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Research Review

Applications of Carbon Nanotubes in Plant Growth and Development: A Review

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Abstract

A subsidiary discipline that arose as a combination of biotechnology and nano-science is of "nano-biotechnology", which involves wide-ranging applications of the physicochemical properties of nanostructures in the agricultural and biomedical domains. Nanotechnology has seen numerous breakthroughs and developments in a short course of time and given rise to newer branches of scientific research. Carbon derived nanomaterials, especially nanotubes (CNTs) have arisen as extremely promising nanostructures with a wide range of applications because of their unique properties. The thoughtful employment of CNTs, in the arena of plant development has resulted in an improvement in the growth parameters of diverse groups of plants. The uptake of carbon nanotubes (CNTs) influences the output of plants, potentially through interactions between the plant DNA and CNTs. The current review suggests the possibility of employing CNTs as a growth stimulating additive when administered in low doses, along with explaining the background of their occurrence and useful attributes. The review focusses on the potential of CNTs in transforming agricultural practices in the near future and providing sustainable solutions to some of the most serious problems plaguing plant growth and development. Finally, it emphasizes on the need for a detailed understanding of the molecular mechanisms which would pave the way for the use of these materials in agriculture, emerging as a novel technology.

***Key words*: Carbon nanotubes, Single walled CNTs, Multi-walled CNTs, Growth, Development, DNA uptake**

It is popularly believed that nanotechnology is a recent development, pioneered by scientists like Richard Feynman, however, its practice has been prevalent since a long time in the form of ancient nanomedicine of Indian origins, Lycurgus Cup of Rome in the 4th century, coloured dyes on monument windows etc. Although, the current scenario is vastly different from its starting point. This scientific domain utilizes formulations at the scale of a nanometre which is of the order of 10^{-9} m which in the form of tubular nanostructure form nanotubes. Their uniquely small size renders them with distinctive properties like high surface area to volume ratio, quantum confinement, super paramagnetism, chemical reactivity, increased bioavailability etc. that is responsible for their applications in multiple areas.

The area of carbon nanotubes or CNTs was initially discovered by Iijima [20], by means of transmission electron microscopy. These nanotubes, having two dimensions in the nanoscale are synthesized from a variety of starting substances like molybdenum, boron etc., but their most functionally diverse version is created from carbon. CNTs are concentric graphite cylinders closed at either end due to the presence of five-membered rings. Such nanotubes can be multi-walled (MWNTs) with a central tubule of nanometric diameter surrounded by graphite layers, or single-walled (SWNTs) having only the tubule and no graphite layers [47]. Nanotubes have been constructed with length to diameter ratio of up to 132,000,000:1; significantly larger than for any other material. The nanotubes range in length from a few tens of nm to several μm, and in outer diameter from about 2.5 to 30 nm [17]. These cylindrical carbon molecules have unusual properties, which are valuable for nanotechnology, electronics, optics and other fields material science and technology.

In recent years, utilization of carbon nanotubes has been investigated in numerous fields. Some of their popular uses are found in the electrical industry in devices like transistors, photovoltaic sensors, supercapacitors, conducting composites,

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and electrodes. Due to their enhanced electrical conductivity and linear geometry, CNTs have also proved to be ideal candidates for connections in molecular electronics. Additionally, the higher aspect ratio of CNTs makes them capable of achieving an optimum conductivity even at lower loadings, unlike other conventional conductive fillers [1]. Their high toughness-to-weight attributes also prove valuable in fuel cells that are where durability is essential for efficient transport. Furthermore, their ability to carry a remarkably high current density is utilized in manufacturing CNT-based displays which show a greater efficiency in comparison to traditional cathode ray tube displays. Biomedical industry also finds significant application of carbon nanotubes due to their high compatibility in the human body. They readily conjugate with various diagnostic and therapeutic agents. CNTs are also being used to transfer DNA, which can easily bond to them. Air and water purification industry has also successfully commercialized the use of CNTs [4], [52].

