Nanotechnology on Breaking Corm Dormancy in Elephant Foot Yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson]

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ABSTRACT

Background: Nanotechnology is a fast developing science which has various applications in agriculture. Nanoparticles are broadly defined as the small particles with at least one-dimension size between 1 and 100 nm in diameter. Nanomaterials have the potential to penetrate the seed coat and enhance the ability of absorption and utilization of water, which stimulates enzymatic system and ultimately improves germination and seedling growth. The present study was undertaken to investigate the effects of nanoparticles and commercially used chemicals on breaking dormancy of *Amorphophallus* corms.

Methods: Silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO_2), cerium oxide (CeO) and zero valent iron (ZVI) nanoparticles were synthesized successfully using chemical method and characterized for its size, shape and properties using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Particle Size Analyzer (PSA) and Raman spectroscopy. Freshly harvested corms of *Amorphophallus* were treated with different concentrations (50 and 100 mg kg^{-1}) of these five nanoparticles and commercially used chemicals *viz*., thiourea (100 and 200 mg kg^{-1}) and potassium nitrate (1000 and 2000 mg kg^{-1}), with three different soaking periods (2, 4 and 6 h).

Result: The analysed data indicated that corms treated with ZnO @ 100 mg kg^{-1} for six hours registered the minimum number of days (47.0 days) for sprout emergence. The maximum sprout length (23.50 cm), sprout diameter (6.35 cm), pseudo stem length (31.00 cm), pseudo stem girth (7.35 cm) and vigour index (1591.7) were recorded in TiO_2 @ 50 mg kg^{-1} for two hours.

Key words: *Amorphophallus*, Breaking dormancy, Chemicals, Nanoparticles, Zinc oxide

INTRODUCTION

Amorphophallus paeoniifolius (Dennst.) Nicolson is a herbaceous perennial belonging to the family Araceae, commonly known as elephant yam or elephant foot yam or suran or sweet yam (Hetterscheid and Ittenbach, 1996). It is basically a crop of South East Asian origin. It serves as a source of carbohydrates (10 g/100 g) and protein (1. 2 g/100 g) and it is rich in minerals (65.6 mg/100 g) and vitamin A (270 IU/100 g). It has long been used as a staple food in many countries such as Philippines, Java, Indonesia, Sumatra, Malaysia, Bangladesh, India, China and South Eastern countries (Chandra, 1984; Sugiyama and Santosa, 2008).

Traditionally, elephant foot yam is propagated through corms and cormels. The main drawback in the propagation of *Amorphophallus* is the prevalence of dormancy. The corms exhibit dormancy for about three to five months after harvest. If the dormancy can be broken by appropriate techniques, it will be possible to plant and harvest the crop throughout the year (CTCRI, 1993). The sprouting percentage in elephant foot yam is 66.6% and thee bottom portion of the corm is not generally used as planting material due to its lower sprouting efficiency (Ravi *et al*., 2011). Chemical substances are extremely used for improvement of seed germination and breakdown of seed dormancy in plants. Application of some nanomaterials can help faster plant germination or production, effective plant protection with reduced environmental impact as compared with the traditional methods (Grover *et al*., 2012).

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The effect of nanoparticles on plant growth varies greatly with the type of nanoparticle, concentration and the plant species used. Further different nanoparticles affect different growth processes of plants. The nanomaterial can enter the plant by binding to carrier proteins, through aquaporin, ion channels, endocytosis, by creating new pores or by binding to the organic chemical in the environmental media (Rico *et al.,* 2011). Hence in the present investigation, a novel approach of utilizing nanotechnology was exploited in order to break the dormancy of *Amorphophallus* corms and to compare the effect of nanoparticles with commercially used chemicals on the dormancy and physiological characters of *Amorphophallus.*

