Journal of Agricultural Science; Vol. 8, No. 9; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Facilitate Seed Germination of the Golden Shower Tree (*Cassia fistula*) *in vitro* Using TiO_2 Nanoparticles and Scarification Treatments

Fatemeh Feizi[1] & Mousa Mousavi[1]

[1] Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran

Correspondence: Mousa Mousavi, Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, P.O. Box 6135743337, Ahvaz, Iran. Tel: 98-613-336-4053. E-mail: mousa_mousawi@yahoo.com

Received: June 11, 2016 Accepted: July 19, 2016 Online Published: August 15, 2016

doi:10.5539/jas.v8n9p168 URL: http://dx.doi.org/10.5539/jas.v8n9p168

Abstract

The main propagation method of *Cassia fistula* is sowing seeds. The seed germination is usually low because of its impermeable hard coat. Therefore, this experiment evaluated the effects of TiO_2 nanoparticles and scarification methods on seed germination and seedling growth *in vitro* condition. The tree seeds were treated with, hot water, H_2SO_4 (36N), and mechanical scarification and culture on ¼ MS salt mixture. The medium was supplemented with different concentrations of TiO_2 nanoparticles. The results showed that the highest percentage and rate of germination was recorded in seeds treated with mechanical scarification. The highest shoot and root dry weight was recorded for seeds treated with mechanical scarification and grown on MS media supplemented with 1.5 mg/ml TiO_2 nanoparticles. TiO_2 nanoparticles did not show any significant effects on the percentage and rate of germination. Different growing soil mixtures had significant effects on the growth of the ex vitro transferred plantlets. Coco peat and peat moss mixture (1:1) was found to be more effective in increasing the number of leaves and root length of the seedlings.

Keywords: scarification, titanium dioxide nanoparticles, *cassia fistula*, dormancy, seedling

1. Introduction

Cassia fistula L. (family Fabaceae), commonly known as the golden shower Indian laburnum, is mainly found in Asian countries such as India, Sri Lanka and China. It is also found in various other countries including Mexico, Mauritius and South Africa (Neelam et al., 2011). The fruits of this plant are cylindrical, brown, septate, 25-50 cm in length, 1.5-3 cm in diameter, and have 25-100 seeds (Danish et al., 2011). In southwest of Iran (Ahwaz and Abadan cities) *Cassia* is cultivated as an ornamental tree. However, the main issues encountered in propagating *Cassia* seedlings is poor seed germination caused by the water-impermeable seed coat, which exerts physical dormancy (Rolston, 1978). Thus the seeds should be given pre-germination treatments before planting.

The recent discovery of interesting properties of nanoparticles has led to its wide variety of applications in the chemical industry, medical field, electronics and agriculture as well (Savithramma et al., 2012). Several experiments have been carried out to evaluate the effects of different nanoparticles on seed germination and seedling growth of many plants. Some of these experiments include effect of ZnO NPs on onion seeds (Raskar & Laware, 2014); wheat, rice, and cucumber (Kumar et al., 2015); TiO_2 NPs on parsley and spinach seeds (Hong et al., 2005a; Dehkourdi & Mousavi, 2013); Silver NPs on *Pennisetum glaucum* seeds (Parveen, 2015); CuO NPs on soybean and chickpea seeds (Adhikari et al., 2012); Fe, Co and Cu NPs on soybean seeds (Ngo et al., 2014); SiO_2 NPs on tomato (Siddiqui & Al-Whaibi, 2014); carbon nanotubes on strawberry seeds (Yosefi et al., 2012); Mo and SiO_2 NPs on rice seeds (Adhikari et al., 2013); and Apatite NPs on soybean (Liu & Lal, 2014). Among the different nanoparticles examined, the TiO_2 NPs showed more effectiveness in increasing plant growth.

The effects of TiO_2 NPs on the germination and growth of spinach seeds involve increased amount of chlorophyll, photosynthesis and Rubisco activity (Zheng et al., 2005). Feizi et al. (2012, 2013) reported that TiO_2 NPs in a suitable concentration could promote the seed germination indices of wheat and *Foeniculum vulgare* in comparison to bulk TiO_2.

Therefore, the aim of this experiment was to investigate the use of TiO_2 nanoparticles along with different scarification methods, including mechanical, acid, and hot water, on the seed germination and growth of *C. fistula* seedlings *in vitro*.

2. Method

This experiment was conducted in the tissue culture laboratory of the Shahid Chamran University of Ahwaz, Iran.

2.1 Seed Scarification and TiO_2 NPs Treatments

Murashige and Skoog (MS1/4) medium was prepared and poured into the glass bottles. Each bottle contained 50 ml of MS media. Different concentrations of TiO_2 NPs were added into the medium, which was sterilized in an autoclave (121 °C/15 psi, for 20 min). Mineralogical structure of anatase titanium dioxide nanoparticles with sol gel solution was prepared by the Physics Department of Sharif University in Iran. Scanning microscopy (TEM) nano-sized titanium dioxide particles were about 30 nm and spindle-shaped.

