Effects of nano-priming, priming time and post-priming maintenance on seed germination of sugar beet

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Abstract

Background/Objectives: Recently reports on the effects of engineered nanomaterials on biological processes and plant growth are on the rise. In this study, the process of sugar beet seed germination under nanopriming treatments was investigated in compare to hydropriming.

Methods/Statistical analysis: Seeds were treated under different concentrations of the nanomaterials, priming times, and post-priming maintenance. Kind of priming reagents with seven levels (water (hydropriming as control); 10, 20, 40 ppm $nTiO_2$; and 10, 20, 40 ppm SWNT), priming time with four levels (5 minutes, 6, 12, 18 hours), and post-priming seed maintenance with two levels (7 and 60 days) were considered as experimental treatments with

three replications. Harvested data for variables root length, shoot length, fresh weight, dry weight, speed of germination, percent of germination, and seedling vigor were to undergo Multivariate and Univariate Analysis Of Variance by using SPSS software.

Results: Nanopriming treatments produced same biomass but less root and shoot lengths compared to hydropriming. According to mean comparisons, nanopriming could improve the sugar beet seed germination quality by increasing speed of germination, germination percentage and vigor of the seedlings significantly. Priming time and post-priming seed maintenance had also significant interactions with nanomaterials on the germination characteristics.

Conclusion: With poorly adverse effects of post-priming storage on seed quality, seed priming with nanoparticles of titanium dioxide as compared to SWNT and hydropriming, caused a significant increase in the speed and percentage of germination.

Keywords: Nanopriming, Seed Germination, Priming Time, Sugar beet.

1. Introduction

The need for increased seed quality has become a priority necessary to face the current demand for high standards in the agricultural market (Paparelle et al. 2015). Seed priming, which allows for the regulation of the water content in the seed, is used to shorten germination time. This seed preparation method is necessary to overcome seed dormancy, in some cases, and is desirable for all plant species. Field emergence of sugar beet (*Beta vulgaris* L.) is generally 60-70% of seed planted, therefore, numerous seed priming treatments in order to reducing mortality in emerging seedling populations have been investigated (Swensen and Murray, 1991). Priming/activation of sugar beet seeds gives many benefits to the grower; as a result of the faster more uniform emergence it provides (Long and Odunlami, 2015). Various methods such as osmopriming (with an osmoticum like PEG), hydropriming (pure water), and drumpriming (water vapor) have been reported for seed priming. Usually materials such as mannitol, potassium nitrate, potassium chloride, and similar materials are also being used to facilitate controlled watering and drying of the seeds (Di Girolamo and Barbanti, 2012; Jisha et al., 2013; Li and Zhang, 2012). Durrant and colleagues (Durrant et al., 1993) showed that with seed priming, sugar beet cultivation was done 10 days earlier and sugar yield increased 48%. In another experiment it was observed that sugar beet seed soaking 2 hours at 0.3 N HCl increased the germination percentage and germination speed (Akeson et al., 1980). Jalilian and Tavakol-Afshari (2004) pretreated seeds of the two monogerm sugar beet varieties with polyethylene-glycol solution (8000 PEG) in different concentrations and different priming times. They found that sugar beet seed priming with 0.5 MPs PEG 8000 for five days increased eight percentage of germination in the cultivars. The priming also reduced the time required to reach 50% of seed germination. Alavi and colleagues (Alavi *et al.*, 2012) check out the effects of hydropriming and

osmopriming with potassium nitrate on sugar beet seed germination of four genotypes. Their results showed that osmopriming compared with hydropriming significantly increased germination percentage, germination speed, root and shoot length, root and shoot dry weight and vigor. Accordance to a survey (Kuppusamy and Ranganathan, 2014), 12-hour hydropriming of the sugar beet seeds showed 31% increase in germination compared to halopriming and osmopriming.

