Physiological and Metabolic Modifications in Response to Nanocarbon in Callus of Indica Rice Cultivar

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Abstract

Nanocarbon has been shown to be implicated in the response of plants to an assortment of positive or negative impacts. In the present research, the effects of nanocarbon on cell growth and secondary metabolite production on rice callus were studied. Different concentrations of nanocarbon (0-1000 mg l^{-1}) were added into callus induction media to investigate the effects on the growth of callus, and secondary metabolite production in indica rice callus (*Oryza sativa* L. cv. Pathumthani1). The growth of callus was measured in terms of the size and weight of callus, and secondary metabolite production was determined based on the levels of total phenolic compounds and flavonoid production. The results showed that the callus induction, the callus growth and the secondary metabolite production in rice callus after treatment with nanocarbon were significantly different when compared to control (without treatment with nanocarbon). The rice callus treated with low (100 and 200 mg l^{-1}) and moderate (400 and 600 mg l^{-1}) level of nanocarbon displayed an enhancement in callus growth, total phenolic compounds and flavonoid production. However, the induction of callus, cell growth, and secondary metabolite production were decreased when rice callus was treated with high concentrations of nanocarbon (800 and 1000 mg l^{-1}). The results of this research showed that the nanocarbon application of 400-600 mg l^{-1} had potential to induce the cell growth and secondary metabolite production. This research suggests further investigation in using different concentrations of nanocarbon on other plant species for potential pharmaceutical application.

Keywords: callus, growth, metabolite, nanocarbon, rice
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1. Introduction

The level of response in plant cells is motivated by various elicitors such as microorganisms, climates, metals and so on. Elicitors from biotic and abiotic stresses are able to activate morphological, physiological and biochemical responses in plant cells [1, 2]. The levels of biochemical responses and secondary metabolite generation and accumulation in plant cells are affected by several elicitors [3, 4]. Generally, the plant cells encompass secondary metabolites such as phenolics and flavonoids which have been considered as the alternative substances for pharmaceuticals, medicinal and supplementary food properties [5]. In recent years, the various types of biotic and abiotic elicitors have been advanced as an application scheme of plant cells that can trigger the generation and accumulation of secondary metabolites [6, 7]. Nanotechnology has gained an increasing attention in agricultural science and attracted remarkable consideration because of the positive and negative effects from the unique properties of nanomaterials and nanoparticles [8-10]. The synthesis and development of nanoparticles could open up the various applications in the field of biological and agricultural science. Several types of nanoparticles have been attracted noteworthy consideration and they have been studied in different organisms to investigate morphological, physiological and biochemical responses [11, 12]. Nanocarbon is one of the comprehensively used nanoparticles for various applications. In recent years, many studies have shown the effects of nanocarbon on plant responses such as micropropagation, plant growth, seed germination and plant metabolites. Positive or negative effects of nanocarbon on plant cells that are diverse among plant species, growth stages, concentrations, size and specific shapes of nanocarbon are also reported. The impacts of nanocarbon on plant response and development have been studied in different plant species such as maize, wheat, tomato and so on. [13-15].

Rice (*Oryza sativa* L.) is the world's important food crop and a model crop for research. Moreover, rice is a main food for more than 50% of the world's population, especially indica rice cultivars which is suitable for studying the impact of nanoparticles in plant responses [16]. The applications of nanoscience and nanotechnology in agricultural technology are an increasingly numerous incidence and enormous challenges that need to be supported for plant productions [17, 18].

The main emphasis of this research was to investigate the impact of nanocarbon on the callus formations (size, fresh weight, dry weight, relative growth rate and callus induction) and the accumulation of secondary metabolites, including total phenolic compound and flavonoid contents, and to evaluate the antioxidant activities of the callus extracts.

2. Materials and Methods

2.1 Plant materials, explants preparation and culture conditions

Mature seeds of indica rice cultivar Pathumthani 1 were obtained from Pathumthani Rice Research Center, Ministry of Agriculture and Cooperatives, Thailand. Mature seeds were dehusked by hand and surface sterilized by immersion in 70% (v/v) ethanol for 2-3 min, followed by immersion in 5% (v/v) commercial bleach (5.25% sodium hypochlorite) for 30 min and followed by immersion in 30% (v/v) commercial bleach (5.25% sodium hypochlorite) for 30 min. The mature seeds were then rinsed 5-6 times with sterile distilled water. The sterilized seeds were placed on sterilized filter papers in a petri dish and cultured on NB basal medium [19] supplemented with 500 mg l^{-1} glutamine, 300 mg l^{-1} casein hydrolysate, 30 g l^{-1} sucrose, 1.5 mg l^{-1} 2,4-D (2,4-Dichlorophenoxy-acetic acid), and 8 g l^{-1} agar for callus induction medium. In order to study the effect of nanocarbon on callus induction and physiological responses, the seeds of indica rice cultivar Pathumthani 1 were

cultured on callus induction medium supplemented with different concentrations (0-1,000 mg l^{-1}) of nanocarbon. The seed cultures were maintained under the experimental conditions at 25 ± 2 ºC under 16/8 h light/dark photoperiod with light intensity of 1,000 lux provided by cool white fluorescent lights.

