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Effect of Multiwalled Carbon Nanotube (MWCNT) on Rice (Pusa Basmati 1121): Insight into their Physiological and Biochemical Parameters during Growth and Development

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ABSTRACT

Nanotechnology has great potential to transform and improve the quality of life through its applications in various fields of science and technologies. From last decade, this technology is becoming very attractive through new innovations and techniques used in agriculture and food industry. The present study focus the effect of multiwalled carbon nanotube (MWCNT type 12) on PB 1121 rice variety by inspection of physiological (germination percentage, shoot length, root length and fresh weight) and biochemical [reactive oxygen species (ROS), indole acetic acid (IAA) and polyphenol oxidase (PPO)] practices during growth and development. A significant increase in seed germination percentage and plant growth was observed against various concentrations of MWCNT supplemented in Murashige and Skoog medium (MS) culture media. On comparing to control, MWCNT treated seedlings were found to be well flourished but remarkably enhanced root system was identified with increased ROS level interestingly. According to IAA and PPO activities, no significant variation was observed in MWCNT treated plants compare to control plants. The carbon nanotube has regulatory effect on seedling and plant growth which resulted in enhanced and improved shoot and root systems. Therefore, the results of this study strengthen our knowledge about MWCNT and also enforce to find out mechanism and relation between the physiological and biochemical parameters for further study.

Key words: *Carbon nanotube, Rice, Physiological parameter and Biochemical activity*

INTRODUCTION

Nanotechnology is unique, innovative and emerging field in the area of science and technology and it is an interdisciplinary field with wide range of applications various area of science and industry such as, medicine, pharmacology, biology, agriculture, biomedical engineering, targeted drug delivery, electronics, cosmetic industry and biosensors[1-5].

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Agriculture is vital source and plays a critical role in livelihood because it supplies food as a primary source, clothing, medicine and employment all over the world. Now it has been associated with fruit cultivation, forestry, bee keeping, poultry, and even dairy farming as modern agriculture practices. Conversely, increasing growth of population and limit of natural resource like land, water, and soil are very concerns that demand high-throughput agriculture development with human friendly environment to preserve the best quality of food safety with reduced environmental risk. Therefore, sustainable growth of agriculture is very important for high tech agricultural development through introduction of new principle and approaches and to transform conventional agricultural system and economic advantages[6-10]. Currently, nanotechnology might be considered as one of the possible alternatives to resolve the problems in the agriculture similar to the biotechnology. In last decade, nanotechnology has gained momentum in agriculture field with the potential to develop immense transition in food and agriculture strengthening. Now a days, diverse range of nano-materials or nano-particles and related technology are available which are being frequently revolutionized and opened a new platform to work with more efficiency which has been successfully utilized in various plant species for desired purpose[11-18].

Moreover, nanotechnology is study of materials having at least one dimension between 1 and 100 nm in size or one billionth of a meter (nanometer)[19]. Nanomaterials comprise both organic and inorganic or metallic and non metallic particles having different shape, size, surface area, charge, and behavior. Due to differential nature of NP, it exhibits extent of diverse characteristics in their mechanical, chemical, electrical, thermal, elastical and optical properties[20,21]. For that reason, NP of same chemical composition having variable sizes and shapes reveal distinguishable effect and this might be possible example to utilize it a new approach to solve complex scientific queries. With rapid advancement in material science, the ample diversity of variable sizes of engineered NP particles are available commercially as well as option of green nanotechnology is also available. Amongst NPs, a broad range of carbon based nanoparticle are available having different structural and functional properties and can be categorized into different dimensional (D) form viz. carbon dots and fullerene come in zero D followed by single-wall carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs) in 1D than graphene, and graphene oxide in 2D[22]. Amongst the carbon nanoparticle (CNP), SWCNTs, MWCNTs and graphene have revealed a significant role in the material science due their physiochemical properties[23]. Lately, CNPs are being evaluated in plant biology due their influential role in plant growth and development[12,15,18,24].

Therefore, uniqueness of physical and chemical properties of NPs makes them attractive due to their ability to coordinate with molecular & cellular processes and play influential role in various biological functions[25]. For instance, NPs are reported to generate variable extent of reactive oxygen species (ROS) which is key factor which transform different metabolic connection for example cell signaling, proliferation, differentiation, cell survival, cell death, oxidative signaling etc.[26-28]**.** Based on the response of NPs, we utilized multiwalled carbon nanotube (MWCNT types 12) to understand the influential role of CNT on Pusa Basmati 1121, a rice variety through the estimation of physiological and biochemical changes. Rice is most important staple crop globally, belongs to monocot of Poaceae family and is considered as model plant for genetic and various stresses mediated studies[29,30]. Rice is cultivated under the diverse range of climate and water-soil relations. The adverse conditions are important constrain to rice growth and production whereas any constrain (biotic and abiotic) prevent to maintain the optimum growth and productivity of rice crop. Various stress such as salinity, heat, cold have adverse effect on the vegetative growth of rice along with their yield production and are more involved in risk of worldwide food safety[31,32]. The goal of present study is to understand the effect of MWCNT on seed germination and development of PB1121 rice along with impact on various physiological and biochemical parameters against various concentration of MWCNT. Here, we have observed a significant positive effect of carbon nanotube on rice plant growth and development that has added to our knowledge to exploit MWCNTs in a particular dosage to increase plant productivity.