Nowadays engineered nanomaterials, as the most important index of the nanotechnology area, have entered all aspects of the human life and their various applications are quickly expanded due to their new characteristics compared to corresponding bulk materials. There are a number of reports in which biological processes have been improved using engineered nanomaterials, despite that their interactions with biological components of the ecosystem is a concern for the nanosafety (Husen and Siddiqi, 2014; Ke and Qiao, 2007). In recent years the use of carbon nanotubes in biology and pharmaceutical sciences has increased significantly. The unique ability of carbon nanotubes is that they easily penetrate into the cell membrane and have shown low toxicity. A promising application of the nanotubes is as vectors for the transfer of biomolecules into biological cells (Srinivasan and Saraswathi, 2010). The use of carbon nanotubes as molecular vectors for plant cells has not yet been fully studied and its mechanism is unknown. Khodakovskaya and colleagues (Khodakovskaya et al., 2009) and Morla and colleagues (Morla et al., 2011) in a separate study on carbon nanotubes found that CNT's have a positive impact on tomato seed germination and seedling optimum growth. They also found that carbon nanotubes enhance cell division and growth by affecting genes responsible for cell growth and also by activation of the water transmission channels into the cell. The results of a research represent carbon nanotube additive effects on seed germination and growth in a variety

of soybeans, barley, and corn (Torre-Roche et al., 2013). Agrawal and Rathore (Agrawal and Rathore, 2014) reported that carbon nanotubes enhance cell growth in some species of plants and it seems that they can be used as channels providing water paths into the cells which lead to faster growth and cell division. Zheng and colleagues (Zheng et al., 2005) studied the effects of titanium dioxide nanoparticles on growth of spinach. They observed that nano titanium dioxide in a 30-day period increased 73% of dry weight, 45 percent chlorophyll a content and the rate of photosynthesis tripled.

Research in the field of seed nano-priming is limited and most of the activities carried out on the effect of nanomaterials on germination process. Salehi and colleagues (Salehi et al., 2009) reported significant effects of rapeseed nanosilver seed priming on germination and plant growth in a comparison with hydropriming. Nanosilver had positive effect on germination percentage, but no effect on seedling growth. The effect of different concentrations of carbon nanotubes, nano titanium and copper nanoparticles on developmental aspects of onion seedling was investigated (Haghighi and Afifi Pour, 2011). Seedling growth characteristics were increased significantly in nano titanium and carbon nanotubes at concentrations of 100 and 10 ppm, respectively, but copper nanoparticles, 40 ppm carbon nanotubes and 400 ppm nano titanium had toxic and reducer effects on growth characteristics. Considering new properties of the engineered nanomaterials, reports about their positive effects on biological processes, and benefits of seed priming for sugar beet, in this study, effects of seed priming with single wall carbon nanotubes and nanoparticles of titanium oxide was investigated.

2. Materials and Methods

2.1. Seeds: ISTA based-qualified seeds of a monogerm sugar beet (cultivar Pars) were provided from the Seed Technology Department of Sugar Beet Seed Institute. Twenty eight bags consisting 50 g seeds each one were picked by divider device and washed in a washing machine for 3 hours at 25-27 °C temperature, then decontaminated in a disinfectant device containing 2000 ppm carboxin thiram solution. Dewatering of seeds was performed on blotting paper for 24 hours in room temperature (RT).

2.2. *Nanomaterials:* Single Wall Carbon Nanotubes (SWNT) and Titanium Oxide nanoparticles (nTiO2) used in this study were prepared from PlasmaChem GmbH. Before applying the treatments, size of the nanomaterials checked out with sonication and Dynamic Light Scattering device (DLS) to ensure the size is less than 100 nanometers.

2.3. *Treatments*: Kind of priming reagents with seven levels (water (hydropriming as control); 10, 20, 40 ppm nTiO2; and 10, 20, 40 ppm SWNT), priming time with four levels (5 minutes, 6, 12, 18 hours), and post-priming seed maintenance with two levels (7 and 60 days) were considered as experimental treatments with three replications. As much as 2 liters of each solution containing different concentrations of SWNT and nTiO2 was prepared and each one was poured in a transparent cylindrical container. Four of the above-mentioned bags (according to the four priming times) were placed in each cylinder and during priming, seeds were also simultaneously aerated. Primed seed bags were washed with running water for three 20-minute cycles, and seeds dewatered by monolayer spreading on blotting paper for 48 hours at RT. The prepared seeds were kept at a temperature of 20 °C until germination experiment.