MATERIALS AND METHODS

The present investigation was conducted at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, during the year 2014-15. The experiments were laid out in factorial completely randomized design (FCRD) with 47 treatments and two replications. The treatments consisted of T_1: Absolute control, T_2: CTCRI technique (corms dipped in cowdung slurry and dried under shade), T_3, T_4, T_5: Water soaking for 2, 4 and 6 hours, T_6, T_7, T_8: Ag @ 50 mg for 2, 4 and 6 hours, T_9, T_{10}, T_{11}: Ag @ 100 mg for 2, 4 and 6 hours, T_{12}, T_{13}, T_{14}: ZnO@ 50 mg for 2, 4 and 6 hours, T_{15}, T_{16}, T_{17}: ZnO@ 100 mg for 2, 4 and 6 hours, T_{18}, T_{19}, T_{20}: TiO_2 @ 50 mg for 2, 4 and 6 hours, T_{21}, T_{22}, T_{23}: TiO_2 @ 100 mg for 2, 4 and 6 hours, T_{24}, T_{25}, T_{26}: CeO @ 50 mg for 2, 4 and 6 hours, T_{27}, T_{28}, T_{29}:CeO @ 100 mg for 2, 4 and 6 hours, T_{30}, T_{31}, T_{32}: ZVI @ 50 mg for 2, 4 and 6 hours, T_{33}, T_{34}, T_{35}: ZVI @ 100 mg for 2, 4 and 6 hours, T_{36}, T_{37}, T_{38}: KNO_3 @ 1000 mg for 2, 4 and 6 hours, T_{39}, T_{40}, T_{41}: KNO_3@ 2000 mg for 2, 4 and 6 hours, T_{42}, T_{43}, T_{44}: Thiourea @ 100 mg for 2, 4 and 6 hours,T_{45}, T_{46}, T_{47}: Thiourea @ 200 mg for 2, 4 and 6 hours. The nanoparticles were synthesized successfully by chemical method and characterized for its size, shape and properties using scanning electron microscope (SEM), transmission electron microscope (TEM), particle size analyzer (PSA) and raman spectroscopy.

Planting material used was corms of *Amorphophallus paeoniifolius* cv. Appakkudal local. The corm pieces of 100 g size each with dormant buds, cut from whole corm were used for the treatments. Freshly harvested and uniform sized corms of *Amorphophallus* were soaked for 2, 4 and 6 h separately in the respective treatment solutions and the treated corms were planted in polythene bags containing sand media. The corms subjected to the treatments were evaluated for their physiological parameters *viz*., days taken for sprouting, sprout length, sprout diameter, pseudostem length, pseudostem girth and vigour index.

RESULTS AND DISCUSSION

Characterization of nanoparticles

Size, shape and functionality of the synthesized nanoparticles (NPs) were characterized using scanning electron microscope (SEM), transmission electron microscope (TEM), particle size analyzer (PSA) and raman spectroscopy.

Scanning electron microscope (SEM)

The surface morphology of the nanoparticles synthesized when examined under SEM revealed that ZnO NPs were in the shape of bunches of flowers (Fig 1a). Each bunch appeared to consist of closely packed nanometer scale rods emerging from central core as radiating structures. The result is consistent with the reports of Moghaddam *et al*. (2009), Senthilkumar (2011) and Sridhar (2012).

Transmission electron microscope (TEM)

The Ag NPs examined under TEM were spherical in shape, with the size ranging from 50 to 100 nm (Fig 1b). Similar results were obtained by Sridhar (2012). TiO_2 NPs were spherical in shape with an average size of 100 nm (Fig 1c). Observation of the present investigation coincides with the results obtained by Park (2005).

Particle size analyser (PSA)

The mean size of five nanoparticles synthe sized when measured in particle size analyzer ranged from 94.6 nm (CeO) to 611.4 nm (ZVI) and Ag, ZnO and TiO_2 NPs measured 163.5, 145.5 and 578.7 nm respectively. The CeO nanoparticle recorded the least size (94.6 nm) compared to the Ag and ZnO, which were 1.72 and 1.53 times larger, while TiO_2 was 6.11 times and ZVI 6.46 times larger. Size and shape of the nanoparticles are naturally expected to

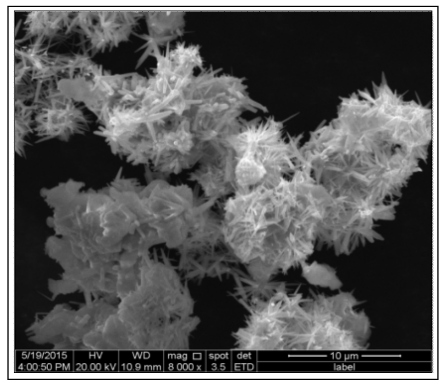

Fig 1a: SEM image of ZnO.

