has increased shoot and root length, leaf surface area, chlorophyll, carbohydrate and protein contents as reported earlier (Salama, 2012). The gold nanoparticle has been wont to induce growth and seed yield in chinese mustard (Arora et al., 2012). Since then, only limited drawbacks were reported for silver and gold nanoparticles mediated toxicity, and still there's huge scope for applying nanoscale manipulated to agriculture use. Horticulture, a number one sector within the field agriculture produces vegetables. Nuts, fruits, flowers, trees and herbs, which covers almost 100% of the daily need by overall population within the world (Priede and Klavina. 2011). This potential sector also has some downside which leads to huge losses in this sector. Among these techniques, root development in plant cutting is quite tedious in spite of using rooting hormones due to two main factors: different rooting abilities and root growth –deteriorating phytopathogens eventually leading to huge loss in horticulture (Steve Dreistadt, 2001). In order to unravel these issues, a singular approach (nano-bullets) was devised to utilize the rooting hormone- stabilized silver nanoparticles both as root promoters and as antimicrobial agent in parallel. In this research work, we've used plant rooting hormones, Indole ethanoic acid (IAA) and Indole-3-Butyric Acid (IBA) as a reducing cum capping agent to synthesis by using silver nanoparticles. Till day it has not been reported the synthesis of nanoparticles by using palnt auxin hormoes. Both indole ethanoic acid (IAA) and indole-3-Butyric Acid (IBA) are native plant growth regulators from auxin family, and specifically play a key role in root development in both ex vitro and in vitro grown plants (Becker and Hedrich, 2002; Stefancic et al., 2005; Tiberia et al., 2011). Therefore, we validated the potential of IAA and IBA stabilized AgNPs in enhancing root growth.

MATERIALS AND METHOD

Synthesis of Silver Nanoparticles from Indole Acetic Acid (IAA)

For the synthesis of silver nanoparticles 1mM of silver nitrate solution was prepared by dissolving 16.9 mg of $AgNO_3$ in 100 ml of distilled water and the solution was kept in an ice bath for half an hour. 10mM of Indole Acetic Acid (IAA) was prepared, at the beginning of the reaction, 5 ml of 1mM ice cold silver nitrate solution was taken in test tubes. Then Indole Acetic Acid (IAA) solution were added and mixed in test tubes containing aqueous $AgNO_3$. The time of addition of Indole Acetic Acid (IAA) in a silver nitrate solution was considered as the starting of the reaction mixtures were subjected to continuous stirring in a water bath at 60-70^{o}C until the transparent solution changed into yellow color. The color change indicates the formation of colloidal nanoparticles stabilized by Indole

Acetic Acid (IAA). The observation of faint yellow color after 24 h of reaction indicated the formation of silver nanoparticles which was characterized by absorbance at 420nm. (R.muthuramalingam thangavelu et al., 2016).

Effect of silver nanoparticle of Indole Acetic Acid (IAA) on seed germination

The Val (*Lablab purpureus)* seeds were collected from agriculture land, Valsad, Gujarat, India. Seeds were surface sterilized with 0.1 % sodium hypochlorite solution for 3 minutes. Ten viable seeds were tested for each silver nano particles from Indole Acetic Acid (IAA) aqueous extract. Seeds, without silver nano particles of Indole Acetic Acid (IAA) extract, served as control. Each petridish contain ten surface sterilized seeds placed on filter paper and moistened with 10 ml of the aqueous extract of silver nano particles of Indole Acetic Acid (IAA). Petri dishes containing seeds with 10 ml of distilled water served as a control. The growth parameters including germination percentage, root length and weight were recorded on the 6th day after incubating seed at 28°C.

Experimental observations on Indole Acetic Acid (IAA) nano-particles

1) Percentage germination (%)

The percentage germination was calculated by dividing number of seeds germinated to the total number of seeds used for soaking.

2) Root length

Root length of ten seedlings in each treatment and replication were measured in centimetre. The average root length were computed and expressed in centimeter.

3) Fresh weight (g) of seedlings

Ten seedlings from each treatment and replication were selected. Fresh weight of the seedlings was taken in gram. The average fresh weight were weighed and expressed in gram. Four most efficient isolates based on their observation of % germination, root length and fresh weight of seedlings were selected for further investigation.

RESULTS AND CONCLUSION

Effect of indole acetic acid nano-particles extract (after 6 days) on germination and growth of *Val (Lablab purpureus*)

The extract of indole acetic acid (IAA) nano-particles showed 100 percent seed germination 10 cm root length and 0.91 gm of fresh root weight compared to the control which showed 70 percent seed germination 3.4 cm root length and 0.49 gm of fresh root weight on the 6th day as shown in Table 1.

Table 1: Effect of nano-particles of Indole Acetic Acid (6th day) on *Val (Lablab purpureus)*

Sample	Seed Germination (%)	Root Length (cm)	Fresh root weight (gm)
Control	70	3.4	0.49

Nano-particles	100	10	0.91

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