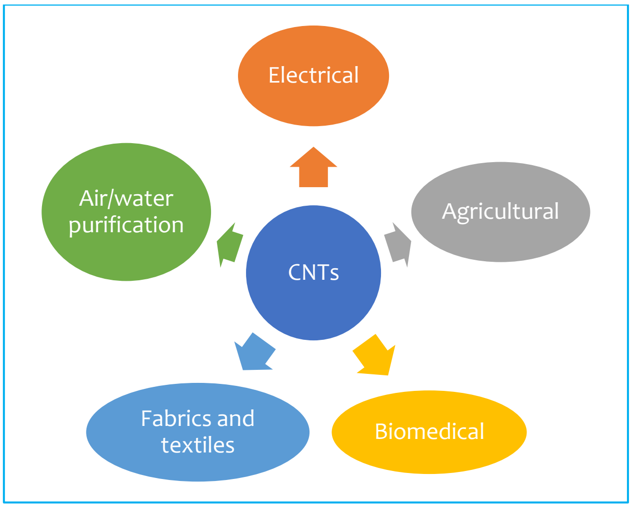

Fig 1 Applications of carbon nanotubes

Today, a number of complex challenges are being faced by the agricultural industry, which mainly include-management of biotic stresses, ensuring efficient use of water, reduction of environmental footprint of agriculture, optimal food production in changing climate, accommodation of bioenergy sources etc. The current research and development scenario depicts a pivotal role of nanotechnology in each of these areas. [49] Most importantly, an ever-increasing world population poses great challenges on the agricultural sector. These challenges can directly be overcome by a simultaneous increase in the yield of crops globally.

A. *Carbon nanotubes- Types and methods of synthesis*

Nano formulations tend to be smaller, faster and more energy efficient choices due to their ability to enter congested, confined spaces while maintaining advantageous properties. Their physical, mechanical, electrical, chemical and optical assets enhance their functionality and such nanomaterials can occur incidentally from natural sources like sea sprays, volcanic ash, mineral composites etc. or through human interventions based on requirement. One such engineered nanomaterial that is the focus of the current review is the carbon nanotube.

CNTs are made of starting material of carbon in the form of fullerenes or graphite with diameters falling in the range of nanometres. These formulations are extremely flexible and strenuous, increasing their potential for usage as a novel class of nanomaterials (Fig 2).

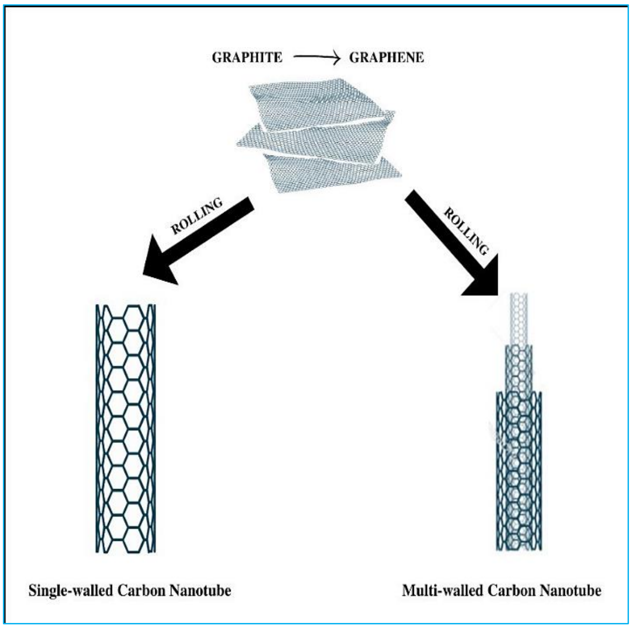

Fig 2 The structure of single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs)

Individual nanotubes naturally align themselves into "ropes" held together by Van-der-Waal's forces, more specifically pi-stacking. CNTs are known to occur in two principal configurations:

Table 1 Techniques for artificially synthesizing carbon nanotubes

S. No.	Methods of CNT Synthesis	Working Principle	Reference
1.	Carbon arc-discharge method	Potential difference between graphite rod anode and cathode results in migration of carbon particles towards cathode at low temperatures for CNT condensation. Transition metals like Ni, Fe, Co favour SWCNT formation. Suitable catalyst increases purity of CNT produced. Yield is 30-90%	[16], [25]
2.	Laser-ablation technique	Vaporization of graphite due to shooting at 1200 °C in a reaction furnace, which is then carried to cold collectors via He or Ar gas. Uniform SWCNTs produced due to Co, Fe and Ni catalysts. Helps is high yield (~70%) and accurate control. Easily tunable diameter of CNTs produced by this method	[30], [46]
3.	Chemical vapor deposition (CVD)	Carbon precursor (fossil-based or botanical hydrocarbon) along with a catalyst form CNTs by decomposition due to injected gas via heat and plasma at 550-1000 °C atm. Yield ranges between 20% to 100%	[19], [46]
4.	Flame synthesis method	Co-flow diffusion flame type. Catalysts: metal nitrate + TiO_2	[31], [46]

Single-walled carbon nanotubes (SWCNTs) can have diameters in the range 1-10 nm and possess only one layer of graphene. These require catalysts for synthesis and have high occurrence of defects during the process of functionalization. SWCNTs are comparatively less pure and their bulk synthesis is tedious because accurate proper control on growth and atmospheric condition. They are don't get accumulated inside the human body easily and can be readily characterized and evaluated. SWCNTs are known to be highly pliable with easy twisting possible.

Multi-walled carbon nanotubes (MWCNTs) have outer diameters which are quite large, depending on the number of multiple graphene layers. Inter-layer separation in multi-walled CNTs is 3.4A which is similar to that in graphite. The central tubule has a diameter of few nano-meters [47]. MWCNTs can be produced without involving catalysts and the probability of defects occurring during functionalization is minimized, however, they are difficult to improve after formation. They are comparatively purer than SWCNTs and have easier method of bulk synthesis. Their accumulation inside bodies is more and their complex multi-layered structure hinders characterization and evaluation, resulting in less flexible and pliable formulations

While CNTs are found occurring in nature [29], they can also be synthesized artificially using different techniques (Ref. Table 1) (Table 1) [46].

They exhibit unique electrical and thermal conductivity properties and are one of the most mechanically strong structures [56]. CNTs with certain surface modifications (called as 'functionalization), Involving interactions like hydrophobicity and adsorption are utilized to increase properties of CNTs like biocompatibility and hydrophilicity (Fig 3).

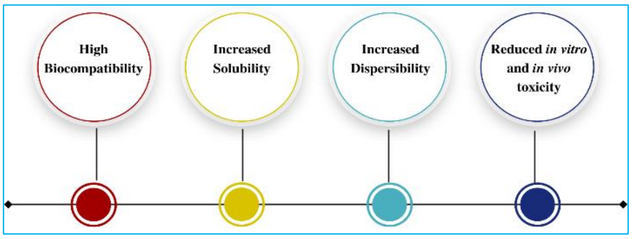

Fig 3 Advantages of using functionalized CNTs

B. *CNTs in the agricultural landscape*

There are a number of challenges being faced by the agricultural sector today, some of them being:

- Management of biotic stresses
- Increasing efficient use of water
- Reduction of environmental footprint of agriculture
- Food production in changing climate
- Accommodation of bioenergy sources
- Maintenance of abundant yields and safe food production

Given the present circumstances of ever-increasing population it is a great challenge to meet the increasing food and nutritional requirements. As a result, the amount of food crops that are being cultivated in the various parts of the world also needs to increase. However, increased and persistent use of chemical fertilizers not only increase the toxicity of the crop on which they are being used but they also render the soil infertile and increase the salinity of the soil leaving it unsuitable for further agricultural purposes. As such the need of the hour is a new "Green Revolution" minus the use of chemical fertilizers for a sustainable increase in food production. Therefore, the use of carbon nanotubes comes aptly into play now given the fact that carbon is already an inherent part of all the biomolecules which might prove to be something ground-breaking in the coming agricultural prospective. The use of carbon nanotubes in plant cultures have shown an increase in the growth parameters in various plant systems. This indicates that the use of carbon nanotubes in enhancing plant growth is an exciting prospect that needs to be explored in detail.