MATERIAL AND METHODS

Plant material and sterilization:

The seeds of *Oryza sativa* L. (Rice) of Pusa Basmati 1121 (PB 1121) variety was used as plant material for the present study. The seed were dehusked manually followed by simple washing. Surface sterilization was performed with 4 % sodium hypochloride (NaClO) solution for 15 min followed by 3 times distilled water washing to remove the trace of disinfectant. Further, The seeds were aseptically surface sterilized with mercuric chloride (0.1% w/v) solution (Merck, India) for 3 min and finally, 3-4 times sterile distilled water washing before the culturing of rice seed in Murashige and Skoog medium (MS) media.

Nano particle parameters and culture conditions:

The effect of engineered 10-20 nm length of multi walled carbon nano tube (MWCNT Type 12) from Sisco Research Laboratories pvt. Ltd. was investigated to access growth and development in Pusa Basmati 1121. The various concentrations of MWCNT Type 12 such as 10 μg/ml, 25 μg/ml, 50 μg/ml, 75 μg/ml, 100 μg/ml, 125 μg/ml and 150 μg/ml was supplemented in MS media (Himedia) without any growth regulators and control was also used without nanoparticle. The dispersion of MWCNT in the MS media supplemented with 3% sucrose (Merck) was made with help of ultrasonic vibration (Yorco) and then medium was adjusted to pH 5.8 before adding 0.8% agar (bacteriological grade; Merck). Further, medium sterilization was achieved at 1.2 kg/cm^2 pressure and 121°C temperature for 20 min. The sterilized PB 1121 seeds were inoculated in Ms medium and kept overnight at dark and further, all the culture were incubate at 25±2°C under 16 h/8 h light/dark photoperiod with 250-300 $\mu mol\ m^{-2}\ s^{-1}$ irradiance level provided by cool white fluorescent tubes (Philips, India). Each treatment consisted of 5 replicates and repeated thrice.

Reactive oxygen species (ROS) determination:

The diphenyl-picrylhydrazyl (DPPH) assay was performed to analyze free radical-scavenging activities for methanolic extract of PB 1121 rice plant which were treated with various concentration of MWCNT Type 12 and ROS was examined after 30 days of experiment. The measurement for radical scavenging capacity is calculated by changing in the colour of DPPH solution. The hydrogen atom donating activity of antioxidant molecule reduce odd electron of nitrogen atom in DPPH to form corresponding hydrazine[33] implying that purple colour of DPPH stable free radical is reduced to yellow colored diphenylpicryl hydrazine. The test was performed with fresh solution of 0.002% DPPH in methanol and methanolic plant extract was used for the analysis. The reaction mixture was kept in dark for 30 min and then absorbance was measured at 517 nm using Shimadzu spectrophotometer and blank was adjusted with 0.002% DPPH methanolic solution. All assays were performed in triplicate. The percentage inhibition of the DPPH radical was calculated according to the formula:

% inhibition of DPPH radical = (1-A sample/A blank) X 100

Where, A blank is the absorbance of the control reaction (containing all reagents except the test sample), and A sample is the absorbance of the Sample/Reference.

Indole acetic acid (IAA) content calculation:

The Indole acetic acid content was estimated in both shoot and root part of PB 1121 rice plant after 30 days treatment of various concentrations of MWCNT Type 12 on the growth and development. The extraction of IAA was made in the salkowaski reagent [0.5 M FeCI3 in 35% HCIO4] and absorbance was recorded at 530 nm after 30 min by spectrophotometer (Shimadzu UV) according to the methods explained by Gordon and Weber[34].

Polyphenol oxidase (PPO) assay:

The 30 days old seedlings of PB 1121 rice were used for PPO estimation in both shoot and root under various concentration of MWCNT treatment. The analysis was made in the 3 ml reaction mixture containing 10mM catechol in 0.05M phosphate buffer pH 7.0.

The process was started by addition of enzyme extract and blank was without substrate. During the reaction, the shifting in colour of reaction mixture takes place due to oxidation of catechol. The absorbance was recorded at 490 nm for one minute at period between 15 seconds using spectrophotometer (Shimadzu UV) [35].

RESULTS AND DISCUSSION

In the present study, the effect of various concentration of multiwall carbon nanotube (MWCNT type 12) was examined against growth and development in PB 1121 rice variety. The in vitro culturing technique was used for the appropriate growth and development of selected rice variety. The 30 days old seedling cultured on MS media supplemented with MWCNT (0, 10, 25, 50, 75, 100, 125, and 150 µg/ml) was used in the present study. Various physiological and biochemical parameters comprising germination percentage, tillering, shoot & root length and their fresh weight, free radical scavenging capacity, indole acetic acid (IAA) and polyphenol oxidase were evaluated in PB 1121 plants. The present study has represented a positive effect of MWCNT on the growth and development of PB1121 rice variety without any toxicity and healthy and improved shoot and root systems have established during the experiment (Fig. 1).