2.2 Determination of callus induction performances

Plant samples were taken 4 weeks after cultivation for callus growth measurement and metabolite analysis because this period was the log phase or growth phase for appropriate growth and metabolite production. After 4 weeks in culture, the number, size, fresh weight and dry weight of embryogenic calli were recorded. The relative growth rate was measured and calculated according to the protocol of Hoffmann and Poorter [20].

2.3 Determination of secondary metabolite accumulations

Four-week-old biomass of callus induction from different concentrations of nanocarbon treatments was used for estimation of secondary metabolites (total phenolic compounds and total flavonoid contents) and antioxidant activities. The biomass of callus from each treatment was extracted with acidic-methanol (methanol:HCl; 99:1) at room temperature for 2 h on a rotary shaker (250 rpm). The homogenates were centrifuged at 8,000 rpm at 4 °C for 20 min. The supernatant was collected and investigated for the total phenolic compound contents, the total flavonoid contents and the total antioxidant activities.

The total phenolic compound contents of the callus extractions were determined by the Folin and Ciocalteu assay according to the protocol of Alothman *et al.* [21]. The total phenolic compound contents were expressed as milligrams of gallic acid equivalents (GAE) per g of sample.

The total flavonoid contents of the callus extractions were determined by the Aluminium-chloride ($AlCl_3$) colorimetric method according to the protocol of Pedro *et al.* [22]. The total flavonoid contents were expressed as milligrams of flavonoids per gram of sample.

The total antioxidant activities of the callus extractions were determined by DPPH method according to the protocol of Brand-Williams *et al.* [23]. The total antioxidant capacities were expressed as % inhibition and micromolar (µM) of Trolox equivalents per gram of sample.

2.4 Statistical analysis

Data were expressed as mean ± SD from five replicates. The experiments were set up in a completely random design (CRD). Data of experimental investigations were analyzed by analysis of variance (ANOVA) and compared by Duncan's New Multiple Range Test (DMRT) ($P \leq 0.05$) using SPSS statistics (SPSS for Windows version 15, SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1 Callus induction performances

The results from this research showed that different concentrations of nanocarbon influenced the performances of callus induction, size, fresh weight, dry weight, relative growth rate in indica rice callus cultivar Pathumthani 1. The mature seeds cultured on callus induction medium supplemented with and without nanocarbon exhibited callus formation after 2 weeks of culture and the data of

these parameters were recorded after 4 weeks of culture. The percentage of callus induction and growth performances (size, fresh weight, dry weight and relative growth rate) induced by nanocarbon at various concentrations after 4 weeks was shown in Figure 1 and Table 1, respectively.

The treatment of 600 mgl^{-1} nanocarbon showed the highest percentage of callus induction (93.33±2.35%) when compared to the other treatments (Figure 1). Callus induction medium containing 600 mgl^{-1} nanocarbon induced the highest size (1.28 ±0.08 cm), fresh weight (80.34 ± 0.65 mg), dry weight (49.56 ±1.52 mg) and relative growth rate (0.0570 ±0.0026 mg d^{-1}) (Table 1). At high concentrations of nanocarbon (800 and 1,000 mgl^{-1}), size, fresh weight, dry weight, and relative growth rate as well as the percentage of callus induction were drastically reduced.

The size, fresh weight, dry weight and relative growth rate of callus were recorded at a regular interval to determine the growth performances for plant growth index [24-26]. Nanocarbon has been used to induce callus tissues and cell culture in many plant species. It had a significant positive effect of nanocarbon on plant germination and growth performances could also be noticed [27-29]. The previous research has reported that the production of biomass and relative growth rate were enhanced by the stimulated process of water uptake in plant cells when exposed to nanocarbon [30]. Nanocarbon has possibly been associated with the motivation of genes and proteins expressions that are related to the plant growth and development [31]. The present research reveals that the use of nanocarbon on callus induction medium was beneficial for callus culture of indica rice cultivar Pathumthani 1. The efficiency of nanocarbon for plant responses was supported by previous studies in various plants [32-34].

