

NANOCARBON INDUCED MODIFICATIONS IN MORPHO-PHYSIOLOGICAL CHARACTERISTICS IN RICE PLANTS [*ORYZA SATIVA* L. CV. BLACK JASMINE RICE (HOM-NIN)]

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Abstract

The aim of this research was to evaluate the potential application of nanocarbon in plant growth and physiological responses in rice plants. This research was designed to evaluate the effects of different concentration of nanocarbon (0-1,000 mg L⁻¹) on growth characterizations and physiological parameters after 1-4 weeks of nanocarbon-treatment. Induction in the growth performances and contents of photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids), antioxidant enzyme activities (catalase and peroxidase) and flavonoids was observed in the rice plants after treated with nanocarbon. The results showed that nanocarbon promoted the plant growth in rice plants cv. Black jasmine rice or Hom-nin by increasing the shoot lengths and dry weights. The significant increase of shoot length and dry weight were found in 200 and 600 mg L⁻¹ nanocarbon-treatments. Additionally, the high contents of photosynthetic pigment concentrations in treated-plants showed significant difference at 600 mg L⁻¹ nanocarbon compared to the control (0 mg L⁻¹ nanocarbon). The antioxidant activities of catalase and peroxidase enzymes in nanocarbon treated-plants were significantly increased compared to the control. Furthermore, the induction of flavonoid contents in treated-plants was increased by 200 and 600 mg L⁻¹ nanocarbon. The results suggested that nanocarbon able to promote the biomass and has benefit to increase the photosynthetic pigments and antioxidant enzymes for plant growth performances. This research provided plant growth and physiological responses evidence on the beneficial effect of nanocarbon in rice plants, especially Black jasmine rice or Hom-nin cultivar.

Keywords: Antioxidant enzymes, flavonoids, nanocarbon, photosynthetic pigments, plant growth, rice

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Introduction

Rice is a major cereal crop in the developing world and an important staple food source for over half of the world's population (Muthayya *et al.*, 2014). The South East Asian countries are the main grown and produced *indica* rice (Arjinajarn *et al.*, 2017). There are many special cultivars of rice containing color pigments such as *Oryza sativa* L (black rice). Reddish anthocyanins or purple flavonoids are reported to be the primary pigments in these rice varieties (Htwe *et al.*, 2010; Min *et al.*, 2012). The bran part of pigmented-rice has been believed to be high in antioxidants and other significant constituents, such as flavonoids, anthocyanins, etc. (Shen *et al.*, 2009; Norkaew *et al.*, 2017). Moreover, Thai black rice contains more anthocyanins or flavonoids than some other rice varieties as a source of healthy food products (Pascual *et al.*, 2013). Plants have improved metabolism pathway for persistence strategies under stress conditions and tolerate them to develop, grow and productivity magnificent. Plants develop many physiological and morphological mechanisms to survive with an unfortunate condition (Shao and Bao, 2015).

Carbon is exchanged between living things, the earth, water and the Earth's atmosphere. Carbon is one element of life. As an element of estimation 50% of the tissue of the organism and in the form of carbon dioxide. The allotropic character of carbon allows for a wide array of nanomaterial morphologies and structures. Nanocarbon have demonstrated efficacy in plant disease suppression and/or plant growth efficiency, but reports are still quite limited (Chutipaijit and Sutjaritvorakul, 2018a). Currently, nanocarbon such as carbon nanotubes (single wall or multiwall), graphene oxide, fullerenes and carbon black have demonstrated potential to affect plant health, which in turn suggests an important disease management tool could be exploited (Chen *et al.*, 2018). It has been essential for the growth of plants. Engineered nanoparticles (1-100 nm) that have demonstrated activity in promoting plant growth or suppressing plant diseases are metalloids,

metallic oxides, nonmetals, and carbon nanomaterials. Nanomaterials have been integrated into disease management strategies as bactericides/fungicides and as nanofertilizers to enhance plant health and growth performances (Chutipaijit, 2015; Hao *et al.*, 2017; Samart *et al.*, 2018). Increasing evidence shows that nanocarbon have a harmful effect on some plants, although in some cases nanocarbon have been shown to improve plant water-retaining capacity, biomass and fruit production (Hao *et al.*, 2016; Chutipaijit and Sutjaritvorakul, 2018b).