The current research and development scenario depicts a pivotal role of nanotechnology in this area. Nanotechnology envisages revolutionizing the agriculture sector and food systems [46]. An exponential growth in nanotube research in the past decade, has led to the emergence of new technologies with novel and exquisite applications of nanotechnology in agriculture sector and food systems. Studies show the applications of nanotechnology in the improvement of nutritional value of food, assessment of nanoscale nutrient delivery systems, harvesting energy and its conversion, livestock reproduction enhancement, sensing technology and enhancement of plant growth that primarily includes the increase in the root and shoot lengths of various agriculturally important crops.

A. Effect of CNTs on plant growth and development

The use of CNTs has a positive impact on plant growth and development, especially during the early stages of growth. *Brassica juncae* and *Phaseolus mungo* were evaluated for the effects of MWCNTs on germination and seedling growth. Both of them showed 100% germination, indicating non-hazardous nature of MWCNTs. *B. Juncae* seedlings showed dramatic increase in vegetative biomass at different CNT concentrations, specifically a 1.5-fold increase at 20µg/ml CNT concentration; and root length was inspired up to 138%, 202% and 135% with CNT concentrations of 10µg/ml, 20 µg/ml and 40 µg/ml respectively, thus suggesting beneficial effects of CNTs on *B. Juncae* seedlings [13], [39].

The finding that carbon nanotubes can penetrate the thick seed coat and support water uptake inside seed was based on the interaction of different concentration of CNTs (10- 40µg/ml) with the tomato seedling showing positive effect on their germination and growth rate. Tomato seeds when grown on MS medium supplemented with CNTs showed accelerated seed germination time and seedling growth. There was also a 2.5-fold increase in vegetative biomass in seedlings along with enhanced water uptake [24]. MWCNTs at 40µg/ml concentration, showed maximum effect [40]. MWCNTs enhance water uptake by activating the gene expression of gene encoding water channel protein (LeAqp1). This was shown in a study with tomato plant where water channel protein was activated on exposure to functionalized MWCNTs. It was also exhibited that more negatively charged CNTs induce seed germination and plant growth significantly [59]. An experiment was carried out showing remarkable change in phenotype of tomato plant with significant increase in plant height, and two times more flowers and fruits as compared to the control at tested concentrations of 50 and 200 µg/mL of MWCNTs [24]. There was a significant increase in total number of fruits by 65% and also, higher fresh and dry weight with 15.5% increase in average fresh weight per fruit and 22.7% increase in average dry weight per fruit [37].

Based on the two-delivery system for multiwalled carbon nanotubes (MWCNTs) i.e., sterile agar medium and deposition on seed surface, in three important agricultural plants (barley, maize and soyabean) showed positive affect of MWCNTs, as early seed germination and activation of growth in exposed seedling was observed. Analytical techniques such as Raman spectroscopy and transmission electron microscopy (TEM) were used to prove the ability of MWCNTs to penetrate the seed coat by detecting nanotube agglomerates. It was shown that expression of seed-located water channel genes (aquaporins) increases significantly by using reverse transcription polymerase chain reaction (RT – PCR) thus supporting the hypothesis about the involvement of carbon nanotubes in low concentration in regulating the activity of water channels in plants exposed to MWCNTs [27]. Barley, soybean and corn when grown hydroponically with MWCNTs treatment exhibited positive phenotypic changes along with enhanced photosynthesis. Corn and barley showed increased shoot length and higher number of leaves [28]. The hypothesis that effectiveness of CNTs may depend on both plant species as well as on their distribution on the testa and root surface was supported by SEM analysis in the experiment conducted to study the effect of CNTs on germination and seedling growth of tomato, turnip, onion and radish.