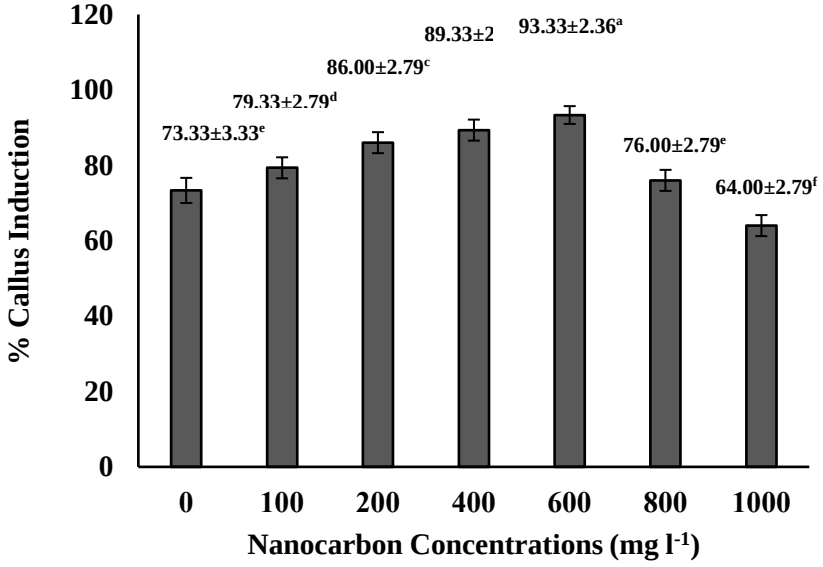

Figure 1. Effect of different concentrations of nanocarbon (0, 100, 200, 400, 600, 800 and 1,000 mg l-1 nanocarbon) on the percentage of callus induction from indica rice cultivar Pathumthani 1. Data show means ±SD (n = 5). Different letters on bars represent statistically significant effects of different concentrations of nanocarbon ($P \leq 0.05$).

Table 1. Effect of different concentrations of nanocarbon (0, 100, 200, 400, 600, 800 and 1,000 mg l^{-1} nanocarbon) on size, fresh weight, dry weight and relative growth rate of callus formations from indica rice cultivar Pathumthani1

Treatments (mg l^{-1} Nanocarbon)	Growth Performances			
	Size (cm)	Fresh weight (mg)	Dry weight (mg)	Relative Growth Rate (mg d^{-1})
0	0.60 ±0.07^{d}	66.52 ±1.45^{d}	30.66 ±1.44^{e}	0.0524 ±0.0019^{bc}
100	0.76 ±0.05^{c}	72.82 ±1.40^{c}	35.26 ±0.63^{d}	0.0534 ±0.0031^{b}
200	0.86 ±0.09^{c}	75.66 ±0.65^{b}	39.44 ±1.06^{c}	0.0541 ±0.0037^{b}
400	1.02 ±0.08^{b}	76.44 ±1.39^{b}	44.08 ±1.17^{b}	0.0555 ±0.0021^{b}
600	1.28 ±0.08^{a}	80.34 ±0.65^{a}	49.56 ±1.52^{a}	0.0570 ±0.0026^{a}
800	0.64 ±0.05^{d}	66.88 ±1.12^{d}	30.80 ±1.04^{e}	0.0524 ±0.0015^{bc}
1000	0.48 ±0.08^{e}	64.06 ±1.02^{e}	25.92 ±1.27^{f}	0.0514 ±0.0056^{c}

Note: Data show means ± SD (n = 5) and different superscripts in the same column mean significant difference ($P \leq 0.05$).

3.2 Secondary metabolite accumulations

After 4 weeks of culture, the application of nanocarbon was tested to determine the effects on the accumulation of total phenolic compound contents, total flavonoid contents and its antioxidant activities in callus extractions of indica rice cultivar Pathumthani 1 (Figures 2-4). The callus extraction of indica rice cultivar Pathumthani 1 showed a significant increase in the total phenolic compound contents and total flavonoid contents as well as antioxidant activities as a response to nanocarbon treatments.

The total phenolic compound and flavonoid contents of acidic-methanol extracts were achieved from the callus formations. Maximum contents of the total phenolic compounds (122.77 ±3.23 mg GAE g^{-1}) and total flavonoids (0.0052 ±0.0001 mg g^{-1}) were obtained from the extracts of callus cultured on callus induction medium supplemented with 600 mg l^{-1} nanocarbon (Figures 2 and 3).