The seeds were sterilized by 70% ethanol for 30 sec, then added to sodium hypochlorite (2.5%) for 5 min and washed 3 times with sterile distilled water. The seeds were treated before sterilization using the following steps:

- Mechanical scarification with damaging of the seed coat (Figure 1);
- Hot Water 80 °C for 3 minutes;
- H_2SO_4 (36N) for 30 minutes.

The non-scarified seeds were used as control.

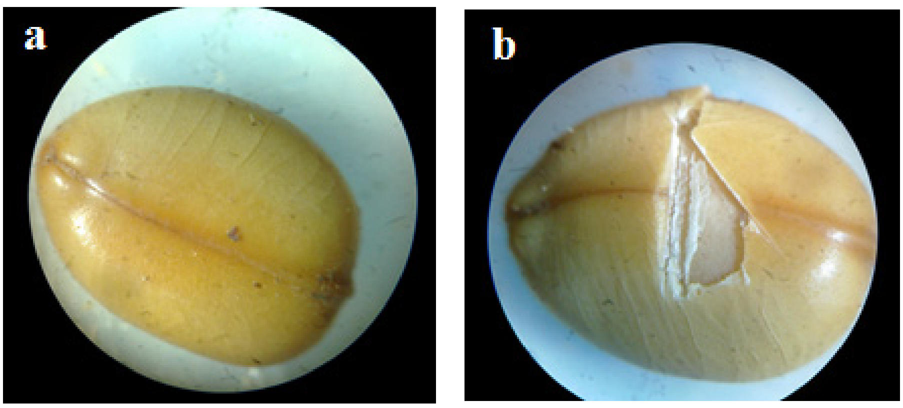

Figure 1. The *Cassia fistula* seed (a) mechanical scarification by damaging the hard seed coat (b)

The seeds were cultured on the medium and incubated at 25 °C with 16 h photoperiod and checked every day.

2.2 Germination Parameters

The number of seeds was counted with visible radicle as sprouted seeds (seeds that have sprouted length root of it considered two millimeters) (Miller & Chapman, 1978). Then it was measured as Equation (1).

$$\% \ GP = \Sigma G/N \times 100 \quad (1)$$

Where, GP is germination percentage, ΣG is the number of seeds germinated on that particular day, and N is the total number of seeds (Hartmann & Kester, 1983). The germination rate was calculated using Equation (2).

$$GR = \Sigma n/\Sigma dn \quad (2)$$

Where, GR is the germination rate, Σn is the number of seeds germinated on the day, and Σdn is the number of days from the start of the experiment (Maguire, 1962).

2.3 Measurement of Seedling Growth Parameters

After 30 days, five randomly selected seedlings were taken from each petri dish to measure their shoot and root lengths using a measuring scale. The measurements were expressed in centimeters. The number of leaves and radicles were also measured. For the fresh and dry weights of the shoots and roots of the seedlings, the fresh weight of each tissue was weighated first. Then the tissues were dried in an oven at 70 °C for 48 hours and their dry weight was measured.

2.4 Plantlets Ex-Vitro Acclimatization

In vitro grown seedlings were ex vitro transferred to acclimatization in a transparent plastic box. Then the agar was washed and disinfected with the fungicide Mancozeb.

The four tested soil mixtures are:

- Cocopeat – perlite (1:1)
- Cocopeat – peatmoss (1:1)
- Peatmoss – perlite (1:1)
- Cocopeat – perlite- peatmoss (1:1:1)

The seedlings were fed with MS1/4 basal salt mixture solution during their growing period. Finally, the shoot and root length, number of leaves, and diagonal shoots were measured.

2.5 Research Design

The experiment was carried out in factorial complete randomized design that included five replicates with five seeds. Factor A included (mechanical scarification, sulfuric acid, hot water, and control). Factor B includes TiO_2 NPs at four concentrations (0, 1.5, 4.5, and 13.5 mg/ml).

The data were analyzed using the MSTATC software. The significant levels of difference for all measured traits were calculated, and the means were compared by the multiple ranges Duncan test at 5% level.

3. Results

The analysis of variance showed that there was a significant difference among all measured indices under different scarification treatments as well as indices affected by TiO_2 NPs except the seed germination percentage, germination rate, and shoot fresh weight in the case of TiO_2 NPs treatments. The interactions between scarification treatments and TiO_2 NPs concentrations also showed the same difference as their individual effects except in the seed germination percentage, germination rate and shoot fresh weight (Table 1).