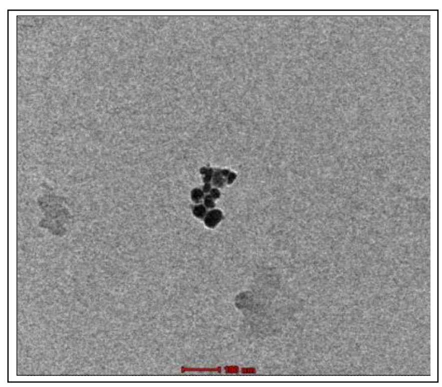

Fig 1b: TEM image of Ag.

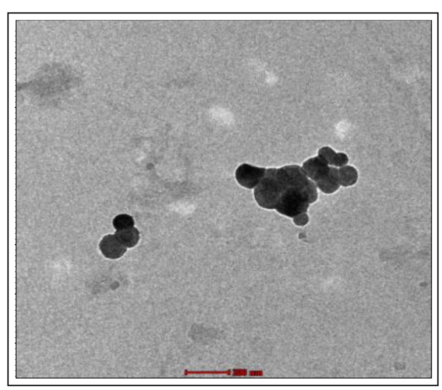

Fig 1c: TEM image of TiO_2.

play major role on the treated *Amorphophallus* corms and smaller sizenano particles are supposed to have greater permeability.

Raman spectroscopy

Raman spectrum was employed to identify the chemical composition and to confirm the five different nanoparticles synthesized by observing the peaks. The peaks were observed at 221, 1039.5, 1354.1 and 1646.6 cm^{-1} for Ag, while at 185.2, 289.3, 368.7 and 797.3 cm^{-1} for ZnO, 195.5, 395.7, 513.8, 635.3 cm^{-1} for TiO_2, 185.2, 289.3, 463.6, 723.9, 797.3 cm^{-1} for CeO and 187.8, 289.3, 660.5, 757.4, 797.3 cm^{-1} for ZVI. The raman spectra peaks for Ag, ZnO, TiO_2, CeO and ZVI nanoparticles confirmed the respective compounds and are supported by the earlier reports of Strelchuk *et al*. (2015), Guo *et al*. (2012) and Madhavi *et al*. (2013) respectively.

Dormancy and physiological characters

Nanoparticles of Ag, ZnO, TiO_2, CeO and ZVI when treated in dif-ferent concentrations *viz*., 50 and 100 mg kg^{-1} significantly outperformed from absolute control (T_1), CTCRI technique (T_2), commercially used chemicals (T_{36} -T_{47}) and water soaking treatments (T_3, T_4, T_5) in terms of sprouting, sprout length, sprout diameter, pseudo stem length, pseudo stem girth and vigour index. Significant differences were also observed between the concentration of nanoparticles and different soaking durations.

Days taken for sprouting

According to the results (Table 1) obtained, it was observed that among the five NPs tested, corms treated with CeO @ 100 mg registered the minimum number of days to sprout (55.7 days), which is significantly lower than other treatments. The duration of soaking profusely decreased the days taken for sprouting of corms. There was a significant reduction in the number of days to sprout from 86.1 to 77.9, when the soaking time extended from 2 to 6 hours. Interaction among the concentration of nanoparticles and soaking duration revealed that ZnO @ 100 mg for 6 hours significantly reduced the days required for sprouting (47.0 days), in comparison to the absolute control (138.0 days) and CTCRI method (117.5 days) of treatments.

In many studies, evidence suggests that zinc oxide nanoparticles (ZnO NPs) increase the plant growth and development. Raskar and Laware (2014) reported that lower concentration of ZnO NPs exhibited beneficial effect on seed germination in onion. The application of ZnO-NPs resulted an enhancement of 37.5% in seed germination in Broccoli. Chlorophyll, phenolic, proline and sugar content were also observed to be increased by 50%, 67.4%, 14.6% and 36.2% respectively. (Awan *et al*., 2021). The beneficial effect of the ZnO NPs in improving the germination could be ascribed to higher precursor activity of nanoscale zinc in auxin production (Kobayashi *et al*., 1970). Apart from this, zinc is one of the essential nutrients required for plant growth. It is

Table 1: Effect of nanoparticles, chemicals and soaking durations on dormancy breaking and early sprouting (days) of *Amorphophallus* corms.

