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RESEARCH PAPER

Customizing zinc oxide nanoparticles for extending seed vigour and viability in tomato (*Lycopersicon esculentum* Mill)

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Abstract : Tomato is one of the important vegetable crops being cultivated throughout India. Rapid deterioration of stored vegetable seeds is a serious problem which occurs at an increasing rate in uncontrolled storage environment. The present study was aimed at investigating the effects of zinc oxide nanoparticles (nano-ZnO) on tomato seeds during aging. The experimental treatments included four concentrations of nano sized ZnO (400, 600, 800, and 1000 mg/kg), and control (without any ZnO) on fresh and aged seeds. Results indicated that the highest and the lowest vigour index (1986 vs. 1521) were obtained in 600mg concentration of nano sized ZnO and control treatments,, respectively in fresh seeds. During aging, vigour index was maintained significantly as 995 with 600mg nano sized ZnO treated seeds when compared with control (495). This study shows that employing ZnO nanoparticles could reduce tomato seed deterioration during aging and promote the seed germination.

Key Words : Nanosized nanoscale ZnO, Germination, Seed vigour, Tomato

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INTRODUCTION

Tomato seeds have a high commercial value, and the loss of seed physiological quality over time is demonstrated by their low storability unless hermetic conditions are used. The ageing process cannot be arrested completely, once commenced. It can be controlled to a certain extent by adoption of new technologies. Controlled storage of seeds would greatly solve the problem of seed deterioration but such facilities are expensive and not available at present for the common farmer. Evolving a cost effective storage technology to counteract seed deterioration would be very useful. Among the various factors responsible for seed deterioration peroxidation of phospholipid (Dadlani and Agarwal, 1983) and free radical accumulation (Ramamoorthy and Basu, 1984 and Wilson and McDonald, 1986) are the major causes for seed aging. Several strategies such as hydration-dehydration

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techniques, halogenations and antioxidant treatment have been tested to prevent deterioration and extending the shelf life of the seed. These techniques are cumbersome and are not being adopted by farmers due to practical difficulties. The use of nanoparticles in commercial products and industrial applications has increased greatly in recent years. However, understanding of the interaction mechanisms at the molecular level between nanoparticles and biological systems is largely unknown (Barrena *et al.,* 2009). Nanotechnology allows wide advances in agricultural research, such as reproductive science and technology, transfer of agricultural and food wastes to energy and other useful by-products through enzymatic nanobioprocessing, disease prevention, and treatment in plants using various nanocides (Carmen *et al.,* 2003). Owing to the small size and larger surface area, metal nanoparticles have an option to efficiently catalyse the chemical reactions. Metal nanoparticles are more efficient in scavenging superoxides (O_2^-) and the scavenging activities of metal nanoparticles are similar to free radical scavenging enzymes superoxide dismutase (SOD) and catalase. In addition metal nanoparticles reduce the accumulation of lipofuscin which is an endogenous autofluorescent marker generated by oxidative degeneration of cellular components that is accumulated with ageing (Halliwell and Gutteridge, 1999; Kajitha and Hikosaka, 2007; Hamasaki and Kashiwagi, 2008; Kim and Takahashi, 2008; Watanabe and Kajita, 2009). Zinc (Zn) is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases) (Auld, 2001; Marschner, 1993; Brown *et al.,* 1993 and Fageria *et al.,* 2002).

Limited studies have been reported on the promotory effects of nanoparticles on plants in low concentrations. Nanoscale titanium dioxide (TiO_2) was reported to promote photosynthesis, and growth of spinach (Hong *et al.,* 2005; Yang *et al*., 2006 and Zheng *et al.,* 2005). Lu *et al*. (2002) have shown that a combination of nanosized SiO_2 and TiO_2 could increase the nitrate reductase enzyme in soybean (*Glycine max*), increase its abilities of absorbing and utilizing water and fertilizer, promote its antioxidant system and in fact accelerate its germination and growth.

Also it has been found that TiO_2 nanoparticles encourage spinach (*Spinacia oleracea*) seed germination and plant growth (Zheng *et al.*, 2005). The presence of nanoscale aluminium (Al) particles did not have a negative effect on the growth of *Phaseolus vulgaris* and *Lolium Perenne* in the tested concentration range (Doshi *et al.,* 2008). Phytotoxicities of nanoparticles on plants were reported by Yang and Watts (2005) (in cabbage and carrot) and Lin and Xing (2007) (radish, rape, and rye grass) at concentrations greater than 2000 ppm. The present study was taken up to investigate the effects of various concentrations of ZnO nanoparticles for the extending vigour and viability in fresh as well as accelerated aged tomato seeds.