Table 1. Analysis of variance the indices of *Cassia fistula* seed germination affected by scarification treatments and TiO_2 NPs dioxide

Sources of variation	df	Percentage germination	Germination rate	Shoot length	Root length	Number leaf	Number root	Fresh weight shoot	Fresh weight root	Dry weight shoot	Dry weight root
Scarification	3	18700*	1041.02*	80.39*	88.23*	38.39*	34.89*	0.270*	0.168*	0.0034*	0.00015*
Nano-TiO_2	3	113.33ns	4.81ns	21.52*	20.66*	36.85*	13.41*	0.102*	0.0299*	0.0011*	0.00006*
Interaction	15	268.88ns	15.13ns	9.79*	4.73*	12.76*	15.64*	0.0178ns	0.0063*	0.00056*	0.00002*
Error	64	5.904	0.234	0.048	0.012	0.57	0.0079	0.0001	0.00006	0.0000001	0.0000003

Note. * Significant at 5 % level.

The comparison of the means as showed in Table 2 indicates that the highest shoot length, number root, root fresh weight, root length, leaf number and dry weight of the shoot and roots of seedling were significantly increased when the seeds were treated with mechanical scarification along with 1.5 mg/ml of TiO_2 NPs. However, no significant difference was observed between 0 and 1.5 mg/ml TiO_2 NPs for root length, leaf number, and dry weight of the shoot and roots of the seedling.

The highest percentage and rate of germination were observed for seeds under mechanical scarification treatments, and the lowest percentage and rate of germination were observed for seeds with no treatment (control) (Figures 2a and 2b). The effects of scarification treatments and TiO_2 NPs concentration on shoot fresh weight are individually showed in Figures 2c and 2d, respectively.

The results obtained from a comparison among different soil mixture showed that they had a significant difference (Table 3). The maximum leaf number and length root were observed for seedlings that had been transplanted in coco peat-peat moss media (Table 4 and Figures 2e, 2f and 3).

Table 2. Effect of scarification methods and nanoparticles of titanium dioxide on growth indices of *Cassia fistula* seedling

Treatment scarification	Nano-TiO_2 (mg/ml)	Shoot length	Root length	Number leaf	Number root	Fresh weight root	Dry weight shoot	Dry weight root
Mechanical	0	7.46^{ab}	6.04^{a}	6.7^{a}	3.18^{b}	0.13^{ab}	0.058^{a}	0.012^{a}
	1.5	7.85^{a}	7.56^{a}	7.28^{a}	8.55^{a}	0.17^{a}	0.057^{a}	0.012^{a}
	4.5	4.27^{cd}	4.38^{b}	2.99^{b}	0.74^{cd}	0.075^{c}	0.039^{ab}	0.007^{b}
	13.5	5.14^{bc}	3.5^{bc}	3.25^{b}	2.5^{bc}	0.054^{c}	0.041^{ab}	0.0059^{bc}
Hot Water	0	1.55^{def}	0.90^{de}	2.20^{bc}	0.68^{cd}	0.058^{c}	0.30^{bc}	0.0062^{bc}
	1.5	2.27^{def}	2.02^{cd}	3.05^{b}	0.98^{cd}	0.052^{c}	0.034^{bc}	0.0058^{bc}
	4.5	2.18^{def}	0.82^{de}	2.40^{bc}	1.18^{cd}	0.031^{cd}	0.041^{ab}	0.0049^{bcd}
	13.5	1.46^{ef}	0.74^{de}	2.40^{bc}	0.80^{cd}	0.037^{cd}	0.038^{ab}	0.0053^{bcd}
H_2SO_4	0	3.66^{cde}	1.31^{de}	2.20^{bc}	1.40^{cd}	0.029^{cd}	0.040^{ab}	0.0031^{cd}
	1.5	1.85^{def}	0.83^{de}	2.90^{b}	1.40^{cd}	0.12^{ab}	0.041^{ab}	0.0051^{bcd}
	4.5	2.18^{def}	0.6^{de}	2.40^{bc}	1.40^{cd}	0.031^{cd}	0.038^{ab}	0.0050^{bcd}
	13.5	2.15^{def}	1.09^{de}	2.80^{b}	1.20^{cd}	0.060^{c}	0.033^{bc}	0.0032^{cd}
Control	0	1.8^{def}	1^{de}	0.80^{cd}	1^{cd}	0.0020^{d}	0.017^{cd}	0.0025^{de}
	1.5	5.86^{abc}	4.13^{b}	6.60^{a}	1.86^{bcd}	0.12^{b}	0.045^{ab}	0.0082^{b}
	4.5	1.5^{def}	0.90^{de}	0.40^{d}	1.60^{bcd}	0.036^{cd}	0.0074^{d}	0.0022^{de}
	13.5	0.5^{f}	0^{e}	0^{d}	0^{d}	0^{d}	0^{d}	0^{e}

Note. Means with different letters at each row have a statistical difference at 5 % level.