The seeds germination percentage was observed within the period of first week of culturing. On comparing to control, the positively synchronized germination was observed from 10 µg/ml to 100 µg/ml of MWCNT supplemented in MS media followed by a down trend on approaching to 150 µg/mli MWCNT concentration was seen (Fig. 2). The average germination percentage was found to be 69 percent ranging from 64% to 84% and maximum was noted at 100 µg/ml carbon nanotube concentration. In the present study, the increased germination may be correlated with increased of water content uptake by the seeds with Carbon based NP in comparison to normal seeds germinations[13]. Seed germination is very important physiological parameter which is essential for the perpetuation of species with the help of three different phase of development[36]. To date, several different types of NPs based studies have been done on various rice varieties for example, Zinc oxide NP[11,37,38], Copper Oxide NP[39], Cerium Oxide NP[16], SiO2 and Mo NP[14], Ag NP[40] etc. Further, the consistency in the number of tiller was observed in CNT treated plants as well as in control plant. Average number of tillers was found to be 3 tillers per plant which were ranged from 3 to 5 tillers around whole experiment respectively. The seedling stage is followed by tillering stage and produces primary, secondary and tertiary tillers which may be defined as varietal characteristics and depend on the duration and morphology of plant[41]. The concept of tillering is known to be high yielding parameter in rice plant[42] and group of modern varieties have been developed which produce 20-25 tillers including primary, secondary and tertiary under controlled environment[43].

On the 30 days old seeding stage, the effect of MWCNT was examined on shoot length and root length of PB 1121 rice variety and comparison was concluded on the basis of treated and untreated practice. Enhanced growth was observed in term of shoot length in MWCNT enriched media but significant differences were not observed between treated and control plants (Fig. 3). The average shoot length was measured around between 17.11 (control) cm to 19.81 cm with an average length of 18.45 cm long. Healthy shoot system was observed without any adverse effect on the seedling growth and the average shoot length was found to be slightly more than control plants. Similarly, increased in root length was observed with increase of MWCNT concentrations supplemented in media and no toxic effect on morphologically of roots was observed (Fig. 3). The average root length of treated plants was 7.87 cm long ranging from 4.94 cm to 10.08 cm long while control length was 3.45 cm long. The present study is in compliance with previous report on effect of different types of carbon nanoparticles on germination and growth of rice plants[13]. Improved growth of seedling has been reported in tomato plant using various concentrations of multiwalled carbon nanotubes[44]. Functionalized and non functionalized carbon nanomaterials have also been examined in edible plants and vegetables which had enhanced of water retaining capacity, biomass and fruit yield in plants[24]. Similarly different plant species have exhibited positive growth and development using various types of carbon nanomaterials[12,15,18,45,46].

The free radical scavenging property of crude methanolic extract of shoot and root was evaluated for PB 1121 rice variety through1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Thirty days old seedlings were employed for estimation of antioxidant properties in both shoot and root against various concentrations of MWCNTs supplemented in the culture media and control plants (Fig. 4). For shoot system, the overall percent inhibition of DPPH was found to be almost similar in both treated and control plant. Similarly for root system, average percent inhibition of DPPH for the MWCNT treated plant was found to be higher at every concentration in respect to control. The DPPH radical scavenging ranged 59.25% to 88.76% with an average of 73.41% in the treated plant and 50.43% was observed in the control plant (Fig. 4). Therefore, the radical scavenging activity of root extract was significantly increased in dosage dependent manner with MWCNT enriched media compared to control, thus indicating the induction of ROS. This result is in agreement with earlier study reported on increased level of ROS in the rice cell suspension through toxicity experiment using various type of multi-walled carbon nanotube[47]. In plant biology, ROSs are thought to be involved not only in signaling reactions but also play necessary in several basic biological processes including cellular proliferation and differentiation[48]. The productions of ROS occurs by various metabolic pathways (NADPH oxidases, lipid catabolism and lipid β-oxidation in the glyoxysomes and mitochondrial respiration) for signaling purpose which regulate the growth and development and also mediate the response in biotic and abiotic stresses. According to recent study, ROSs have been involved in various physiological process such as, seed germination/development, root/shoot development, flowering process and throughout the plant life cycle[49]. Regarding the root growth and development, earlier studies have documented the relation between ROS and phytohormone which mediate important role in the root shapes and their structural design and are also reported to manage the root growth and development[50,51].

Indole acetic acid (IAA) estimation was measured at 30 days old seedling stage in both shoot and root of PB1121 rice variety. The average of IAA distribution within shoot and root of treated plants was found to be lower compared to control plants whereas no significant variation was observed in both shoot and root of treated plant (Fig. 5). This lower of IAA level is in accordance to earlier report on two rice varieties (sensitive and tolerant) against fast regulation of hormone metabolism and significant changes using salt stress[52]. However, the much reduced level of IAA was observed in the root of treated plant along with control plants than shoot respectively. IAA (or auxin) a phytohormone, is very crucial factor as it play an influential role in plant not only morphogenesis comprising tropistic growth, root patterning, vascular tissue differentiation, auxiliary bud formation, and flower organ development[53] but is also involved in responses to various abiotic stress which results in phenotypic changes that are related to alterations in auxin level and distribution[54].