Conc. of NPs and chemicals	Days taken for sprouting				
	Soaking duration (h)				
	0 h	2 h	4 h	6 h	Mean
Absolute control	138.0	-	-	-	138.0
CTCRI technique	117.5	-	-	-	117.5
Water soaking treatment	-	112.5	108.0	106.5	109.0
Ag @ 50 mg	-	101.5	114.0	105.0	106.8
Ag @ 100 mg	-	56.5	76.5	48.5	60.5
ZnO @ 50 mg	-	85.0	56.5	94.0	78.5
ZnO @ 100 mg	-	92.5	75.0	**47.0**	71.5
TiO_2 @ 50 mg	-	79.5	75.0	85.5	80.0
TiO_2 @ 100 mg	-	50.5	103.0	78.0	77.2
CeO @ 50 mg	-	85.0	98.5	105.5	96.3
CeO @ 100 mg	-	61.0	55.5	50.5	55.7
ZVI @ 50 mg	-	105.0	75.0	94.5	91.5
ZVI @ 100 mg	-	89.5	77.5	51.5	72.8
KNO_3 @ 1000 mg	-	96.0	98.5	72.5	89.0
KNO_3 @ 2000 mg	-	95.5	102.5	76.5	91.5
Thiourea @ 100 mg	-	117.5	89.0	76.0	94.2
Thiourea @ 200 mg	-	63.5	89.5	77.5	76.8
Mean	127.8	86.1	86.3	77.9	
		SE (d)		CD (0.05)	
Soaking duration (h): D		3.3		6.6	
Conc. of NPs and Chemicals: C		7.1		14.3	
Interaction (D×C)		12.3		24.8	

an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Zinc oxide nanoparticles also have positive effect on the reactivity of phytohormones especially indole acetic acid (IAA), which is involved in the phytostimulatory actions. Zinc-rich ZnO nanoparticles could increase the level of IAA in the roots, which in turn can increase growth rate of seedlings (Pandey *et al*., 2010).

Sprout length and sprout diameter (cm)

The results pertaining to sprout length and diameter revealed that the corms treated with ZVI @ 100 mg and TiO_2 @ 50 mg registered the maximum sprout length (18.33 cm) and sprout diameter (5.15 cm), respectively. Among the chemical treatments tested, KNO_3 @ 2000 mg recordedthe maximum sprout length and diameter (16.08 cm and 5.03 cm). There was no significant variation between two and four hours soaking durations. Interaction among the concentration of nanoparticles and duration of soaking suggests that TiO_2 @ 50 mg for 2 hours significantly increased the sprout length (23.50 cm) and diameter (6.35 cm) in comparison to absolute control and CTCRI method of treatments (Fig 2). This results are in accordance with the report of Mahmoodzadeh *et al.* (2013), who observed that TiO_2 NPs enhanced seed germination and promoted radicle and plumule growth of canola seedlings. Raskar and Laware (2013) observed that TiO_2 NPs increased the shoot length and total seedling height in onion.

Vigour index

Data with respect to vigour index clearly indicated that corms treated with TiO_2 @ 50 mg registered the maximum vigour index (1591.7), which is significantly higher than other treatments. Other NPs such as Ag @ 50 mg (1339.0) and ZVI @ 100 mg (1325.2) were also found to increase vigour index compared to the commercial chemicals. Among the chemicals tested, Thiourea @ 100 mg registered higher vigour index of 1053.4. There was a significant reduction in vigour index from 1043.7 to 1036.2 when the soaking time extended from 2 to 6 hours. Interaction among the concentration of nanoparticles and duration of soaking revealed that TiO_2 @ 50 mg for 2 hours has significantly increased the vigour index (1933.2), in comparison to absolute control (508.3) and CTCRI method (240.0) of