Table 3. Analysis of variance the effects of different soil mixture on growth of *Cassia fistula* seedling *ex vitro*

Sources of variation	df	Number leaf	Shoot length	Root length	Diagonal shoot
Soil mixture	3	38.20^{*}	3.19^{ns}	0.78^{*}	0.19^{ns}
Error	12	0.126	0.020	0.0073	0.0036

Note. * Significant at 5 % level.

Table 4. Effects of the different soil mixture on growth of *Cassia fistula* seedling

Soil mixture	Number leaf	Root length (cm)
Cocopeat – perlite	6.20^{bc}	1.62^{b}
Cocopeat – peatmoss	10.60^{a}	4.61^{a}
Peatmoss- perlite	7.60^{ab}	2.30^{b}
Cocopeat – perlite- peatmoss	4^{c}	1.38^{b}

Note. Means with different letters at each row have a statistical difference at 5 % level.

4. Discussion

The results obtained in this study showed that the mechanical scarification treatment of *C. fistula* seeds significantly enhanced seed germination percentage from 20 to 80 percent. Similar to the seeds of many leguminous species, *C. fistula* seed have impermeable hard coat dormancy, which may be due to impermeability of testa to water. Damaging the coat or breaking dormancy, facilitates germination of the seeds as it helps in supplying adequate water to their inner tissues and starts the germination process. Compared to hot water and sulfuric acid treatments, mechanical scarification effectively increases the germination with fewer side effects on the seed tissues. Pant and Chauhan (2013) reported that soaking of *Cassia tora* seeds in water for 24 h after mechanical scarification (by rubbing the seeds with sand paper) showed a significant level of improved germination (76.88%) when compared to sulfuric acid scarification, hot water and alcohol treatments. However, Karaboon et al. (2005) found that scarification the *Cassia fistula* seeds with sulfuric acid for 15-20 min represented the best treatment for enhancing germination, but mechanical scarification (by piercing the seeds at the part of cotyledon) showed slightly lower effects (statistically significant). Recently Wu et al. (2014) reported that TiO_2 nanoparticles effectively electrospray (using single-capillary electrospray) dispersed nanoparticles for breaking the seed coat to enhance seed germination percentage of lettuce seeds. However, this method may be not simple to use due to the requirement of a technical apparatus and absence of adequate information on its efficiency in seeds with hard coat dormancy.

On the other hand, the presence of TiO_2 NPs in the germination medium did not further improve the seed germination percentage and rate but showed significantly enhanced seedling growth indices. To the best of our knowledge, there is no report on the application of TiO_2 NPs to enhance *C. fistula* seed germination or seedling growth. Feizi et al. (2012) found similar results for wheat seed germination. They showed that the treatment of wheat seeds with bulk and nanosized TiO_2 could not significantly enhance germination percentage and rate compared to control but the nanosized TiO_2 increased shoot and seedling lengths at 2 and 10 ppm concentrations which was higher than those of the untreated control and bulk.

Zheng et al. (2005) reported that nanosized TiO_2 helped in water absorption by the spinach seeds and enhanced the germination of the seeds. Hatami et al. (2014) set an experiment to determine the effects of different concentrations of the TiO_2 NPs on germination indices and seedling vigor of five medicinal plants, namely, *Alyssum homolocarpum*, *Salvia mirzayanii*, *Carum copticum*, *Sinapis alba*, and *Nigella sativa*. They concluded that the nanosized TiO_2 treatments in appropriate concentrations accelerates the germination characteristics of the reference plant seeds and increases their vigor. Maroufi et al. (2011) reported that priming the *Vigna radiata* seeds with TiO_2 NPs increased germination percentage, seedling dry weight and seedling vigor. Seedling emergence losses in the soil are not generally due to germination failure, but the failure of seedlings to grow and emerge above the soil surface (Halmer & Bewley, 1984).