2.4. *Seed germination*: The seeds were decontaminated again with sodium hypochlorite 0.025% for 15 minutes at RT and cast on a filter paper. After adding 10 ml of water the filter paper was gently roll into a tube and placed into a cylindrical tube to avoid flattening of the paper. The cylindrical tubes with a paper in which transferred to transparent plastic containers with lid and 300 ml of distilled water was poured into each container. The samples were placed in dark germinator with a temperature of 22-25 °C. Germination characteristics speed of germination (Coefficient of Velocity), germination percentage; seedling vigor, root length, shoot length, fresh weight, and dry weight were measured and calculated during and at the end of the seventh day after culture. The Coefficient of Velocity (CV) was calculated as:

$$CV = 100[\sum N_i / \sum N_i T_i]$$

where N_i is the number of newly emerged seedlings on day i and T_i is the number of days after planting (Campbell and Enz, 1991; Scott *et al.*, 1984). To calculate the seedling vigor, percentage of seed germination was multiplied by the total length of seedling as follows:

Seedling Vigor = Germination percentage × (Root length + Shoot length)

When all the seeds germinate, germination percentage is equal to one, vigor to the highest number that is equal to total length of seedling. The experiment was conducted in Completely Randomized Design with three replications and harvested data for variables root length, shoot length, fresh weight, dry weight, speed of germination, percent of germination, and seedling vigor were to undergo Multivariate and Univariate Analysis Of Variance by using SPSS software.

3. Results

Kind of priming reagents with seven levels (water (hydropriming as control); 10, 20, 40 ppm $nTiO_2$; and 10, 20, 40 ppm SWNT), priming time with four levels (5 minutes, 6, 12, 18 hours), and post-priming seed maintenance with two levels (7 and 60 days) were entered into a Multivariate Analysis Of Variance (MANOVA) as independent variables with the dependent variables root length, shoot length, fresh weight, dry weight, speed of germination, percent of germination, and seedling vigor.

According to the results of the multivariate tests considering Wilk's Lambda (λ), significant multivariate effects were found for all independent variables and their two and three-factor interactions (summarized in table 1). As can be seen in Table 2, most of the evaluated traits were significantly affected by priming reagents, priming time and post-priming maintenance and their interactions. Germination rate, which is the most important attribute in priming studies, was the least affected by the main and interaction effects of factors than other attributes. Post-priming factor and the interactions covering this factor had no significant effect on germination rate.

Table 1- Multivariate Tests for main and interaction effects of priming reagent, priming time, and post-priming maintenance factors on sugar beet seed germination (Results of the Pillai's Trace, Hotelling's Trace, and Roy's Largest Root tests are not shown).

Effect	Wilks' Lambda Value	F	Hypothesis df	Error df	Sig.
Priming Reagents(A)	.062	9.618	42	500.636	.000
Priming Time(B)	.216	10.248	21	304.925	.000
Post-Priming Maintenance(C)	.395	23.196	7	106.000	.000
A * B	.033	3.838	126	706.383	.000
A * C	.281	3.700	42	500.636	.000
B * C	.475	4.300	21	304.925	.000
A * B * C	.077	2.674	126	706.383	.000

Table 2- Full factorial MANOVA for sugar beet seed germination characteristics under different priming reagents, priming times, and post-priming seed maintenance.