This research was done to evaluate potential application of nanocarbon in plant growth and physiological responses for rice plants cv. Black jasmine rice (Hom-nin). This research was designed to investigate the effects of different concentration of nanocarbon (0-1,000 mg L⁻¹) on growth characterizations and physiological parameters of Black jasmine rice. This research provided nanocarbon and detailed assessment of rice seedling effect on plant growth, photosynthetic pigments and antioxidant enzyme activities in Black jasmine rice (Hom-nin) plants for the rice response knowledge.

Materials and Methods

Plant Materials, Growth Conditions and Determination of Plant Growth Parameters

Rice seeds (*Oryza sativa* L. cv. Black jasmine rice or Hom-nin) were germinated in distilled water for 2 days at room temperature. After germination, germinated-rice seeds were placed on seedling trays containing soil. Irrigation water was monitored daily with in a seedling trays. Rice seedlings (14-day-old) were irrigated with water supplemented with different concentrations of nanocarbon (0, 200, 600 and 1,000 mg L⁻¹). The rice plants were treated with different concentrations of nanocarbon every 7 days. Black jasmine rice plants were harvested at 1, 2, 3, and 4 weeks after nanocarbon treatments and the growth parameters were measured in terms of the

shoot length and dry weight. Moreover, photosynthetic pigment contents and antioxidant enzyme activities and flavonoid contents were determined for physiological analysis.

Determination of photosynthetic pigment content performances

The photosynthetic pigment contents (chlorophyll A, chlorophyll B and the total carotenoids concentrations; mg g^{-1} FW) were estimated. Rice fresh leaves (100 mg) were homogenized in 3 mL of acetone. Then, homogenized leaves were stored at 4°C for 48 h and measured the photosynthetic pigment contents by spectrophotometric method at wavelengths of 470, 644, and 662 nm wavelengths. The pigment content was calculated by using the equation according to Lichtenthaler (1987) and Shabala *et al.* (1999).

Determination of Antioxidant Enzyme Activity Performances

The catalase and peroxidase enzymes were determined as antioxidant enzyme activities of Black jasmine rice. The peroxidase enzyme activity was measured at 460 nm wavelength according to Beffa *et al.* (1990). The catalase

enzyme activity was determined at 280 nm wavelength by following Aebi (1984).

Determination of Flavonoid Content Performances

Flavonoid contents was used to investigate the effect of nanocarbon on the plant metabolites of rice that were exposed with and without nanocarbon. To investigate the effect of nanocarbon on the plant metabolites from rice, flavonoid contents of rice exposed with and without nanocarbon was analyzed. Four grams of rice plants were extracted with acidic-methanol (methanol: HCl; 99:1). After extraction, the samples were centrifuged at 8,000 rpm for 15 min and measured the flavonoid contents by spectrophotometric method at wavelengths of 374 and 535 nm wavelengths. The contents of flavonoids were determined according to Pedro *et al.* (2016). The results were expressed as mg of flavonoids per g of fresh weight sample (mg g^{-1} FW).

Statistical Analysis

The experiment was arranged in a Completely Randomized Design (CRD). Data were expressed as mean $\pm$ SD, based on 5 replications ($n=5$). The Mstat software (Mstat for Windows, V. 1.42, Michigan State University, USA) was used to analyze the significance of changes between different treatments at probability levels of 0.05. The significance of differences between the mean values of control and samples concentrations i.e., 0, 200, 600, and 1,000 mg L^{-1} nanocarbon.

Results and Discussion

Plant Growth Performances

As shown in Figure 1, the plant growth performances were significantly enhanced in shoot length and dry weight parameters from Hom-nin rice plants when exposure to nanocarbon. After 1-4 weeks of treating, the highest change rates of shoot length and dry weight were 111-121% and 110-116% increment when exposed to 600 mg L^{-1} nanocarbon as

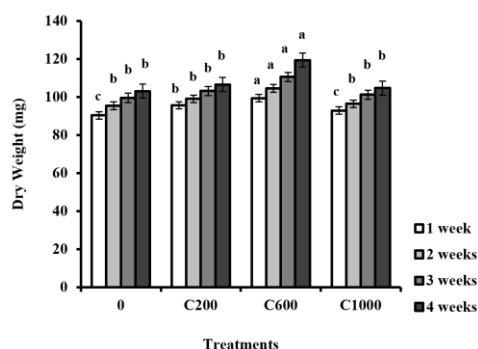


Figure 1. Effects of different concentrations (0, 200, 600, 1,000 mg L^{-1}) of nanocarbon on shoot length (A) and dry weight (B) in rice seedlings of *indica* rice cv. Black jasmine rice or Hom-nin). The values presented means ($\pm$ SD) following different letters indicated significant differences at $P < 0.05$ among different treatments in each week