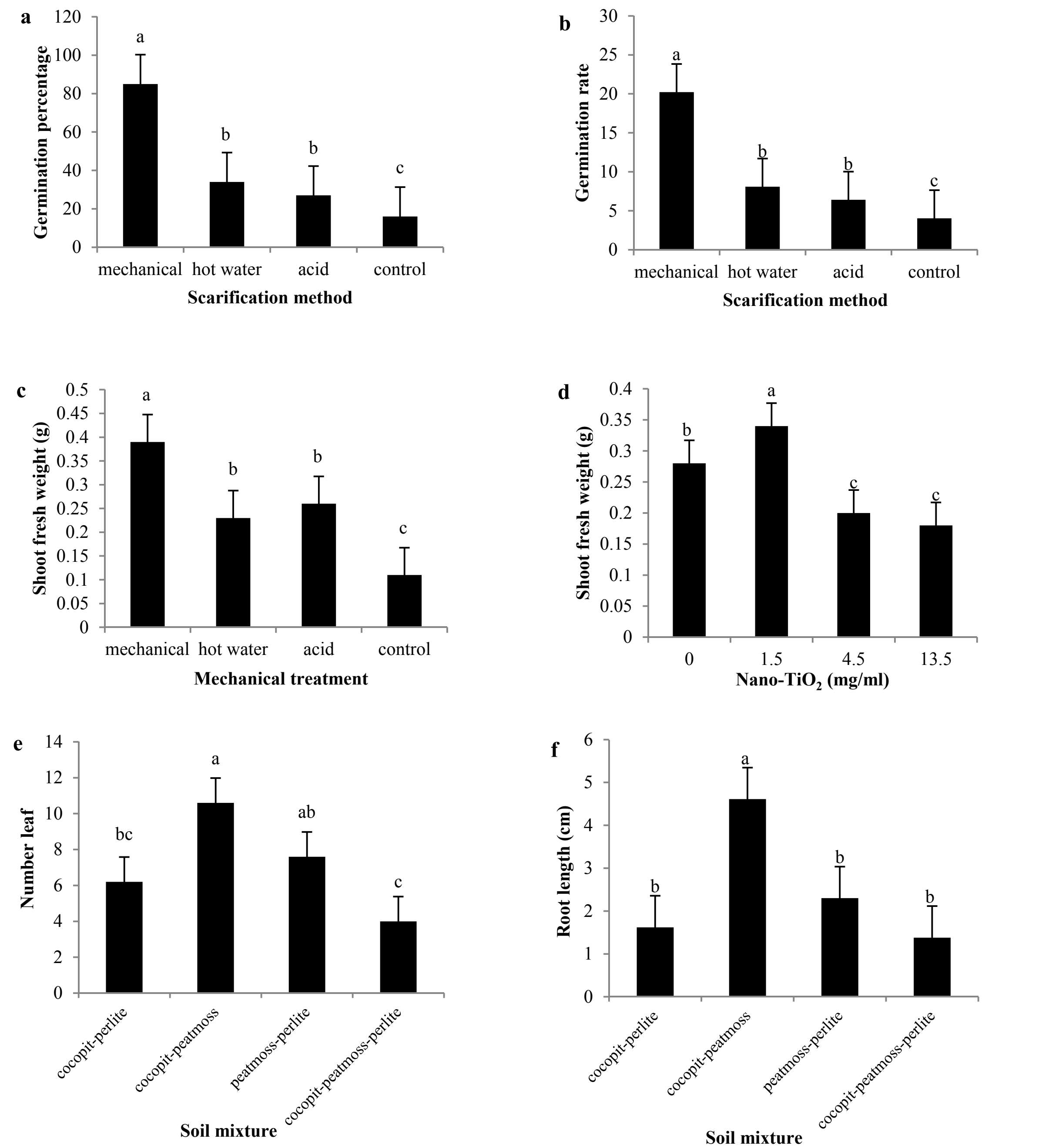

Figure 2. Effect of different scarification methods on *Cassia fistula* seed germination percentage (a) ; germination rate (b) and shoot fresh weight (c); effect of TiO_2 NPs on shoot fresh weight (d); effect of different soil mixture on number leaf (e) and root length (f)

TiO_2 NPs significantly enhanced the *C. fistula* seedling root growth indices including length, number, fresh and dry weight by facilitating water uptake and further enhancement of shoot and leaf growth. Farooq et al. (2005) demonstrated that priming of the tomato seed increased the rate of cell division in the root tips of seedlings. Primed seeds might have better plasma membrane structure by slow hydration (Jett et al., 1996).

The photosynthesis process starts after *C. fistula* seedling emerges above the soil surface and its expanded green leaves are exposed to the light. TiO_2 NPs promotes the photosynthesis rate by changing the light energy to electron energy and then to active chemistry energy. Sayama et al. (2002) demonstrated that the mechanism to water splitting into H_2 and O_2 by TiO_2 under the visible light irradiation was similar to the Z-scheme reaction in the natural photosynthesis.

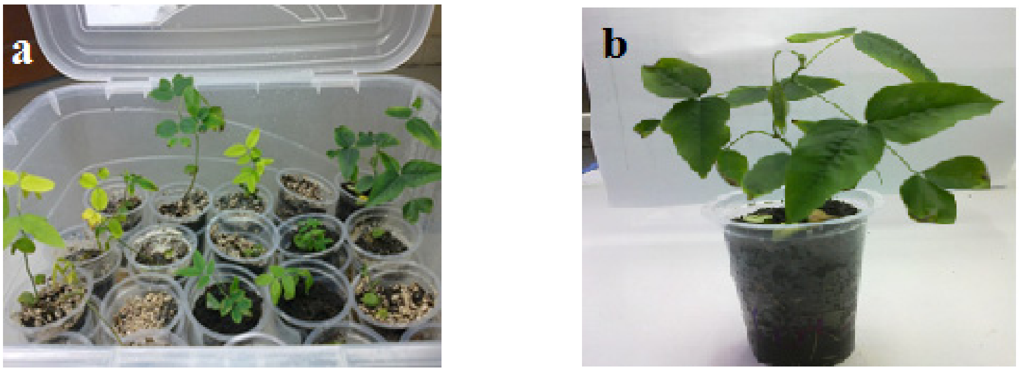

Figure 3. Ex vitro grown seedlings in different soil mixtures in the acclimatization box (a); seedling of *Cassia fistula* grown from seed after mechanical scarification and nano-TiO_2 treatment *in vitro* (b)