Dependent Variable	Source of Variation															
	Priming Reagents(A)		Priming Time(B)		Post-Priming Seed Maintenance (C)		A*B		A*C		B*C		A*B*C		Error	
	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	Mean square
Root Length	6	6.86^{**}	3	0.79^{ns}	1	4.44^{*}	18	2.95^{**}	6	1.36^{ns}	3	4.65^{**}	18	1.17^{ns}	112	0.61
Shoot Length	6	10.82^{**}	3	18.57^{**}	1	11.17^{**}	18	10.00^{**}	6	5.58^{**}	3	3.85^{*}	18	5.32^{**}	112	5.19
Fresh Weight	6	1.89^{ns}	3	0.024^{ns}	1	73.03^{**}	18	3.91^{**}	6	3.58^{**}	3	7.99^{**}	18	4.72^{**}	112	0.08
Dry Weight	6	2.71^{*}	3	2.83^{*}	1	0.44^{ns}	18	2.28^{**}	6	3.08^{**}	3	2.92^{*}	18	2.76^{**}	112	0.00
Speed of Germination	6	19.78^{**}	3	11.69^{**}	1	0.01^{ns}	18	2.06^{*}	6	0.01^{ns}	3	0.01^{ns}	18	0.01^{ns}	112	0.73
Percent of Germination	6	13.94^{**}	3	2.93^{*}	1	4.82^{*}	18	1.50^{ns}	6	2.48^{*}	3	1.85^{ns}	18	1.95^{*}	112	40.00
Vigor	6	22.95^{**}	3	51.82^{**}	1	19.98^{**}	18	9.22^{**}	6	5.49^{**}	3	8.38^{**}	18	3.05^{**}	112	433.42

Because the most important question to be answered was whether nanopriming is better than hydropriming for sugar beet seed germination, and on the other hand, interactions were less important than main effects, all experimental data were classified in two groups of hydropriming and nanopriming for a One-Way MANOVA as independent variable and dependent variables were the same as above analysis. There was a statistically significant effect between hydropriming and nanopriming on the combined dependent variables, $F(7, 160) = 24.554$, $p=.004$; Wilks' $\lambda= .482$. Univariate analysis showed that effects of nanopriming and hydropriming on sugar beet seed germination in this study are significantly different for root length, shoot length, speed of germination, percent of germination and seedling vigor, but not for fresh weight and dry weight (results are not shown). Figure 1 indicates results of the pairwise comparisons among means of dependent variables root and shoot length together and 3 other significantly different variables separately.

Since the random effects of the treatments were in question, data grouping and group mean comparisons were performed in order to extract information and choose the most effective treatments.

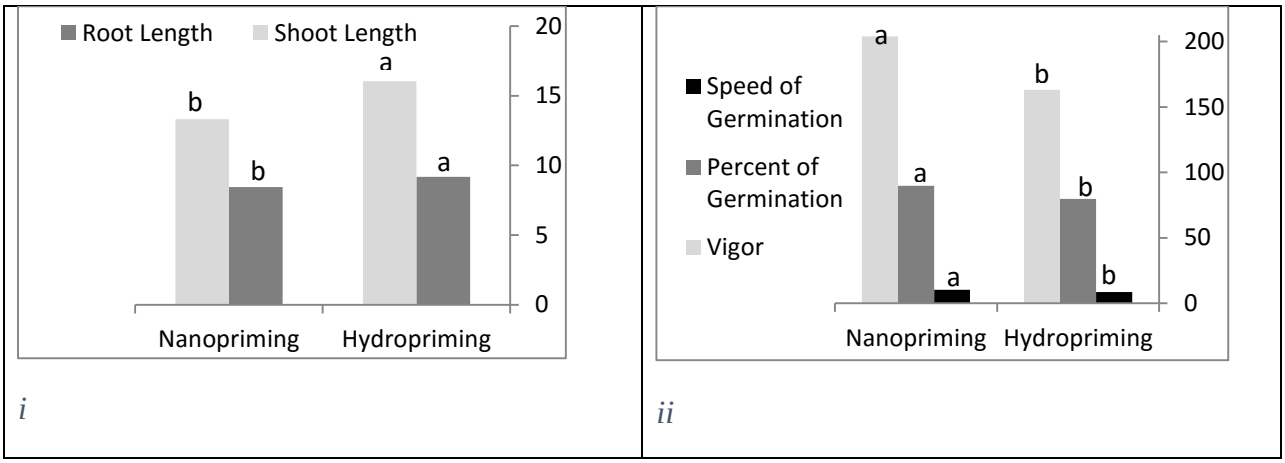

Figure 1. Diagrams showing effects of hydropriming and nanopriming on sugar beet seed germination characteristics root and shoot length (i), and speed of germination, percent of germination and vigor (ii). Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by Tukey's test).