The maximum percentage of radical scavenging activity (% inhibition) and antioxidant activities (µM Trolox g^{-1}) was found in the extract of callus cultured on callus induction medium supplemented with 600 mg l^{-1} nanocarbon. The callus extracts from the treatment of 600 mg l^{-1} nanocarbon also showed the percentage of inhibition and antioxidant activities which were 41.38 ±0.90 % and 6.85 ±0.24 µM Trolox g^{-1}, respectively. These were more than the extract from the other treatments (Figure 4).

Callus extracts have been used effectively for the production of plant secondary metabolites for several applications [35-37]. In this research, nanocarbon treated callus at optimum concentrations could induce higher total phenolic compound and flavonoid contents than untreated callus. Under abiotic conditions such as nanomaterials and nanoparticles, this stress could trigger a sequence of modifications in physiological and biological processes and products in the accumulation of reactive oxygen species (ROS) in plant cells. Non-enzymatic antioxidant compounds including phenolic compounds and flavonoids contribute in ROS scavenging to defend

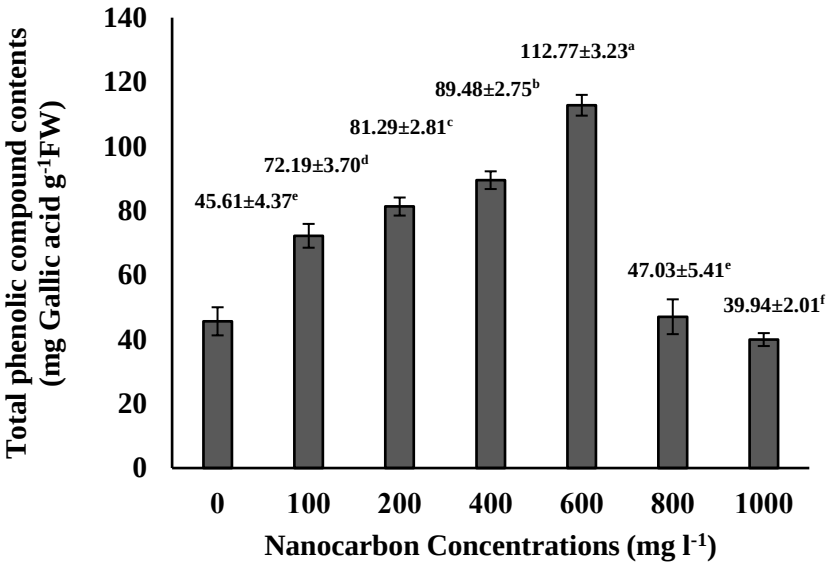

Figure 2. Effects of different concentrations of nanocarbon (0, 100, 200, 400, 600, 800 and 1,000 mg l^{-1} nanocarbon) on the contents of total phenolic compounds from callus extraction in indica rice cultivar Pathumthani 1. Data show means ± SD (n = 5). Different letters on bars represent statistically significant effects of different concentrations of nanocarbon ($P \leq 0.05$).

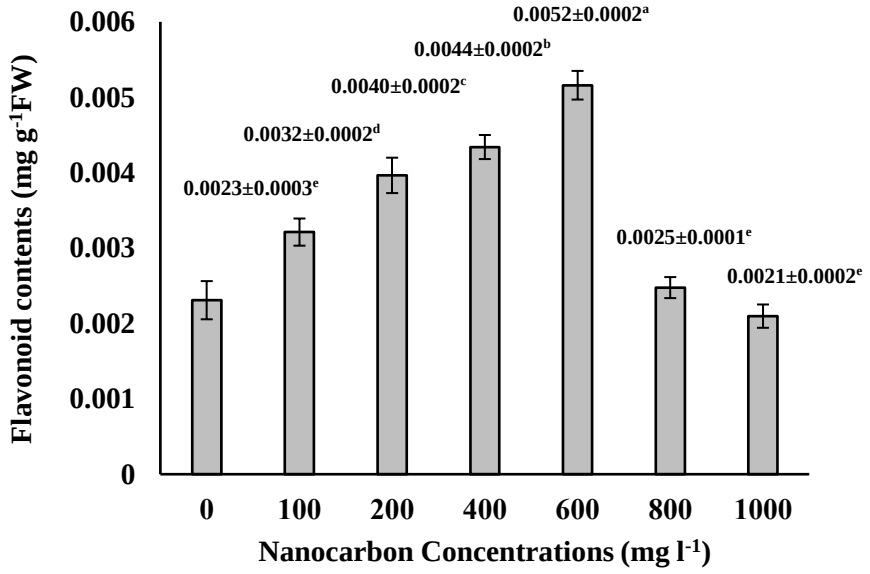

Figure 3. Effects of different concentrations of nanocarbon (0, 100, 200, 400, 600, 800 and 1,000 mg l^{-1} nanocarbon) on the contents of total flavonoids from callus extraction in indica rice cultivar Pathumthani1. Data show means ± SD (n = 5). Different letters on bars represent statistically significant effects of different concentrations of nanocarbon ($P \leq 0.05$).