This characteristic of nanosized TiO_2 can also provide a low-cost, environmentally friendly solar-hydrogen production (Ni et al., 2007). Qi et al. (2013) reported that the net photosynthetic rate, conductance to H_2O, and transpiration rate of tomato leaves increased after the application of TiO_2 NPs. Elghniji et al. (2014) suggested that photocatalytic degradation characteristic of the TiO_2 helped in removing the cytotoxicity of 4-CP in irrigation wastewater and enhance seed germination and seedling growth of tomato, turnip and onion when they were exposed to sunlight.

It was reported that the TiO_2 NPs strongly absorbed light not only in the UV region, but also in the visible region from 400 to 800 nm (Zheng et al., 2007). Zheng et al. (2005) reported that TiO_2 NPs treatments markedly promoted seed vigor, chlorophyll biosynthesis, and improvement of growth and development in spinach but bulk TiO_2 treatments had comparatively little effects. Hong et al. (2005a) reported that photosynthesis promoted by TiO_2 NPs might be related to the activation of a photochemical reaction of chloroplasts of spinach. The TiO_2 NPs treatment could protect chloroplasts from aging for long-time illumination by enhancing the activities of the enzymes, such as catalase, peroxidase, and superoxide dismutase, lowering the accumulation of free radicals of the reactive oxygen and the level of malondialdehyde, and maintaining the stability of membrane structure of chloroplast under light (Hong et al., 2005b). Hong et al. (2005c) showed that the TiO_2 NPs could improve spinach growth through increased chlorophyll contents and photosynthetic rate by enhance light absorbance, accelerating the electron transport and efficient transformation of the light energy. Gao et al. (2006) reported that TiO_2 NPs could not only improve the light absorbance and the transformation from light energy to electron energy and active chemical energy but also promoted carbon dioxide assimilation of spinach. In this mechanism the TiO_2 NPs actives Rubisco and promotes Rubisco-carboxylation, which increases the rate of photosynthetic carbon reaction.

5. Conclusion

The results obtained in this study demonstrated that the scarification mainly increased the germination percentage and germination rate and the TiO_2 NPs treatment markedly enhanced the seedling growth. Therefore, the combination of the scarification with 1.5 mg/ml TiO_2 NPs treatment provided a good strategy for the propagation of *C. fistula* through seed.

References

Adhikari, T., Kundu, S., Biswas, A. K., Tarafdar, J. C., & Rao, A. S. (2012). Effect of copper oxide nanoparticle on seed germination of selected crops. *Journal of Agricultural Science Technology, A2*, 815-823. Retrieved from http://www.davidpublishing.com/davidpublishing/Upfile/7/22/2012/2012072283786577.pdf

Adhikari, T., Kundu, S., & Rao, A. S. (2013). Impact of SiO_2 and Mo nanoparticles on seed germination of rice (*Oryza sativa* L.). *International Journal of Agriculture and Food Science Technology, 4*(8), 809-816. Retrieved from http://www.ripublication.com/ijafst_spl/ijafstv4n8spl_17.pdf

Danish, M., Singh, P., Mishra, G., Srivastava, S., Jha, K. K., & Khosa, R. L. (2011). *Cassia fistula* Linn. (Amulthus)-An important medicinal plant: A review of its traditional uses, phytochemistry and pharmacological properties. *Journal of Natural Product and Plant Resources, 1*(1), 101-118. Retrieved from http://scholarsresearchlibrary.com/JNPPR-vol1-iss1/JNPPR-2011-1-1-101-118.pdf

Dehkourdi, E. H., & Mousavi, M. (2013). Effect of anatase nanoparticles (TiO_2) on parsley seed germination (*Petroselinum crispum*) *in vitro*. *Biological trace element research, 155*(2), 283-286. http://dx.doi.org/10.1007/s12011-013-9788-3

Elghniji, K., Sabrine, S., Ben Mosbah, M., Elimame, E., & Moussaoui, Y. (2014). Detoxification of 4-chlorophenol in TiO_2 sunlight system: Effect of raw and treated solution on seed germination and plants growth of various sensitive vegetables. *Toxicological and Environmental Chemistry, 96*(6), 869-879. http://dx.doi.org/10.1080/02772248.2014.983511