There was no significant difference between hydropriming and nanopriming in terms of biomass production (fresh weight and dry weight), although the average length of root and shoot were significantly higher in hydropriming. Considering the importance of speed and percentage of germination, the results indicated that sugar beet seed nanopriming can improve the quality of seed germination in comparison to hydropriming. Mean of speed of germination, germination percentage and vigor in nanoprimming group were significantly higher than the corresponding values in the group of hydropriming.

Effects of $nTiO_2$ and SWNT on root and shoot lengths was significantly different from each other. Priming with $nTiO_2$ was more beneficial to root length but SWNT was more in favor of shoot length (Figure 2-i). As shown in Figure 2-ii diagram, there was no significantly differences between $nTiO_2$ and SWNT for percent of germination and vigor, but speed of germination was significantly higher in $nTiO_2$ nanopriming than SWNT nanopriming.

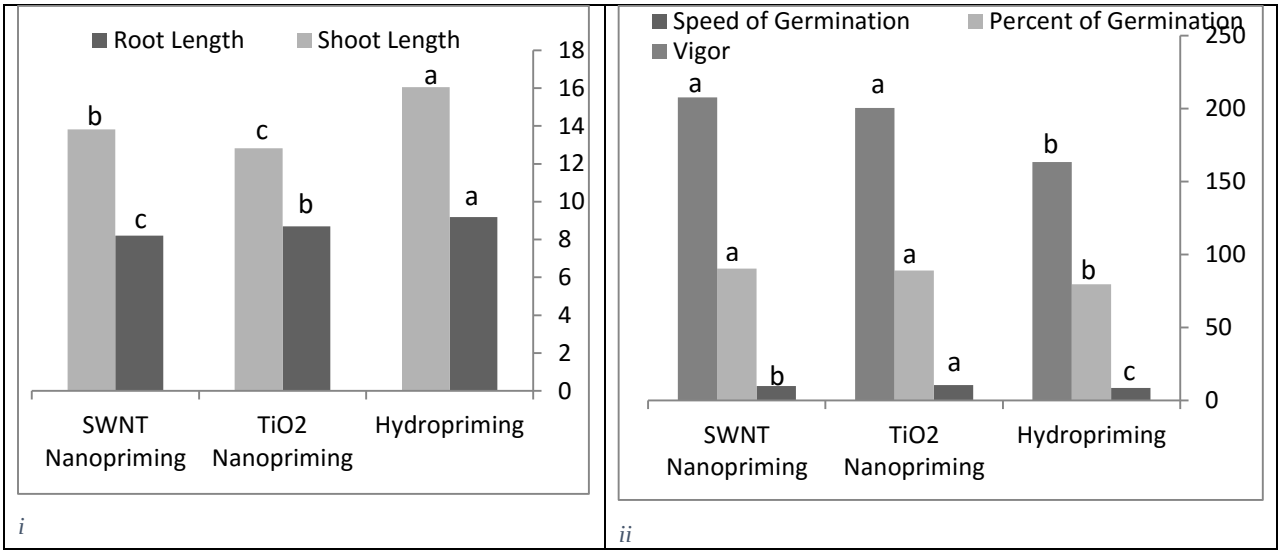

Figure 2. Diagrams showing effects of hydropriming, nTiO2 and SWNT nanopriming on sugar beet seed germination characteristics root and shoot length (i), and speed of germination, percent of germination and vigor (ii). Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by Tukey's test).