Effect of SWCNTs on *Zea mays* roots varies with tissue type. SWCNT treatment increases seminal root growth whereas it decreases the root hair growth [63]. MWCNTs are seen to enhance the germination of maize at low concentrations by improving water delivery [57]. CNTs exhibit both stimulatory and inhibitory effects on the growth of bean sprouts at a concentration of 100 and 1200 µg/mL respectively. Thus, it is inhibitory in an increasing dose-dependent manner along with altering pH of water even at low concentrations [32]. SWCNTs enhance length and growth of roots and stem in *Ficus carica* in low concentrations [11].

Correlation between the activation of cell growth and up-regulation of the genes involved in cell division and cell wall formation as well as water transport was found via a study on tobacco cell culture when exposed to MWCNTs. Tobacco aquaporin (NtPIP1) gene and protein expression significantly increases in cells exposed to MWCNTs compared to control cells or the cells exposed to activated carbon which stimulated cell growth only at low concentration; along with an up-regulation of marker genes for cell division (CycB) and cell wall extension (NtLRX1) [23].

When mesophyll cells of *Arabidopsis thaliana* were exposed to single walled carbon nanotubes, it showed dual phase regulation. In the low concentration that is less than or equal to 50 µg/ ml, SWCNTs stimulated plant cell to grow out trichome clusters on their surface. On the other hand, at concentration more than 50µg/ml, SWCNTs exhibit toxic effects such as increasing generation of reactive oxygen species (ROS), changing green leaves into yellow, inducing changes to protoplast morphology and inducing protoplast cell necrosis and apoptosis [66].

MWCNTs promote seedling growth of hybrid Bt-cotton both *in vitro* and *in vivo*. Seeds when grown in exposure to MWCNTs *in vitro* showed highest root and shoot length at a concentration of 60µg/ml; whereas *in vivo*, a significant increase was observed in plant height and number of leaves along with 2.8-fold increase in number of bolls per plant and 1.85-fold increase in boll size at a concentration of 100µg/ml, thereby increasing the yield of Bt-cotton plant [42].

Aseptic culturing of leaf segments of *Satureja khuzestanica* with different MWCNTs' concentrations showed significant improvement in growth of calli with increasing MWCNTs concentration, highest being around 50 µg/ml and decreasing at high concentrations. Moreover, at certain concentrations, MWCNTs could promote *in vitro* biosynthesis of valuable secondary metabolites and anti-oxidant drugs [14].

In *Ricinus communis* L. (castor plant), MWCNTs enhanced percentage of seed germination maximally at concentrations 50 and 100µg/ml. The length of radical and seedling, number of rootlets, seedling vigour, wet weight and dry weight were all found to be maximum at 100µg/ml concentration of MWCNTs [10].

A medicinal multipurpose plant, *Dodonaea viscose* L. (hopbush) showed a dramatic improvement in seed germination percentage, mean germination time, length of stem and root as well as their respective fresh and dry weight on exposure to MWCNTs. In increased drought stress conditions, MWCNTs significantly enhanced seed germination and other growth parameters at 50 and 10 mg/L concentrations [65]. Likewise, *Hibiscus sabdariffa* L., MWCNTs exhibited positive results in growth parameters. A significant increase was also found in bioactive constituents of water extracts from calyces. Untreated plants produced only four active compounds. On treatment with MWCNTs at a concentration of 500 mg/L, the plants produced 12 active compounds and at a concentration of 1000mg/L, the plants produced 41 active compounds [2].

Bioenergy crops namely sorghum and switchgrass showed positive results on treatment with CNTs in seed germination. In switchgrass, there was a 20% and 19.6% increase in germination at concentration 50µg/mL and 200µg/mL respectively. In sorghum, there was an increase of 21.89% in germination at 50µg/mL CNT. Under salt stress, CNTs can reduce the suppression of seed germination, root and shoot length of switchgrass seeds [45]. Seed priming of wheat by MWCNTs influences its growth positively by causing early seed germination in addition to enhanced grain number, biomass, vascular bundle size and stomatal density. Larger root length and denser root hair were also observed, facilitating increased water and mineral uptake, thus resulting in faster growth and higher yield of wheat plant [22].