Farooq, M., Basra, S. A. M., Hafeez, K., & Ahmad, N. (2005). Thermal hardening: A new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology, 47*(2), 187-193. http://dx.doi.org/10.1111/j.1744-7909.2005.00031.x

Feizi, H., Moghaddam, P. R., Shahtahmassebi, N., & Fotovat, A. (2012). Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. *Biological Trace Element Research, 146*(1), 101-106. http://dx.doi.org/10.1111/j.1744-7909.2005.00031.x

Feizi, H., Kamali, M., Jafari, L., & Moghaddam, P. R. (2013). Phytotoxicity and stimulatory impacts of nanosized and bulk titanium dioxide on fennel (*Foeniculum vulgare* Mill). *Chemosphere, 91*(4), 506-511. http://dx.doi.org/10.1016/j.chemosphere.2012.12.012

Jett, L. W., Welbau, G. E., & Morse, R. D. (1996). Effect of matric and osmotic priming treatments on broccoli seed germination. *Journal of the American Society for Horticultural Science, 121*(3), 423-429. Retrieved from http://journal.ashspublications.org/content/121/3/423.full.pdf

Halmer, P., & Bewley, J. D. (1984). A physiological perspective on seed vigor testing. *Seed Science and Technology, 12*, 561-575.

Hartmann, H. T., & Kester, D. E. (1983). *Plant propagation principles and practice*. NewJersey, Prentice Hall.

Hatami, M., Ghorbanpour, M., & Salehiarjomand, H. (2014). Nano-anatase TiO_2 modulates the germination behavior and seedling vigority of some commercially important medicinal and aromatic plants. *Journal of Biodiversity and Environmental Sciences, 8*(22), 53-59. Retrieved from http://dergipark.ulakbim.gov.tr/jbes/article/viewFile/5000161121/5000145365

Hong, F., Zhou, J., Liu, C., Yang, F., Wu, C., Zheng, L., & Yang, P. (2005a). Effect of nano-TiO_2 on photochemical reaction of chloroplasts of spinach. *Biological Trace Element Research, 105*(1-3), 269-280. http://dx.doi.org/10.1385/BTER:105:1-3:269

Hong, F., Yang, F., Liu, C., Gao, Q., Wan, Z., Gu, F., … Yang, P. (2005b). Influences of nano-TiO_2 on the chloroplast aging of spinach under light. *Biological Trace Element Research, 104*(3), 249-260. http://dx.doi.org/10.1385/BTER:104:3:249

Hong, F., Yang, P., Gao, F., Liu, C., Zheng, L., Yang, F., & Zhou, J. (2005c). Effect of nano-TiO_2 on spectral characterization of photosystem II particles from spinach. *Chemical Research in Chinese Universities, 21*(2), 196-200. Retrieved from https://www.google.com/?gws_rd=ssl#q=Effect+of+nano-TiO2+on+spectral+characterrization+of+photosystem+II+particles+from+spinach

Gao, F., Hong, F., Liu, C., Zheng, L., Su, M., Wu, X., … Yang, P. (2006). Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach. *Biological Trace Element Research, 111*(1), 239-253. http://dx.doi.org/10.1385/BTER:111:1:239

Karaboon, S., Ripona, S., Thanapornpoonpong, S. N., Pawelzik, E., & Vearasilp, S. (October, 2005). *Breaking dormancy and optimum storage temperature of golden shower (Cassia fistula) seeds.* In Conference on International Agriculture Research for Development. Stuttgart-Hohenheim, Tropentag. Retrieved from http://www.tropentag.de/2005/abstracts/full/258.pdf

Kumar, S., Patra, A. K., Datta, S. C., Rosin, K. G., & Purakayastha, T. J. (2015). Phytotoxicity of nanoparticles to seed germination of plants. *International Journal of Advanced Research, 3*(3), 854-865. Retrieved from http://www.journalijar.com/article/3696/phytotoxicity-of-nanoparticles-to-seed-germination-of-plants

Liu, R., & Lal, R. (2014). Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (*Glycine max*). *Scientific Reports, 4*(5686), 1-6. http://dx.doi.org/10.1038/srep05686

Maguire, J. D. (1962). Speed of germination-Aid in selection and evaluation for seedling emergence and vigor. *Crop Science, 2*(2), 176-177. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x

Maroufi, K., Farahani, H. A., & Moradi, O. (2011). Evaluation of nano priming on germination percentage in green gram (*Vigna Radiata* L.). *Advances in Environmental Biology, 5*(11), 3659-3663. Retrieved from http://www.aensiweb.com/old/aeb/2011/3659-3663.pdf