Similarly, the polyphenol oxidase (PPO) activity was checked in PB 1121 rice variety on 30 days old seedling stage in response to their growth and development against treatment of MWCNT enriched in culture media. PPO is a nuclear encoded, plastid copper-containg enzyme and is very known to play crucial role in browning reaction. This enzyme catalyze the oxidation of monophenols/o-diphenols to o-diquinones then o-diquinones can lead to the generation of reactive oxygen species (ROS) and protein complexes, commonly observed as brown melanin pigments[55]. In the present study, decreased level of PPO was observed in both shoot and no significant changes were observed against the treatment of MWCNT (Fig. 6). The contemporary study on up-and-down level of PPO level has been reported in seedling and callus of *Trigonella foenum-graecum* and *T. aphanoneura* against treatment of various NaCl concentrations supplemented in MS media[56]. To date, several studies have been reported in various plant species which documented the role of PPO in relation to plant immunity or defense mechanism which reflect its promising involvement in physiological process under biotic and abiotic stress. For example, PPO activities was measured under the host–pathogen interaction in *Jasminum officinale* and *Uromyces hobsoni*[57] against *Aphis craccivora* infestation in *Vicia faba*[58], in healthy and viral infected sunflower[59], in fungal infection[60], against NaCl treatment[56,61], inspection of PPO (*Os*PPO) expression under drought and salt stress[62] etc.

In conclusion, the effect of multi walled carbon nanotube (MWCNT) was examined on Pusa Basmati 1121 (PB 1121) rice variety against their growth and development. The physiological and biochemical parameters were measured insight through germination percentage, tillering, shoot & root length and their fresh weight, free radical scavenging capacity, indole acetic acid (IAA) content and polyphenol oxidase level. During experiment, the healthy seeds of PB 1121 were used for culturing on half MS media supplemented with different concentrations of MWCNT and measured physiological and biochemical parameters on 30 days old seedling stage. A significant increased in germination percentage was seen in MWCNT treated seeds compared to untreated seeds. Also, rice seedlings were successfully established with enhanced root system at every MWCNT concentration compared to control and no negative effect was seen on shoot growth but slightly improved shoot growth was obtained than control. The considerable distributions of shoot and root fresh weights were recorded in MWCNT treated and untreated plants respectively. MWCNT promoted growth with better root system and enhanced biomass in the whole study. According to the metabolic interaction of MWCNT, the skewness in the signaling molecules was observed and transformed values of DPPH, IAA and PPO were estimated. In the present study, it is presumed that the penetration of carbon nanotubes in the plant system might play an important role in the growth and development of PB 1121 rice variety with the stimulation of certain biochemical changes through certain metabolic signaling which requires indepth study.

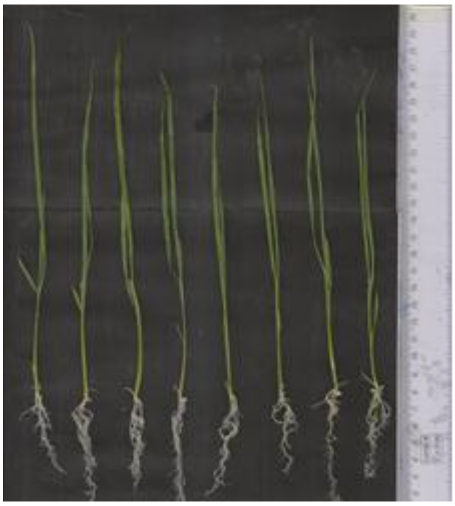

Fig. 1: This image represented healthy shoot and root system of PB 1121 rice variety against treatment of Multiwalled carbon nanotube (MWCNT type 12) supplemented in half MS culturing media. On moving right to left, MWCNT concentration is increasing while control is at first position adjacent to measurement scale

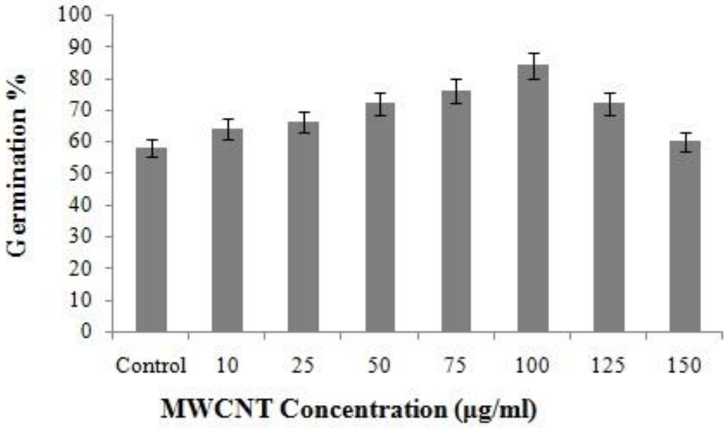

Fig. 2: Average germination percentage in PB 1121 rice variety with various concentrations of multiwalled carbon nanotube (MWCNT type 12) supplemented in MS media