Differences in plant responses to functionalized and non-functionalized CNTs were studied on six crop species (cabbage, carrot, cucumber, lettuce, onion and tomato). Root elongation was enhanced in onion and cucumber by CNTs; whereas it was inhibited in tomato and lettuce by CNTs and functionalized CNTs (fCNTs) respectively. No significant effect was observed in cabbage and carrots on exposure to either nanotube [6]. In contrast, based on the study of *Zea mays* L. exposed to different concentrations of SWCNTs spiked soil for 40 days showed that the uptake of different concentration of SWCNTs (0, 10, 100 mg/kg) was independent of their functionalities (non-functionalized, OH functionalized or surfactant stabilized) as in all the cases, SWCNTs accumulated mostly in roots (0 – 24 µg/g), with minimal accumulation in stem and leaves as studied via microwave induced heating method. Similarly in different study on corn to determine plant physiological stress by measuring photosynthetic rate in plant, exposed to 10 mg/kg SWCNTs under optimum growth or water deficit conditions showed no significant difference between control and SWCNTs treatment [7].

In a research study to investigate the effect of carboxylic acid functionalized water-soluble carbon nanotubes on *Cicer arietinum* plant it was observed that with increased concentration of fCNTs, an increase in growth occurred, as in a control setup only 2 out of 5 plants survived while the plant grown in 100 µL and 200 µL solution showed 3 out of 5 and 4 out of 5 survival rate respectively. An increase in the rate of water absorption can be considered as one of the reasons of this positive response since plant grown in distilled water showed least water absorption while plants grown with CNTs absorbed more water. Root length was also found to be highest in plants treated with CNTs [58].

Carboxylic acid functionalized MWCNTs promote plant growth and biomass production significantly, along with accelerating the process of germination in wheat, maize, peanut and garlic at a concentration of 50µg/ml [53]. –OH functionalized CNTs increased germination percentage, germination index, plant biomass, root and shoot length, and number of leaves substantially in wheat and brinjal [26], [38].

Carbon nanotubes at a concentration of 100µg/ml promote rice seed germination and root growth and inhibit at higher concentrations [21]. Both SWCNTs and MWCNTs promote rice seedling growth. On exposure to CNTs, primary root length increased five-fold and crown root length increased by twice. An astonishing effect was observed in lateral roots whose length increased by 80% on being exposed to SWCNTs and by 700% on being exposed to MWCNTs for 7 days [62]. Use of f-MWCNTs in urea fertilizer for rice induces higher plant growth and increases number of panicles, grain yield, total dry weight and nitrogen absorption in comparison to pristine MWCNTs [64].

In an experiment to determine the effect of exposure of oxidized multiwalled carbon nanotubes (o-MWCNTs) with a length of 50 – 630 nm on development and physiology of wheat plant, it was found that o-MWCNTs significantly promote cell elongation and dehydrogenase activity resulting in faster root growth and higher content of biomass production. On examining the wheat plant exposed to different concentration of o-MWCNTs (10 to 160µg/ml) for 7 days an increase in vegetative biomass and faster root growth was observed but seed germination and stem length did not show any difference as compared with control. Analysis by transmission electron microscopy showed 1.4-fold increase in cell length of root zone and significant concentration dependent increase in the dehydrogenase activity [60].

Taunit, engineered nanoparticle containing multiwalled carbon nanotubes was found to enhance the growth of *Onobrychis arenaria* roots and stems and also augment the peroxidase activity in the seedling [51].

Interaction between commercial MWCNTs and 100mM NaCl treated broccoli plant, results in biochemical changes and promotes water uptake by retaining Na+ ions in the root. MWCNTs also induced changes in rigidity, permeability, lipid composition of root plasma as well as enhanced aquaporin transduction in salt stressed plants thus improving water uptake and transport [35].