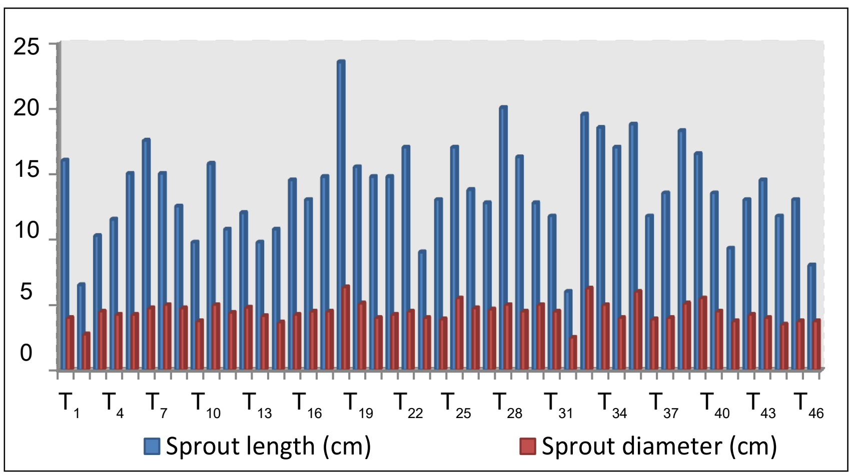

Fig 2: Effect of nanoparticles and chemicals on sprout length and diameter (cm).

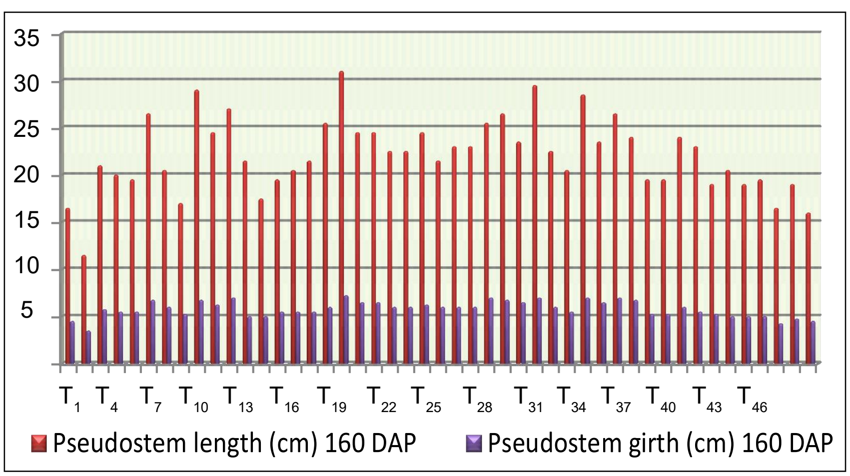

Fig 3: Effect of nanoparticles and chemicals on pseudostem length and girth (cm).

treatments. The results are in confirmation with the findings of Zheng *et al*. (2005), who reported that nanoscale TiO_2 particles enhanced the seedling vigour in spinach (*Spinacia oleracea*). TiO_2 NPs at lower concentration enhances seed germination, promptness index and seedling growth in onion. These results point out the possible use of TiO_2 NPs in onion to promote seed germination and early seedling growth to have healthy and sturdy seedling stock for plantation in the field (Raskar and Laware, 2013).

Pseudostem length and girth (cm)

The achieved results showed that among the five NPs tested, corms treated with TiO_2 @ 50 mg registered the maximum pseudostem length (25.30 cm) and girth (6.60 cm), which is significantly higher than other treatments. Among the chemicals used, KNO_3 @ 2000 mg registered the maximum pseudostem length and girth (20.80 cm, 5.30 cm). The interaction among the concentration of nanoparticles and duration of soaking revealed that TiO_2 @ 50 mg for 2 hours significantly increased the pseudostem length (31.00 cm) and girth (7.35 cm), in comparison to absolute control and CTCRI method of treatment (Fig 3).

CONCLUSION

In the present study, a novel approach of utilizing nanotechnology was exploited in order to break the dormancy of *Amorphophallus* corms. The *Amorphophallus* corms treated with synthesized nanoparticles outperformed in enhancing sprouting of corms, sprout length, sprout diameter, pseudostem length, pseudostem girth and vigour index compared to the commercially used chemicals. It may be due to the enhancement of physiological and biochemical properties of corms resulting in improved sprouting and vigour.

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