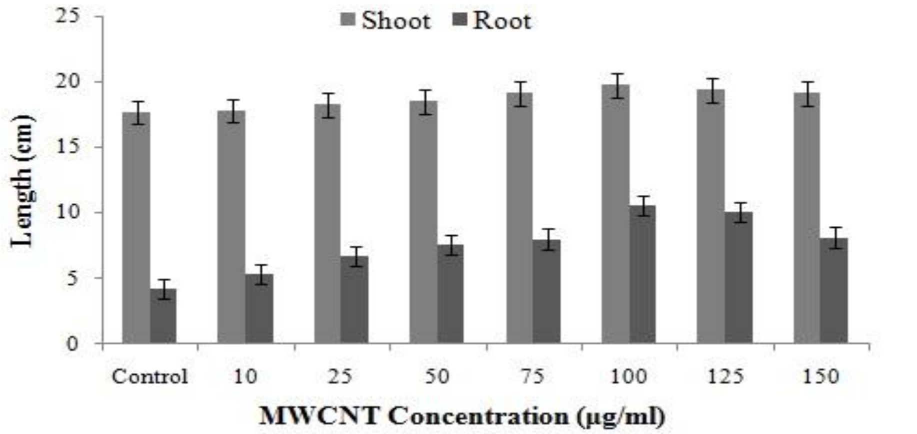

Fig. 3: Mean value of shoot and root length with respect of various concentration of MWCNT enriched in culture media

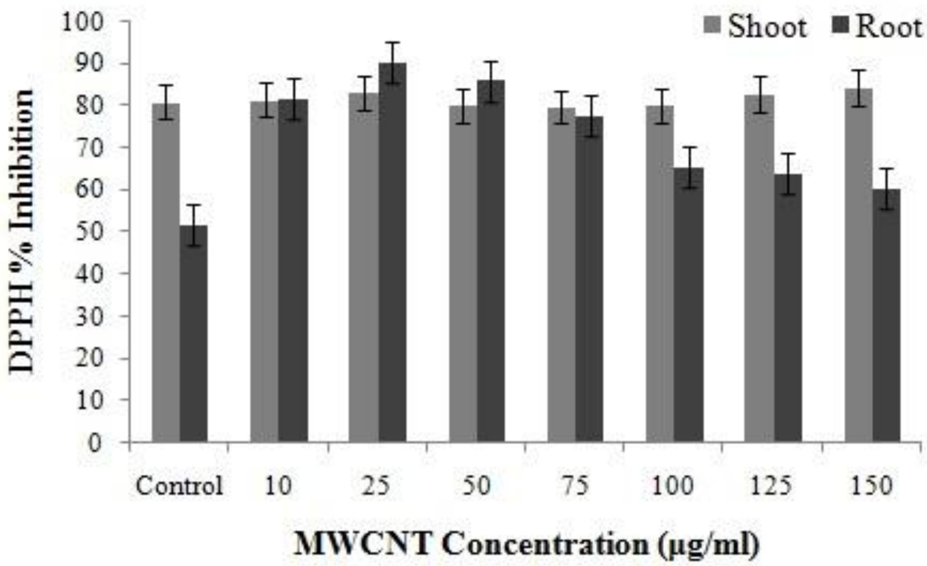

Fig. 4: Average radical scavenging activity of methanolic extract of PB 1121 rice variety which was treated with different concentration of MWCNT

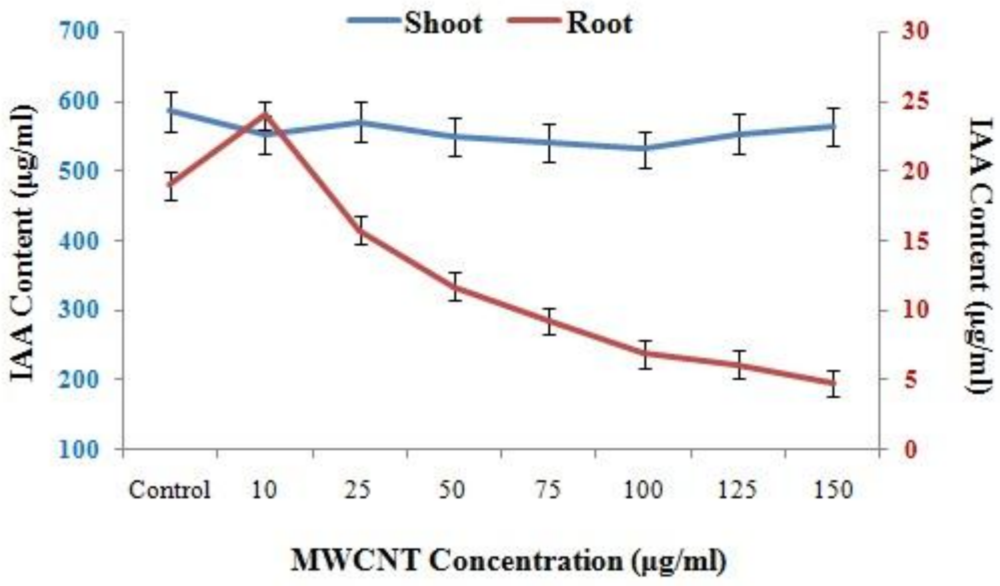

Fig. 5: Indole acetic acid (IAA) content in shoot and root of PB 1121 rice variety under the effect of different concentration of MWCNT