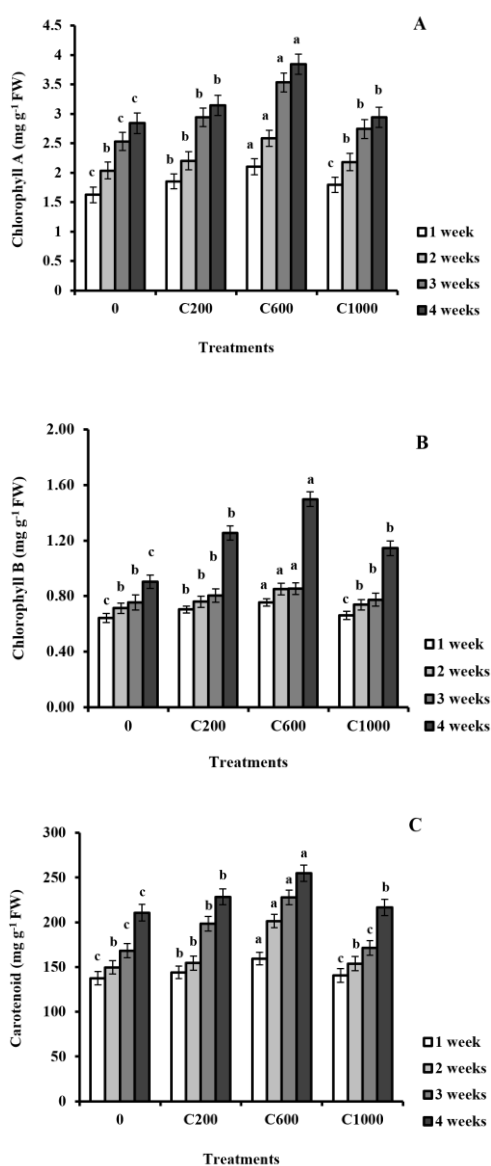


Figure 2. Effects of different concentrations (0, 200, 600, 1,000 mg L⁻¹) of nanocarbon on the contents of chlorophyll A (A), chlorophyll B (B) and carotenoids (C) in rice seedlings of *indica* rice cv. Black jasmine rice or Hom-nin). The values presented means ($\pm$ SD) following different letters indicated significant differences at $P < 0.05$ among different treatments in each week

compared to the control (0 mg L⁻¹ nanocarbon), respectively.

The previous researches have been revealed that the nanomaterials suspensions able to increase the growth rate, plant height and biomass as well as the germination rate (Arora *et al.*, 2012; Numana *et al.*, 2018; Samart and Chutipaijit, 2018). The beneficial effects of nanocarbon have been reported in other previous researches. They suggested that nanocarbon has positive effect to the plant growth performances by enhancing in plant metabolism and stimulation of biochemical reactions in plant cells (Chen *et al.*, 2012; Ye *et al.*, 2019).

Photosynthetic Pigment Content Performances

As shown in Figure 2, the results showed the significant induction in the contents of chlorophyll A and chlorophyll B as well as carotenoid contents, when exposed to nanocarbon in Hom-nin rice plants during 1-4 weeks of treated-period. At 600 mg L⁻¹ nanocarbon concentration, the inductions in the contents of chlorophyll A and chlorophyll B carotenoids were presented the highest rate (127-140%, 113-166% and 116-135%, respectively) when compared to the control (0 mg L⁻¹ nanocarbon).

The contents of chlorophyll A, chlorophyll B and carotenoids, as also noticed in previous studied about the effect on affects the plant's ability such as photosynthetic rate and plant metabolites *via* Calvin cycle (Hu *et al.*, 2014; Alexandra and James, 2016). The earlier reports were suggested that nanocarbon induced the water adsorption in plant cells and it had an effect on significantly stimulating the photosynthesis process as well as advancing the plant growth rate in the next step (Tilman *et al.*, 2011; Wang *et al.*, 2014).

Antioxidant Enzyme Activity and Flavonoid Content Performances

In Figures 3 and 4, seedlings of Hom-nin rice plants when exposed to nanocarbon at 1-4 weeks of treated-plants induced a significant increase in antioxidant enzyme activities (catalase and peroxidase enzymes) and non-

enzymic antioxidants (flavonoid content). The results indicated that antioxidant enzyme activities and non-enzymic antioxidants were a significant increase in catalase and peroxidase enzyme activities, and flavonoid content from Hom-nin rice plants when exposed to nanocarbon compared to the control (0 mg L⁻¹ nanocarbon). The 600 mg L⁻¹ nanocarbon treatment were presented the highest increase in the activities of catalase enzyme (140-253%), peroxidase enzyme (137-193%) and the content of flavonoid (140-213%) in plant seedlings when compared to the control and

found relatively to the results of the plant growth and photosynthetic content performances.