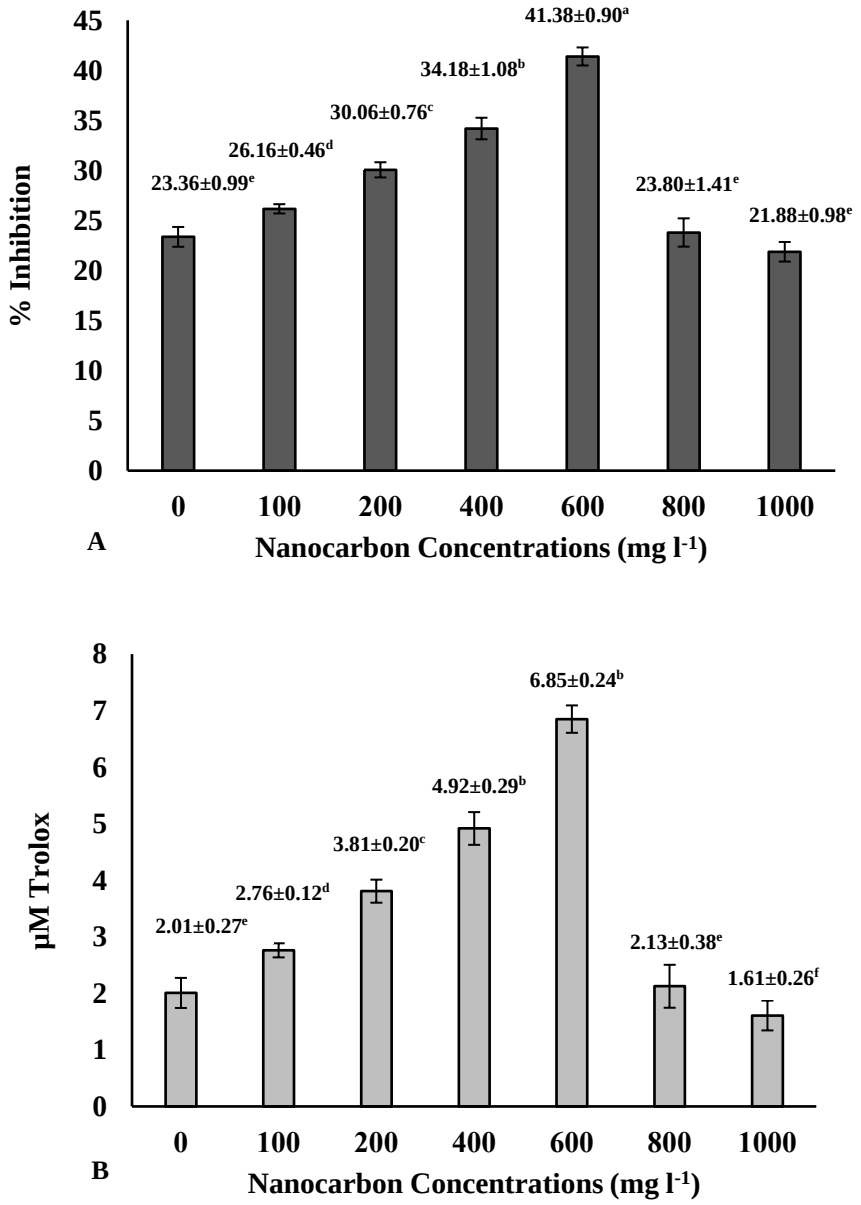

Figure 4. Effects of different concentrations of nanocarbon (0, 100, 200, 400, 600, 800 and 1,000 mg l^{-1} nanocarbon) on the percentages of inhibition (A) and Trolox equivalent antioxidant capacities (B) from callus extraction in indica rice cultivar Pathumthani 1. Data show means ± SD (n = 5). Different letters on bars represent statistically significant effects of different concentrations of nanocarbon ($P \leq 0.05$).

plant cells from stress conditions [38-40]. Phenolic compounds and flavonoids are essential for plant growth, development and plant protection [41]. Previous studies reported that application of nanomaterials and nanoparticles at appropriate concentrations could act as elicitors to induce plant metabolic responses, in particular the secondary metabolite induction by plant tissue culture for various plant species [42-48]. The phenolic compounds and flavonoids are quintessential components because of their antibacterial, anti-inflammatory, antiviral and free radical scavenging activities which have attracted considerable attention by various researchers [49-51].