MATERIAL AND METHODS

The ZnO nanoparticles were synthesized in Department of Nano Science and Technology, and accelerated ageing study was carried out at the Department of Seed Science and Technology, Tamil Nadu Agriculture University. The seed samples of COTH2 tomato hybrid was obtained from the Department of Vegetables.

Synthesis of nanoparticle :

ZnO nanoparticles were synthesized using 0.45 M aqueous solution of zinc nitrate $Zn(NO_3)_2.4H_2O_3$) and 0.9 M aqueous solution of sodium hydroxide (NaOH). The beaker containing sodium hydroxide solution was heated at temperature of about 55^{0}C. The $Zn(NO_3)_2$ solution added drop by drop (slowly for 40 min) to the above heated solution under high-speed stirring. The beaker sealed at this condition for 2h. The precipitated ZnO NPs cleaned with Millipore water and ethanol and then dried in air atmosphere at about 60^{o}C. Morphology of the synthesized nanoparticle was investigated using Scanning Electron Microscope (SEM). The SEM images demonstrate that a bulk quantity of flower-like bunches exist. Each bunch is gathered of closely packed nanometer scale rods and it forms radiating structures (Plate 1).

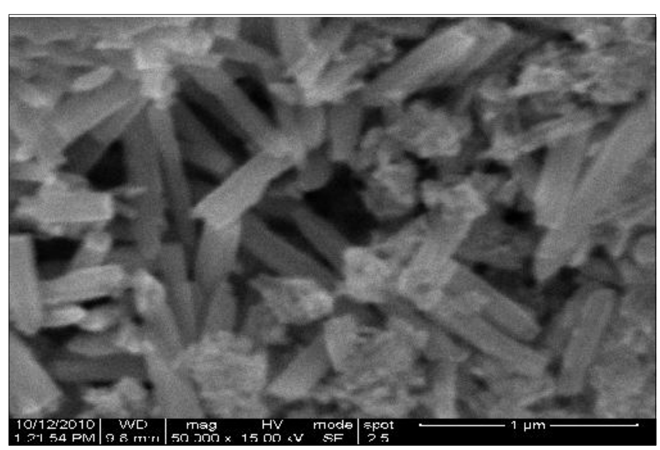

Plate 1: SEM image of ZnO nanoparticles

Standardization of ZnO nanoparicles on the maintenance of vigour and viability of tomato :

The synthesized nanoparticles (ZnO) were used for treating fresh tomato seeds in various concentrations as detailed below and the untreated seeds were used as control for this experiment.

Dosage :

Treatments	Concentrations (mg/kg)
T_0	Control
T_1	400
T_2	600
T_3	800
T_4	1000

The seeds were mixed with nanoparticles in air tight narrow mouthed glass bottle and vigorously shaken well for 10 min to ensure the entry of nanoparticles in the seeds. Treated seeds were subjected to germination test. Part of the treated and control seeds were subjected to accelerated aging for that the seeds were packed in perforated butter paper bags and placed in a desiccation glass jar containing water instead of silica gel to maintain relative humidity at 98 ± 2 per cent. Then the glass jar was kept inside a hot air oven maintained at 40 ± 1° C. Germination test was carried out immediately as well as after ageing.

Seedling evaluation :

The germination test was carried out in modified roll towel method using eight replicates of 25 seeds each were germinated in a germination room at 25 ± 2ºC temperature and 95 ± 3 per cent RH. At the end of fourteen day, the number of normal seedlings in each replication were counted and expressed in percentage. At the time of germination count, all the normal seedlings from each replication were used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root. The mean values were recorded and expressed in centimetre. The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the point of attachment of seed to tip of the leaf and the mean values were recorded and expressed in centimetre.

Vigour index :

Vigour index values were computed using the following formula as suggested by Abdul-Baki and Anderson (1973) and the mean values were expressed in whole number.