Multivariate tests showed a statistically significant effect of priming time on the combined dependent variables, $F(21, 454.241) = 3.806$, $p = .004$; Wilks' $\lambda = .628$. One-Way ANOVA showed that shoot length, speed of germination and vigor responses to different priming times but the rest of the properties did not respond to this variable (Figure 3).

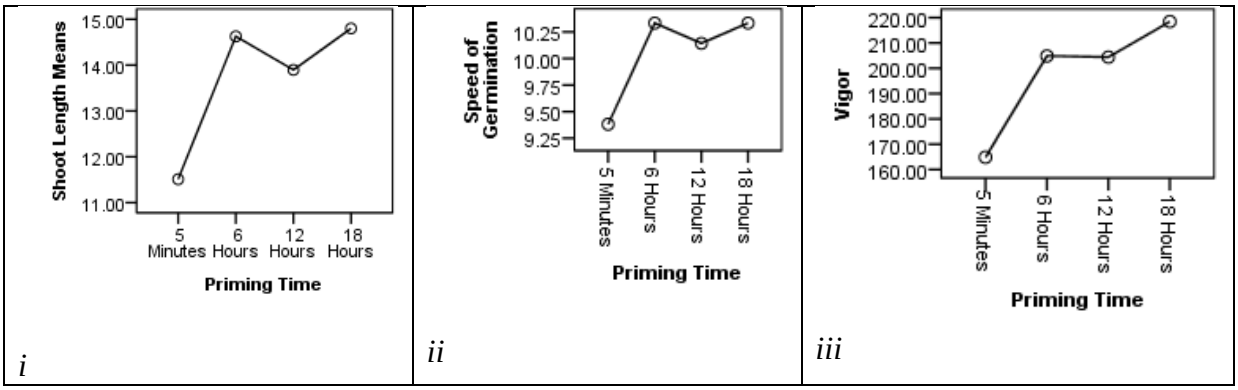

Figure 3. Diagrams showing effects of priming time on sugar beet seed germination characteristics shoot length (i), speed of germination (ii), and vigor (iii). In all three graphs, mean of the 5 minutes priming treatment is minimal.

Reaction patterns to priming time was similar in shoot length and speed of germination (figure3-i and –ii), and as seen in the figure, 5 minutes priming treatment had generally the least averages showing that in order to effectiveness of priming, sugar beet seeds should be primed for at least a few hours. There were no significant differences between the priming times 6, 12, and 18 hours.

According to results of the On-Way ANOVA for post-priming seed maintenance (7 and 60 days), only two attributes fresh weight and vigor reacted to the post-priming maintenance and, in fact, this factor was the weakest factor affecting sugar beet seed germination. Average value for fresh weight at 7-day was higher than the corresponding value at 60-day, and vice versa for vigor.

4. Discussion

Nanopriming is a new method for quality improvement of plant seed germination and in addition to the lack of information on biological behavior of engineered nanomaterials, there are a restricted number of reports on the plant seed nanopriming. Our results on nanopriming and

hydropriming comparisons were nearly similar to results that Mahakham and colleagues (2016) achieved. In their research, gold nanoparticles as nanopriming agent was used to activate the germination and early seedling growth of maize aged seeds. Priming with 5 ppm gold nanoparticles showed the best effects on promoting emergence percentage (83%) compared to unprimed control (43%) and hydroprimed groups (56%). Seed priming at both 5 and 10 ppm gold nanoparticles also enhanced seedling vigor index by 3 times over the control (Mahakham *et al.*, 2016). In contrast, Salehi and colleagues (2009) evaluated the germination behavior of canola RGS cultivar treated with silver nanoparticles in concentrations of 0, 20, 40 ppm and ascorbate priming in three levels 100, 200, 400 ppm and hydropriming with distilled water. Their results on silver nanoparticles were in contrast with the results obtained in our study. Treatment with Nanocide (trade name of a nanosilver product) resulted in growth improvement and successful seed establishment, although percentage and rate of seed germination reduced (Salehi *et al.*, 2009).