4. Conclusions

The results of this research indicated that the induction of callus formation, total phenolic compound contents, total flavonoid contents and antioxidant activities from indica rice cultivar Pathumthani 1 could be enhanced when cultured on media supplemented with suitable concentrations of nanocarbon. The results substantiated the presence of nanocarbon substance in indica rice, which may be used as an alternative for plant metabolites production. The influence of nanocarbon significantly changed the growth performance and metabolite response of the callus. The results obviously demonstrated that nanocarbon enhanced the callus formations as well as secondary metabolite accumulations of indica rice cultivar Pathumthani 1. The synergistic effect of nanocarbon was found to be the optimal concentrations for the induction of the total phenolic compounds and flavonoids. It is interesting to characterize a reliable application of nanocarbon for the production of potential secondary metabolites (total phenolic compounds and flavonoids) through the callus culture of indica rice cultivar Pathumthani 1.

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References

[1] Mandal, C., Bera, S., Dey, N. and Adak, M., 2016. Physiological alterations of *Salvinia natans* L. exposed to aluminium stress and its interaction with polyamine. *Plant Science Today*, 3, 195-206.

[2] Wang, W., Yang, C., Tang, X., Gu, X., Zhu, Q., Pan, K. and Ma, D., 2014. Effects of high ammonium level on biomass accumulation of common duckweed *Lemna minor* L. *Environmental Science and Pollution Research*, 21, 14202-14210.

[3] Ardebili, N.O., Saadatmand, S., Niknam, V. and Khavari-Nejad, R.A., 2014. The alleviating effects of selenium and salicylic acid in salinity exposed soybean. *Acta Physiologiae Plantarum*, 36, 3199-3205.

[4] Habibi, G., 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. *Acta agriculturae Slovenica*, 101, 31-39.

[5] Ozkan, G., Sagdic, O., Gokturk, R.S., Unal, O. and Albayrak, S., 2010. Study on chemical composition and biological activities of essential oil and extract from *Salvia pisidica*. *LWT-Food Science and Technology*, 43, 186-190.
[6] Shaw, A.K., Ghosh, S., Kalaji, H.M., Bosa, K., Brestic, M., Zivcak, M. and Hossain, Z., 2014. Nano-CuO stress induced modulation of antioxidative defense and photosynthetic performance of Syrian barley (*Hordeum vulgare* L.). *Environmental and Experimental Botany*, 102, 37-47.
[7] Asgari-Targhi, G., Iranbakhsh, A. and Ardebili, Z.O., 2018. Potential benefits and phytotoxicity of bulk and nano-chitosan on the growth, morphogenesis, physiology, and micropropagation of *Capsicum annuum*. *Plant Physiology and Biochemistry*, 127, 393-402.
[8] Gogos, A., Knauer, K. and Bucheli, T.D., 2012. Nanomaterials in plant protection and fertilization, current state, foreseen applications, and research priorities. *Journal of Agricultural and Food Chemistry*, 60, 9781-9792.
[9] Pandey, A.C., Sanjay, S.S. and Yadav, R.S., 2010. Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*. *Journal of Experimental Nanoscience*, 5, 488-497.
[10] Dimkpa, C.O., Latta, D.E., McLean, J.E., Britt, D.W., Boyanov, M.I. and Anderson, A.J., 2013. Fate of CuO and ZnO nano and micro particles in the plant environment. *Environmental Science and Technology*, 47, 4734-4742.
[11] Adhikari, T., Kundu, S., Biswas, A.K., Tarafdar, J.C. and Rao, A.S., 2012. Effect of copper oxide nano particle on seed germination of selected crops. *Journal of Agricultural Science and Technology*, 2, 815-823.
[12] Rahmani, F., Peymani, A., Daneshvand, E. and Biparva, P., 2016. Impact of zinc oxide and copper oxide nano-particles on physiological and molecular processes in *Brassica napus*. *Indian Journal of Plant Physiology*, 21, 122-128.
[13] Zaytseva, O. and Neumann, G., 2016. Carbon nanomaterials: production, impact on plant development, agricultural and environmental applications. *Chemical and Biological Technologies in Agriculture*, 3, 17.
[14] Peng, Q., Dearden, A.K., Crean, J., Han, L., Liu, S., Wen, X. and De, S., 2014. New materials graphyne, graphdiyne, graphone, and graphane: review of properties, synthesis, and application in nanotechnology. *Nanotechnology, Science and Applications*, 7, 1-29.
[15] Chutipaijit, S. and Sutjaritvorakul, T., 2018. Application of activated charcoal and nanocarbon to callus induction and plant regeneration in aromatic rice (*Oryza sativa* L.). *Chemical Speciation and Bioavailability*, 30, 1-8.
[16] Ata-Ul-Karim, S.T., Liu, X.J., Lu, Z.Z., Yuan, Z.F., Zhu,Y. and Cao, W.X., 2016. In-season estimation of rice grain yield using critical nitrogen dilution curve. *Field Crops Research*, 195, 1-8.
[17] Adams, J., Wright, M., Wagner, H., Valiente, J., Britt, D. and Anderson, A., 2017. Cu from dissolution of CuO nanoparticles signals changes in root morphology. *Plant Physiology and Biochemistry*, 110, 108-117.
[18] Yang, Z., Chen, J., Dou, R., Gao, X., Mao, C. and Wang, L., 2015. Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.). *International Journal of Environmental Research and Public Health*, 12, 15100-15109
[19] Li, L., Qu, R., De Kochko, A., Frauquet, C. and Beachy, R.N., 1993. An improved rice transformation method using the biolistic method. *Plant Cell Reports*, 12, 250-255.
[20] Hoffmann, A. and Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. *Annals of Botany*, 80, 37-42.