Vigour index=Germination(%)×Total seedling length(cm)

Statistical analysis :

Experiment was carried out with eight replication in Completely Randomized Block Design. The analysis of variance for all the characters was worked out as suggested by Panse and Sukhatme (1967). The critical difference (CD) was worked out at 5 per cent probability level. Experiments were analyzed using CRD (Completely Randomized Design).

RESULTS AND DISCUSSION

Tomato seeds responded variably towards the treatment at various concentrations of ZnO. In fresh seeds among the different concentration of ZnO nanoparticles tested both the 600mg and 800 mg concentration recorded the highest germination of 86 per cent followed by 400 mg and 1000 mg recorded 84 per cent germination over control (78%). While the highest vigour index (1986) was observed in 600 mg followed by 800 mg (1961) over control (1521) (Table 1). Similar results were observed by Zheng *et al*. (2005) when *Spinacia oleracea* seeds were treated with nanoscale TiO_2 particles, an increase of germination rate and the vigour indices was noticed at 0.25-4 per cent concentration. Ruffini and Cermonini (2009) suggested that nanoparticles can explain their actions depending on both the chemical compound and on the size and/or shape of the particles.

The effect of ZnO nano particles on the vigour of tomato hybrid after aging for three days were presented in Table 2. The results indicated that the seed treatment with 1000 mg of ZnO significantly increased the germination to 58 per cent over control (30 %), corresponding increase in vigour index (957). Highest vigour index obtained in case of 600 mg. Such promotory effect of nanoscale SiO_2 and TiO_2 on germination was reported in soya bean (Lu *et al.*, 2002), in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. These increases in seed vigour parameters ascertained that 600 mg of ZnO nano particles can be used as presowing treatment of aged seeds (Table 2). Owing to the lager surface area, metal nano particles have an option to increase the catalytic

Table 1: Effect of ZnO nano particles on seed germination and seedling characters of fresh seeds of tomato hybrid COTH 2

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Vigour index
Control	78.00	12.80	6.30	1521.00
400 mg	84.00	15.60	6.60	1865.00
600 mg	86.00	16.10	7.00	1986.00
800 mg	86.00	16.00	6.80	1961.00
1000 mg	84.00	15.80	6.80	1898.00
Mean	83.60	15.26	6.70	1846.20
S.E. ±	2.48	0.45	0.20	53.95
C.D. (P=0.05)	5.52*	0.93**	0.41*	120.21**

* = significant at 95% ** = significant at 99%

Table 2 : Effect of ZnO nano particles on seed germination and seedling characters of tomato hybrid COTH 2 after aging for three days

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Vigour index
Control	30.00	11.30	5.20	495.00
400 mg	48.00	11.50	5.50	816.00
600 mg	55.00	12.00	6.10	995.00
800 mg	55.00	11.50	5.10	913.00
1000 mg	58.00	11.00	5.50	957.00
Mean	49.20	11.46	5.48	835.20
S.E. ±	1.43	0.34	0.17	24.39
C.D. (P=0.05)	3.18**	0.76	0.37**	54.34**

* = significant at 95% ** = significant at 99%

activity and they act as antioxidants for scavenging the reactive oxygen species (Hong *et al.*, 2005).

It is known that the vigor or strength of seeds decreases as a function of the storage duration. The decrease in seed vigor is possibly the result of the accumulation of free radicals. It has been shown that pretreatment of seeds before sowing could significantly reverse the effects of aging (Ramamoorthy and Basu, 1984). Immediately after treatment there was not much difference among the treatment and control seeds in terms of germination and vigour. But after aging germination, seedling length, vigour index of nano-particle treated seeds had increased with the increasing concentration upto 1000mg in nano-particles. Nanoparticulate formulations have great potential as novel properties with high specificity and improved functions. Nanoparticles treatment could activate superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) and remove ROS in the aged seeds (Hong *et al.*, 2005).

Conclusion :

Controlled storage of seeds would greatly solve the problem of seed deterioration but such facilities are expensive and no available at present for the common farmer. Rapid deterioration of stored vegetable seeds is a serious problem which occurs at an increasing rate in uncontrolled storage environment. Nano-particles would be effectively counteracting seed deterioration during storage of tomato seeds. In this study results suggest that ZnO nano-particles have the potential to sequester reactive oxygen thereby protecting the tomato seed from deterioration. Further studies can be explored to understand the mechanisms associated with the protective role of nano-particles on restoring seed viability.

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