Miller, T. R., & Chapman, S. R. (1978). Germination responses of tree forage grasses to different concentration of six salts. *Journal of Range Management, 31*(2), 123-124. http://dx.doi.org/10.2307/3897659

Neelam, C., Ranjan, B., Komal, S., & Nootan, C. (2011). Review on *Cassia fistula*. *International Journal of Research in Ayurveda and Pharmacy, 2*(2), 426-430. Retrieved from http://www.ijrap.net/admin/php/uploads/435_pdf.pdf

Ni, M., Leung, M. K. H., Leung, D. Y. C., & Sumathy, K. (2007). A review and recent developments in photocatalytic water-splitting using TiO_2 for hydrogen production. *Renewable and Sustainable Energy Reviews, 11*, 401-425. http://dx.doi.org/10.1016/j.rser.2005.01.009

Ngo, Q. B., Dao, T. H., Nguyen, H. C., Tran, X. T., Van Nguyen, T., Khuu, T. D., & Huynh, T. H. (2014). Effects of nanocrystalline powders (Fe, Co and Cu) on the germination, growth, crop yield and product quality of soybean (*Vietnamese species* DT-51). *Advances in Natural Sciences: Nanoscience and Nanotechnology, 5*(1), 015016(7pp). http://dx.doi.org/10.1088/2043-6262/5/1/015016

Pant, G., & Chauhan, U. K. (2013). Germination behavior of *Cassia tora* seeds in various pre-sowing treatment methods. *International Journal of Pharma and Bio Sciences, 4*(3), 773-778. Retrieved from http://www.ijpbs.net/cms/php/upload/2598_pdf.pdf

Parveen, A., & Rao, S. (2015). Effect of nanosilver on seed germination and seedling growth in *Pennisetum glaucum*. *Journal of Cluster Science, 26*(3), 693-701. http://dx.doi.org/10.1007/s10876-014-0728-y

Qi, M., Liu, Y., & Li, T. (2013). Nano-TiO_2 improves the photosynthesis of tomato leaves under mild heat stress. *Biological Trace Element Research, 156*(1), 323-328. http://dx.doi.org/10.1007/s12011-013-9833-2

Rolston, M. P. (1978). Water impermeable seed dormancy. *The Botanical Review, 44*, 365-398. http://dx.doi.org/10.1007/BF02957854

Raskar, S. V., & Laware, S. L. (2014). Effect of zinc oxide nanoparticles on cytology and seed germination in onion. *International Journal of Current Microbiology and Applied Sciences, 3*(2), 467-473. Retrieved from http://ijcmas.com/vol-3-2/S.V.Raskar%20and%20S.L.Laware.pdf

Savithramma, N., Ankanna, S., & Bhumi, G. (2012). Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. *Nano Vision, 2*, 61-68. Retrieved from http://www.nano-journal.org/dnload/N...S.../NANO-JOURNAL-NANV02I123P0061.pdf

Sayama, K., Mukasa, K., Abe, R., Abe, Y., & Arakawa, H. (2002). A new photocatalytic water splitting system under visible light irradiation mimicking a Z-scheme mechanism in photosynthesis. *Journal of Photochemistry and Photobiology A: Chemistry, 148*(1-3), 71-77. http://dx.doi.org/10.1016/S1010-6030(02)00070-9

Siddiqui, M. H., & Al-Whaibi, M. H. (2014). Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.). *Saudi Journal of Biological Sciences, 21*(1), 13-17. http://dx.doi.org/10.1016/j.sjbs.2013.04.005

Wu, S. G., Huang, L., Head, J., Ball, M., Tang, Y. J., & Chen, D. R. (2014). Electrospray facilitates the germination of plant seeds. *Aerosol and Air Quality Research, 14*, 632-641. http://dx.doi.org/10.4209/aaqr.2013.06.0212

Yosefi, R., Mousavi, M., Moalemi, N., & Ghafarian Mogharab, M. H. (May, 2012). *Investigation the effect of carbon nanotubes on in vitro germination of strawberry seeds (Fragaria ananassa cv. Paros and Camarosa)* (pp. 1-7). Paper presented in 1st Nano Technology and its Application in Agriculture and Natural Resources Conference. Tehran University. Retrieved from http://www.merid.org/en/Content/News_Services/Nanotechnology_and_Development_News/Articles/2012/Feb/01/Tehran_confernece.aspx

Zheng, L., Hong, F., Lu, S., & Liu, C. (2005). Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research, 104*(1), 83-91. http://dx.doi.org/10.1385/BTER:104:1:083

Zheng, L., Mingyu, S., Xiao, W., Chao, L., Chunxiang, Q., Liang, C., Huang, H., … Hong, F. (2007). Effect of nano anatase on spectral characteristics and distribution of LHCLL on the thylakoid membranes of spinach. *Biological Trace Element Research, 120*, 273-280. http://dx.doi.org/10.1007/s12011-007-8025-3