[21] Alothman, M., Bhat, R. and Karim, A.A., 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, 115, 785-788.
[22] Pedro, A.C., Granato, D. and Rosso, N.D., 2016. Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. *Food Chemistry*, 191, 12-20.
[23] Brand-Williams, W., Cuvelier, M.E. and Berset. C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28, 25-30.
[24] Ivanova, K., Dimitrova, V., Georgieva, T. and Markovska, Y., 2015. Effect of soil salinity on growth, gas exchange and antioxidant defence of two Paulownia lines. *Genetics and Plant Physiology*, 4, 163-173
[25] Gupta, S.D. and Jatothu, B., 2013. Fundamentals and applications of light emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnology Reports*, 7, 211-220.
[26] Samart, S. and Chutipaijit, S., 2018. Modifications of morphological and physiological characteristics of pigmented-rice seedlings by application of titanium dioxide nanoparticles. *AIP Conference Proceedings*, 2010, 020003.
[27] Wang, X., Han, H., Liu, X., Gu, X., Chen, K. and Lu, D., 2012. Multi-walled carbon nanotubes can enhance root elongation of wheat (*Triticum aestivum*) plants. *Journal of Nanoparticle Research*, 14, 1-10.
[28] Tiwari, D.K., Dasgupta-Schubert, N., Villasenor Cendejas, L.M., Villegas, J., Carreto Montoya, L. and Borjas Garcia, S.E., 2014. Interfacing carbon nanotubes (CNT) with plants: enhancement of growth, water and ionic nutrient uptake in maize (*Zea mays*) and implications for nanoagriculture. *Applied Nanoscience*, 4, 577-591.
[29] Husen, A. and Siddiqi, K.S., 2014. Carbon and fullerene nanomaterials in plant system. *Journal of Nanotechnology*, 12, 1-10.
[30] 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, 3221-3227.
[31] Villagarcia, H., Dervishi, E., de Silva, K., Biris, A.S. and Khodakovskaya, M.V., 2012. Surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants. *Small*, 8, 2328-2334.
[32] Canas, J.E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M., Ambikapathi, R., Lee, E.H. and Olszyk, D., 2008. Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and Chemistry*, 27, 1922-1931.
[33] Flores, D., Chacón, R., Alvarado, L., Schmidt, A., Alvarado, C. and Chaves, J., 2014. Effect of using two different types of carbon nanotubes for blackberry (*Rubus adenotrichos*) *in vitro* plant rooting, growth and histology. *American Journal of Plant Sciences*, 5, 3510-3518.
[34] Khodakovskaya, M.V., Kim, B.S., Kim, J.N., Alimohammadi, M., Dervishi, E., Mustafa, T. and Cernigla, C.E., 2013. Carbon nanotubes as plant growth regulators: effects on tomato growth, reproductive system, and soil microbial community. *Small*, 9, 115-123.
[35] Kumari, A., Baskaran, P. and Van Staden, J., 2016. *In vitro* propagation and antibacterial activity in *Cotyledon orbiculata*: a valuable medicinal plant. *Plant Cell, Tissue and Organ Culture*, 124, 97-104.
[36] Liao, D.Y., Chai, Y.C., Wang, S.H., Chen, C.W. and Tsai, M.S., 2015. Antioxidant activities and contents of flavonoids and phenolic acids of *Talinum triangulare* extracts and their immunomodulatory effects. *Journal of Food and Drug Analysis*, 23, 294-302.
[37] Mahboubi, A., Kamalinejad, M., Ayatollahi, A.M. and Babaeian, M., 2014. Total phenolic content and antibacterial activity of five plants of *Labiatae* against four foodborne and some other bacteria. *Iranian Journal of Pharmaceutical Research*, 3, 559-566.