The oxidative damages have been presented in plant cells under abiotic stress or nanomaterials exposure. Plant defense mechanisms are induced to increase the antioxidant activities and non-enzymic antioxidants, such as catalase, peroxidase, ascorbic acid, glutathione, flavonoid, etc. (Michalak, 2006; Pham-Huy *et al.*, 2008; Hatami and Ghorbanpour, 2014; Fu-Ming *et al.*, 2017). These mechanisms are stimulated for the ability of plant resistance and adaptation of the plant survivals as well as prevent the plant cells from reactive oxygen species damaged (Desikan *et al.*, 2001; Yan *et al.*, 2007). Similar consequences have been reported in the various plant species under abiotic stress and nanomaterials conditions (Ma, 2004; Khodakovskaya *et al.*, 2011; Saxena *et al.*, 2016; Singh *et al.*, 2016). The results were expected that the applications of nanocarbon have been attributed to imposing advance of antioxidant process.

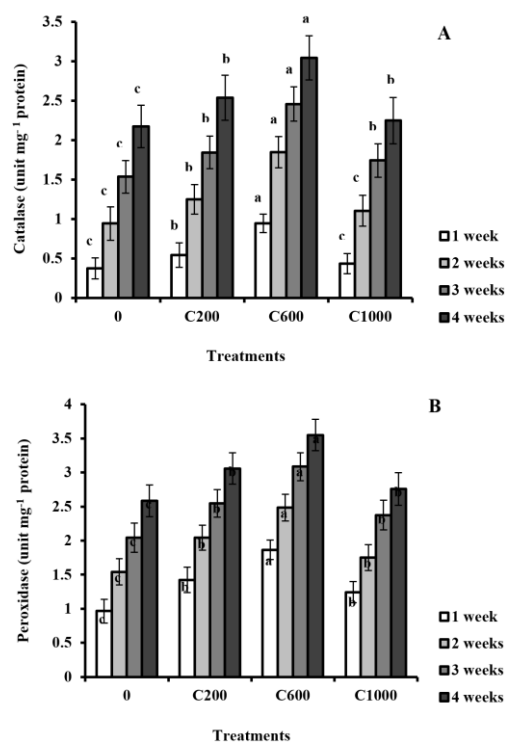


Figure 3. Effects of different concentrations (0, 200, 600, 1,000 mg L⁻¹) of nanocarbon on the activities of catalase enzyme (A) and peroxidase enzyme (B) in rice seedlings of *indica* rice cv. Black jasmine rice or Hom-nin). The values presented means ($\pm$ SD) following different letters indicated significant differences at $P < 0.05$ among different treatments in each week

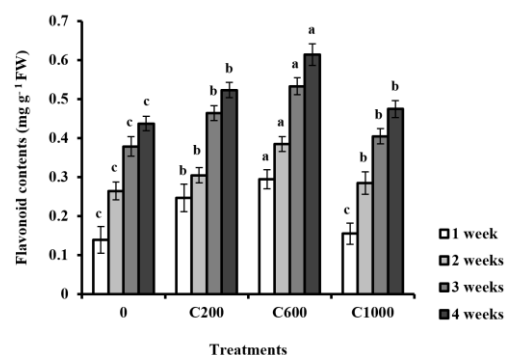


Figure 4. Effects of different concentrations (0, 200, 600, 1,000 mg L⁻¹) of nanocarbon on the contents of flavonoids in rice seedlings of *indica* rice cv. Black jasmine rice or Hom-nin). The values presented means ($\pm$ SD) following different letters indicated significant differences at $P < 0.05$ among different treatments in each week

Conclusions

In this research, the results pointed out that nanocarbon stimulated a positive effect on plant growth and photosynthetic content performances of treated-seedlings in *indica* rice cv. Black jasmine rice or Hom-nin. Moreover, this condition indicated that induced the incidence of oxidative stress. Nanocarbon was able to increase obviously the activities of antioxidant enzymes (catalase and peroxidase enzymes) and non-enzymatic antioxidant (flavonoid content), therefore the plant defense against oxidative stress by the high production of antioxidant system and funding positively to stress tolerance. The results suggested that a potential application of nanocarbon for *indica* rice growth and productivity

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Disclosure Statement

The authors declare that they have no conflict of interest.

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