Although choosing of the correct timepoint to stop the priming treatment and dehydrate the seed is a critical step still difficult to monitor (Paparella *et al.*, 2015), but results of the priming time in our experiment showed that it may have a minimum threshold point (5 minutes in this study). Like cardinal temperatures case for growth and development of an organism, species specific cardinal times based on the pure water may be determined for seed priming.

Reduction of seed longevity is a well-reported disadvantage of seed priming (Chiu *et al.*, 2002), and in some cases, desiccation can alter the beneficial effects of priming, which are lost during storage (Heydecker and Gibbins, 1978), but results we obtained from 7 and 60 days post-priming maintenance of the sugar beet seeds do not match these reports on loosing seed longevity.

5. Conclusion

Seed quality is one of the major agricultural issues on which many researchers worldwide are interested, and 'seed priming', a well-known practice, is used to improve the quality of seed germination. Results of this study showed that using nanopriming for sugar beet seed preparation can improves the seed quality and germination. Basically, increasing of speed and percentage of germination is main purpose of the seed priming and it significantly happened in nanopriming. Based on the linear scale, hydropriming was better than nanopriming, but no significant differences were observed between the two groups in terms of biomass production content at all. Furthermore, $nTiO_2$ nanopriming caused better responses, especially for speed of germination, than SWNT. With respect to the significant interactions between priming time and priming reagents, it should be in mind that the time scale of priming in sugar beet should be at hour scale. Decrease in storage capability of primed seeds which has been reported for many plant species, did not happen for sugar beet in 60 day time period.

There are a limited number of reports on plant seed nanopriming, but considering the intensity of reports about effects of engineered nanomaterials on seed germination, a question arises whether the effects of nanomaterials in nanopriming occurs at the priming time or happens because of their presence at the time of seed germination?

It should be noted that many of the mechanisms of interactions between plants and nanoparticles are still not known. These physiological effects and interactions, which represent entry and activity of nanoparticles in the cell, if known, can be used to regulate plant growth and development.

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7. References

- Agrawal S. and Rathore P. 2014. Nanotechnology Pros and Cons to Agriculture: A review. *International Journal of Current Microbiology and Applied Sciences*, 3(3):43-55.
- Akeson W.R., Henson M.A., Freytage A.H. and Westfall D.C. 1980. Sugar beet fruit germination and emergence under moisture and temperature stress. *Crop Science*, 20: 735-739.
- Alavi Z.S., Roushanfekr H., Hasibi P., and Mesgarbashi M. 2012. Effect of salinity stress on the germination of six genotypes of sugar beet (*Beta vulgaris* L.). *Proceedings of the Second Conference of Seed Science and Technology*- Mashhad, Iran.
- Campbell L.G. and Enz J.W. 1991. Temperature effects on sugar beet seedling emergence. *Journal of sugar beet research*, 28:129-140.
- Chiu K.Y., Chen C.L., Sung J.M. 2002. Effect of priming temperature on storability of primed sh-2 sweet corn seed. *Crop Science*, 42:1996–2003.
- Di Girolamo G., Barbanti L. 2012. Treatment conditions and biochemical processes influencing seed priming effectiveness. Italian J Agr 7:e25.