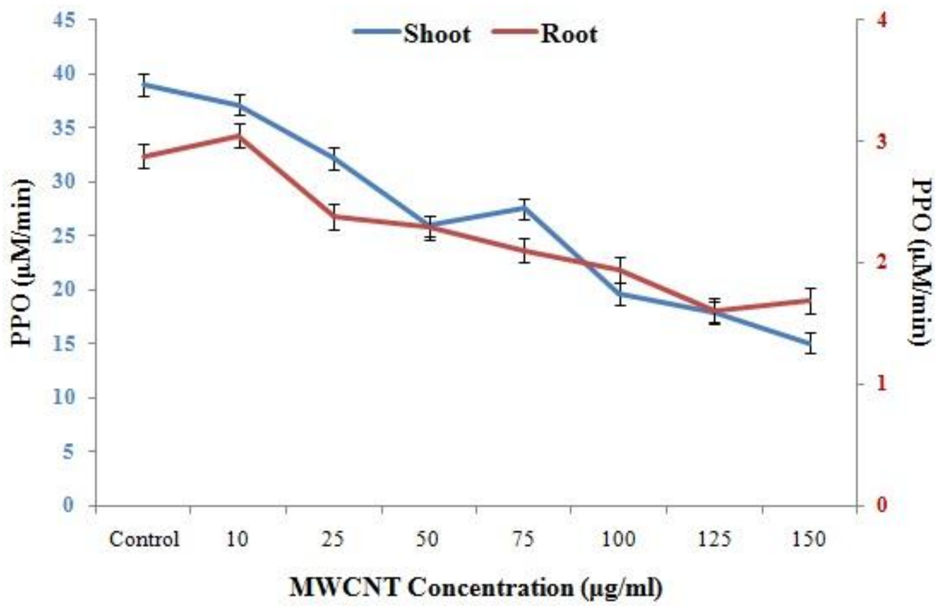

Fig. 6: Mean value of polyphenol oxidase (PPO) in shoot and root of PB 1121 rice variety which was treated with different concentration of MWCNT

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REFERENCES

1. Silva, G. A., Neuroscience nanotechnology: progress, opportunities and challenges, *Nat. Rev. Neurosci.* **7:** 65 (2006).
2. Ayodele, A. T., Valizadeh, A., Adabi, M., Esnaashari, S. S., Madani, F., Khosravani, M.,Adabi, M., Ultrasound nanobubbles and their applications as theranostic agents in cancer therapy: A review, *Biointerface Res. Appl. Chem.* **7:** 2253-2262 (2017).

3. Kaphle, A., Navya, P., Umapathi, A.,Daima, H. K., Nanomaterials for agriculture, food and environment: applications, toxicity and regulation, *Environ. Chem. Lett.* **16:** 43-58 (2018).
4. Hussain, C. M.,Mishra, A. K., *Nanotechnology in Environmental Science*, 2 (Vol. 1), John Wiley & Sons, 2018.
5. Teleanu, D. M., Chircov, C., Grumezescu, A. M.,Teleanu, R. I., Neurotoxicity of Nanomaterials: An Up-to-Date Overview, *Nanomaterials* **9:** 96 (2019).
6. Lyons, K., Scrinis, G.,Whelan, J., Nanotechnology, agriculture, and food, CRC Press, 146-169 (2011).
7. Prasad, R., Kumar, V.,Prasad, K. S., Nanotechnology in sustainable agriculture: present concerns and future aspects, *African J. Biotechnol.* **13:** 705-713 (2014).
8. Scott, N. R., Chen, H.,Cui, H., Nanotechnology applications and implications of agrochemicals toward sustainable agriculture and food systems, ACS Publications, 6451-6456 (2018).
9. Khan, Z., Khan, S. H., Ghouri, M. Z., Shahzadi, H., Arshad, S. F., Waheed, U., Firdous, S.,Ahmad, A., Nanotechnology: An Elixir to Life Sciences, *Preprints*, 2019020235 (2019).
10. Cassman, K. G., Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture, *Proc. Natl. Acad. Sci.* **96:** 5952-5959 (1999).
11. Boonyanitipong, P., Kumar, P., Kositsup, B., Baruah, S.,Dutta, J. in *International Conference on Environment and Bioscience*, 172-176 (2011).
12. Khodakovskaya, M. V., De Silva, K., Biris, A. S., Dervishi, E.,Villagarcia, H., Carbon nanotubes induce growth enhancement of tobacco cells, *ACS nano* **6:** 2128-2135 (2012).
13. Nair, R., Mohamed, M. S., Gao, W., Maekawa, T., Yoshida, Y., Ajayan, P. M.,Kumar, D. S., Effect of carbon nanomaterials on the germination and growth of rice plants, *J. Nanosci. Nanotechnol.* **12:** 2212-2220 (2012).
14. Adhikari, T., Kundu, S.,Rao, A. S., Impact of SiO2 and Mo nano particles on seed germination of rice (*Oryza sativa* L.), *Intl. J. Agri. Food Sci. Technol.* **4:** 809-816 (2013).
15. Lahiani, M. H., Dervishi, E., Chen, J., Nima, Z., Gaume, A., Biris, A. S., Khodakovskaya, M. V., Impact of carbon nanotube exposure to seeds of valuable crops, *ACS Appl. Mater. Interfaces* **5:** 7965-7973 (2013).
16. Rico, C. M., Hong, J., Morales, M. I., Zhao, L., Barrios, A. C., Zhang, J.-Y., Peralta-Videa, J. R.,Gardea-Torresdey, J. L., Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and in vivo fluorescence imaging, *Environ. Sci. Technol.* **47:** 5635-5642 (2013).
17. Almutairi, Z. M., Alharbi, A., Effect of silver nanoparticles on seed germination of crop plants, *J. Adv. Agric* **4:** 283-288 (2015).
18. Oloumi, H., Mousavi, E. A.,Nejad, R. M., Multi-Wall Carbon Nanotubes Effects on Plant Seedlings Growth and Cadmium/Lead Uptake In Vitro, *Russ. J. Plant Physiol.* **65:** 260-268 (2018).
19. Lövestam, G., Rauscher, H., Roebben, G., Klüttgen, B. S., Gibson, N., Putaud, J.-P., Stamm, H., Considerations on a definition of nanomaterial for regulatory purposes, *JRC. Ref. Rep.* 80004-80001 (2010).
20. Lin, P.-C., Lin, S., Wang, P. C., Sridhar, R., Techniques for physicochemical characterization of nanomaterials, *Biotechnol. Adv.* **32:** 711-726 (2014).
21. Khan, I., Saeed, K., Khan, I., Nanoparticles: Properties, applications and toxicities, *Arab. J. Chem.* (2017).
22. Han, X., Li, S., Peng, Z., Al-Yuobi, A.-R. O., Bashammakh, A. S. O.,Leblanc, R. M., Interactions between carbon nanomaterials and biomolecules, *J. Oleo Sci.* **65(4):** 365-365 (2016).
23. Lalwani, G., Patel, S. C., Sitharaman, B., Two-and three-dimensional all-carbon nanomaterial assemblies for tissue