[38] Sergeant, K., Kieffer, P., Dommes, J., Hausman, J.F. and Renaut, J., 2014. Proteomic changes in leaves of poplar exposed to both cadmium and low-temperature. *Environmental and Experimental Botany*, 106, 112-123.
[39] Song, S.Y., Chen, Y., Chen, J., Dai, X.Y. and Zhang, W.H., 2011. Physiological mechanisms underlying *OsNAC5*-dependent tolerance of rice plants to abiotic stress. *Planta*, 234, 331-345.
[40] Chutipaijit, S., 2016. Changes in physiological and antioxidant activity of *indica* rice seedlings in response to mannitol-induced osmotic stress. *Chilean Journal of Agricultural Research*, 76, 455-462.
[41] Ghorbanpour, M., 2015. Major essential oil constituents, total phenolics and flavonoids content and antioxidant activity of *Salvia officinalis* plant in response to nano-titanium dioxide. Indian *Journal of Plant Physiology*, 20, 249-256.
[42] Aghajani, Z., Pourmeidani, A. and Ekhtiyari, R., 2013. Effect of nano-silver on stages of plant growth and yield and composition of essential of *Thymus kotchyanus* Boiss. & Hohen. *African Journal of Agricultural Research*, 8, 707-710.
[43] Hatami, M., Ghorbanpour, M. and Salehiarjomand, H., 2014. Nanoanatase TiO_2 modulates the germination behavior and seedling vigority of the five commercially important medicinal and aromatic plants. *Journal of Biological and Environmental Sciences*, 8, 53-59.
[44] Ghorbanpour, M. and Hatami, M., 2015. Changes in growth, antioxidant defense system and major essential oils constituents of *Pelargonium graveolens* plant exposed to nano-scale silver and thidiazuron. *Indian Journal of Plant Physiology*, 20, 116-123.
[45] Yang, H., Liu, C., Yang, D., Zhang, H. and Xi, Z., 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. *Journal of Applied Toxicology*, 29, 69-78.
[46] Remya, N., Saino, H.V., Baiju, G.N., Maekawa, T., Yoshida, Y. and Sakthi, K.D., 2010. Nanoparticulate material delivery to plants. *Plant Science*, 179, 154-163.
[47] Oloumi, H., Soltaninejad, R. and Baghizadeh, A., 2015. The comparative effects of nano and bulk size particles of CuO and ZnO on glycyrrhizin and phenolic compounds contents in *Glycyrrhiza glabra* L. seedlings. *Indian Journal of Plant Physiology*, 20, 157-161.
[48] Mohammadia, R., Maali-Amiria, R. and Mantri, N.L., 2014. Effect of TiO_2 nanoparticles on oxidative damage and antioxidant defense systems in chickpea seedlings during cold stress. *Russian Journal of Plant Physiology*, 61, 768-775.
[49] Nagarajan, A., Arivalagan, U. and Rajagurua, P., 2011. *In vitro* root induction and studies on antibacterial activity of root extract of *Costusigneus* on clinically important human pathogens. *Journal of Microbiology and Biotechnology Research*, 1, 67-76.
[50] El-Bakry, A.A., Mostafa, H.A.M. and Alam Eman, A., 2014. Antioxidant and antibacterial activity of callus and adventitious root extracts from *Rumex vesicarius* L. *Journal of Medicinal Plants Research*, 8, 479-488.
[51] Baskaran, P., Kumari, A., Naidoo, D. and Van Staden, J., 2015. *In vitro* propagation and biochemical changes in *Aloe pruinosa*. *Industrial Crops and Products*, 77, 51-58.