- Durrant M.J., Mash S.J. and Jaggard K.W. 1993. Effects of seed advancement and sowing date on establishment, bolting and yield of sugar beet. *The Journal of Agricultural Science*, 121(3), pp. 333–341. doi: 10.1017/S002185960008552X.
- Haghighi M., and Afifi Pour Z. 2011. The effect of priming with carbon nanotubes, nano-titanium and copper nanoparticles on germination characteristics of onion. *Proceedings of the First National Congress of Science and New Technologies of Agriculture*, Zanjan, Zanjan University.
- Heydecker W., Gibbins B.M. 1978. The priming' of seeds. Acta Horticulturae 83:213–215.
- Husen A. and Siddiqi K.S. 2014. Carbon and fullerene nanomaterials in plant system. Journal of Nanobiotechnology 12:16.
- ISTA. 1995. Understanding seed vigour. In: *The ISTA Handbook of Vigour Test Methods*, ISTA, Zurich, Switzerland.
- Jalilian A., and Tavakol-Afshari R. 2004. Osmopriming effect on sugar beet seed germination under drought stress. *Journal of Agriculture*, 27 (2): 23-36.
- Jisha K.C., Vijayakumari K., Puthur J.T. 2013. Seed primming for abiotic stress tolerance: an overview. Acta Phsiol Plant 35:1381-1396.
- Ke P.C., and Qiao R. 2007. Carbon nanomaterials in biological systems. *Journal of Physics: Condensed Matter*, 19:373101–373125.
- Khodakovskaya M., Dervishi E., Mahmood M., Xu Y., Li Z., Watanabe F. and Biris A.S. 2009. Carbon Nanotubes Are Able To Penetrate Plant Seed Coat and Dramatically Affect Seed Germination and Plant Growth. *ACS Nano*, 3 (10), pp 3221–3227.

- Kuppusamy N. and Ranganathan U. 2014. Storage potential of primed seeds of okra (*Abelmoschus esculentus*) and beet root (*Beta vulgaris* L.). *Australian Journal of Crop Science*, 8(9) 1290-1297.
- Li X., Zhang L. 2012. SA and PEG-induced priming for water stress tolerance in rice seedling. Inf Tech Agr 134:881-887.
- Long J. and Odunlami B. 2015. Effect of priming intensity on the storability of sugar beet seed. *Abstracts of Papers, 75TH IIRB CONGRESS, 16-17 FEBRUARY*, BRUSSELS (B).
- Mahakham W., Theerakulpisut P., Maensiri S., Phumying S., Sarmah A.K. 2016. Environmentally benign synthesis of phytochemicals-capped gold nanoparticles as nanopriming agent for promoting maize seed germination. *Science of the Total Environment*, Vol. 573, 15 December 2016, Pages 1089–1102.
- Morla S., Ramachandra C.S.V. and Chakrapani R. 2011. Factors Affecting Seed Germination and Seedling Growth of Tomato Plants cultured in Vitro Condition. *Journal of Chemical, Biological and Physical Sciences*, Vol.1, N0.2, Sec. B.328-334.
- Paparella S., Arau´jo S.S., Rossi G., Wijayasinghe M., Carbonera D., Balestrazz A. 2015. Seed priming: state of the art and new perspectives. *Plant Cell Reports*, 34: 1281. doi:10.1007/s00299-015-1784-y.
- Salehi M., Tamaskony F., Ehsani M. and Arefi M. 2009. Effect of priming on germination and seedling growth of canola compared with nanosilver under salt stress. *Journal of Plant Science Research, Number*, 16, No. 4. pp 57-52.
- Scott S., Jones R.A., and Williams W.A. 1984. Review of data analysis methods for seed germination. *Crop Science*. 24: 1192-1199.

- Srinivasan C. and Saraswathi R. 2010. Nano-agriculture – carbon nanotubes enhance tomato seed germination and plant growth. *Current Science*, vol. 99, No. 3, 10.
- Swensen J.B. and Murray G.A. 1991. Optimal Priming Conditions and Persistence of Enhanced Emergence in Osmotically Primed Sugar beet Seed. *Journal of Sugar Beet Research*, Vol 28, No 1 &2, 31-39.
- Torre-Roche R., Hawthorne J., Deng Y., Xing B., Cai W., Newman L.A., Wang Q., Ma X., Hamdi H. and White J.C. 2013. Multiwalled carbon nanotubes and C60 fullerenes differentially impact the accumulation of weathered pesticides in four agricultural plants. *Environmental Science & Technology*, Volume 47(21), pp 12539-12547.
- Zheng L., Hong F., Lu S., Liu C. 2005. Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research*, 104(1):83–91.