engineering and regenerative medicine, *Ann. Biomed. Eng.* **44:** 2020-2035 (2016).

24. Husen, A., Siddiqi, K. S., Carbon and fullerene nanomaterials in plant system, *J. Nanobiotechnol.* **12:** 16 (2014).
25. Gatto, F., Bardi, G., Metallic Nanoparticles: General Research Approaches to Immunological Characterization, *Nanomaterials* **8:** 753 (2018).
26. Murphy, M. P., Holmgren, A., Larsson, N.-G., Halliwell, B., Chang, C. J., Kalyanaraman, B., Rhee, S. G., Thornalley, P. J., Partridge, L.,Gems, D., Unraveling the biological roles of reactive oxygen species, *Cell Metab.* **13:** 361-366 (2011).
27. Li, R., Jia, Z.,Trush, M. A., Defining ROS in biology and medicine, *Reactive oxygen species (Apex, NC)* **1:** 9 (2016).
28. Abdal Dayem, A., Hossain, M., Lee, S., Kim, K., Saha, S., Yang, G.-M., Choi, H.,Cho, S.-G., The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles, *Int. J. Mol. Sci.* **18:** 120 (2017).
29. Das, P., Nutan, K. K., Singla-Pareek, S. L.,Pareek, A., Understanding salinity responses and adopting 'omics-based'approaches to generate salinity tolerant cultivars of rice, *Front. Plant Sci.* **6:** 712 (2015).
30. Hoang, T., Tran, T., Nguyen, T., Williams, B., Wurm, P., Bellairs, S.,Mundree, S., Improvement of salinity stress tolerance in rice: challenges and opportunities, *Agronomy* **6:** 54 (2016).
31. Pareek, A., Sopory, S. K.,Bohnert, H., Abiotic stress adaptation in plants, Springer, 2009.
32. Mantri, N., Patade, V., Penna, S., Ford, R.,Pang, E., Abiotic stress responses in plants: present and future. In Abiotic stress responses in plants, Springer, New York, 1-19 (2012).
33. Contreras-Guzman, E., Strong III, F. C., Determination of total tocopherols in grains, grain products, and commercial oils, with only slight saponification, and by a new reaction with cupric ion, *J. Agric. Food Chem.* **30:** 1109-1112 (1982).
34. Gordon, S. A.,Weber, R. P., Colorimetric estimation of indoleacetic acid, *Plant Physiol.* **26:** 192 (1951).
35. Malik, C. P., Singh, M., A text manual plant enzymology and histo-enzymology, Navin Shandara, New Delhi, 66-71 (1980).
36. Nonogaki, H., Bassel, G. W., Bewley, J. D., Germination—still a mystery, *Plant Sci.* **179:** 574-581 (2010).
37. Salah, S. M., Yajing, G., Dongdong, C., Jie, L., Aamir, N., Qijuan, H., Weimin, H., Mingyu, N., Jin, H., Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress, *Sci. Rep.* **5:** 14278 (2015).
38. Upadhyaya, H., Shome, S., Sarma, R., Tewari, S., Bhattacharya, M. K., Panda, S. K., Green Synthesis, Characterization and Antibacterial Activity of ZnO Nanoparticles, *Am. J. Plant Sci.* **9:** 1279 (2018).
39. Shaw, A. K., Hossain, Z., Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings, *Chemosphere* **93:** 906-915 (2013).
40. Thuesombat, P., Hannongbua, S., Akasit, S.,Chadchawan, S., Effect of silver nanoparticles on rice (Oryza sativa L. cv. KDML 105) seed germination and seedling growth, *Ecotoxicol. Environ. Saf.* **104:** 302-309 (2014).
41. Mohanan, K., Mini, C., Relative contribution of rice tillers of different status towards yield, *Intl. J.Plant Breed. Genet.* **2:** 9-12 (2008).
42. Mohanan, K., Pavithran, K., Chronology of tiller emergence and tiller orientation in rice (Oryza sativa L.), *ORYZA-An Intl. J. Rice* **44:** 307-310 (2007).
43. Khush, G. S., New plant types of rice for increasing the genetic yield potential, *Rice Breed. Genet.* 99-108 (2000).
44. Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F.,Biris, A. S., Carbon nanotubes are able

to penetrate plant seed coat and dramatically affect seed germination and plant growth, *ACS nano* **3:** 3221-3227 (2009).

45. Cañas, J. E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M., Ambikapathi, R., Lee, E. H.,Olszyk, D., Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species, *Environ. Toxicol. Chem.* **27:** 1922-1931 (2008).
46. Wang, X., Han, H., Liu, X., Gu, X., Chen, K., Lu, D., Multi-walled carbon nanotubes can enhance root elongation of wheat (Triticum aestivum) plants, *J. Nanoparticle Res.* **14:** 841 (2012).
47. Tan, X.-m., Lin, C., Fugetsu, B., Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells, *Carbon* **47:** 3479-3487 (2009).
48. Mittler, R., ROS are good. *Trends Plant Sci.* **22:** 11-19 (2017).
49. Mhamdi, A.,Van Breusegem, F., Reactive oxygen species in plant development, *Development* **145:** dev164376 (2018).
50. Tsukagoshi, H., Control of root growth and development by reactive oxygen species, *Curr. Opin. Plant Biol.* **29:** 57-63 (2016).
51. Du, Y., Scheres, B., Lateral root formation and the multiple roles of auxin, *J. Exp. Bot.* **69:** 155-167 (2017).
52. Formentin, E., Barizza, E., Stevanato, P., Falda, M., Massa, F., Tarkowskà, D., Novák, O., Lo Schiavo, F., Fast Regulation of Hormone Metabolism Contributes to Salt Tolerance in Rice (Oryza sativa spp. Japonica, L.) by Inducing Specific Morpho-Physiological Responses, *Plants* **7:** 75 (2018).
53. Zhao, Y., Auxin biosynthesis and its role in plant development, *Annu. Rev. Plant Biol.* **61:** 49-64 (2010).
54. Pasternak, T., Potters, G., Caubergs, R.,Jansen, M. A., Complementary interactions between oxidative stress and auxins control plant growth responses at plant, organ, and cellular level, *J. Exp. Bot.* **56:** 1991-2001 (2005).
55. Steffens, J. C., Eitan, H.,Michelle, D. H., Polyphenol oxidase." In Genetic engineering of plant secondary metabolism, *Springer, Boston, MA,* 275-312 (1994).
56. Niknam, V., Razavi, N., Ebrahimzadeh, H., Sharifizadeh, B., Effect of NaCl on biomass, protein and proline contents, and antioxidant enzymes in seedlings and calli of two Trigonella species, *Biol. Plant.* **50:** 591-596 (2006).
57. Kuvalekar, A., Redkar, A., Gandhe, K., Harsulkar, A., Peroxidase and polyphenol oxidase activities in compatible host–pathogen interaction in Jasminum officinale and Uromyces hobsoni: insights into susceptibility of host, *N. Z. J. Bot.* **49:** 351-359 (2011).
58. Soffan, A., Alghamdi, S., Aldawood, A., Peroxidase and polyphenol oxidase activity in moderate resistant and susceptible Vicia faba induced by Aphis craccivora (Hemiptera: Aphididae) infestation, *J. Insect Sci.* **14:** 285 (2014).
59. Papaiah, S., Narasimha, G., Peroxidase and polyphenol oxidase activities in healthy and viral infected sunflower (Helianthus annuus L.) leaves, *Biotechnol.* **9:** 01-05 (2014).
60. Jia, H., Zhao, P., Wang, B., Tariq, P., Zhao, F., Zhao, M., Wang, Q., Yang, T.,Fang, J., Overexpression of polyphenol oxidase gene in strawberry fruit delays the fungus infection process, *Plant Mol. Biol. Rep.* **34:** 592-606 (2016).
61. Ali, R., Abbas, H., Response of salt stressed barley seedlings to phenylurea, *Plant Soil and Environment* **49:** 158-162 (2003).
62. Akhtar, W., Mahmoo, T., Response of rice polyphenol oxidase promoter to drought and salt stress, *Pak. J. Bot.* **49:** 21-23 (2017).