SWCNTs at low concentrations could also alleviate drought-stress induced reduction in seed germination and plant growth through promoting water uptake and triggering plant's defence system. This conclusion was reached by a study on *Hyoscyamus niger* wherein its seeds were exposed to different concentrations of SWCNTs resulting in increase of rate of germination by 22.8% and root length by 26.2% [18].

Heavy metal stress of Cd and Pb on *Brassica napus* seedlings can be alleviated by treatment with MWCNTs. MWCNTs treated seedlings show significantly higher root and shoot lengths which is otherwise inhibited with Pb and Cd treatments alone. These heavy metal treatments also lowered the fresh weight, dry weight and chlorophyll concentrations which was reversed by MWCNT treatment [43], [44].

The bacterial growth and root development of plant in rhizobium-legume symbiosis system of *Lotus japonicas* and *Mesorhizobium loti* was inhibited by SWCNTs under non-symbiotic condition. MWCNTs, on the other hand, enhanced the number of nodules in plants by 39% at 14 days post-inoculation (dpi) and 41% at 28 dpi at 100µg/mL concentration. Increase is nodulation by MWCNTs was correlated with and increased expression of *NIN* gene that regulates nodule development by up to 2-fold. Biological N fixation of nodules was also enhanced by 10% at 100µg/mL of MWCNTs [67]. Since *rhizobium*-legume symbiosis system plays a critical role in nitrogen cycle balance in agriculture, this study may stimulate more research on use of CNTs in agriculture from this perspective.

MWCNTs promoted the growth of maize plant, its same concentration inhibited the growth of soybean. Transpiration of water and dry biomass was found to have increased in maize, on exposure to MWCNTs. In addition, the uptake and translocation of these MWCNTs clearly exhibited cellular, charge, and size selectivity in maize and soybean, which could be important properties for nano transporters [68].

B. Nano-particulate uptake and transport in plants

As seen in the previous section, due to the exceptional physical, chemical, and mechanical capabilities of nanotubes, they have proven to have an impact on plant growth and development parameters. As nanoparticles have small diameters, they can pass through the cell through their apoplastic route [41]. MWCNTs can activate the aquaporin genes which translate into water channel proteins that facilitate the transport of water in plants [36], [48]. Additionally, a high level of CNT functionalization results in a marked reduction of its harmful effects on plant cells. MWCNTs, on the other hand, have been discovered to have penetrating properties that increased the germination and growth rates of tomato seeds without exhibiting toxicity. Short functionalized MWCNTs (f-MWCNTs) were distributed in a size-dependent manner inside cell organelles, according to experiments done on the *Catharanthus roseus* plant. While the shorter ones were seen inside the nucleus, plastids, and vacuoles, the longer ones were predominantly located inside subcellular structures like the endoplasmic reticulum and mitochondria. Additionally, it was found that these MWCNTs did not enter the cell through endocytosis but rather directly penetrated the plasma membrane [50].

Confocal fluorescence micrographs have shown that SWCNT isothiocyanate and SWCNT/DNA conjugates are taken up by cells, indicating that SWCNTs have a lot of potential as nano transporters for walled plant cells. Additionally, it has been proposed that SWCNTs might convey various payloads to various organelles within plant cells. It has been demonstrated that CNTs can go through plant cell walls and cell membranes [33].

C. Carbon nanomaterials in plant genetic transformation: A novel approach

Gene transfer is an important part of genetic engineering-based plant improvement. Several methods have been used in the past for this purpose. Nanomaterials for the delivery of biomolecules and are being successfully used in animal cells. However, the study on plant systems is at its infancy. However, they seem to be a promising area of research in the near future [61]. Nowadays, there is significant interest in the prospect of employing carbon nanotubes as nanocarriers to carry genetic material inside a cell. In one study on *Nicotiana tabacum* low concentrations of both SWCNTs and MWCNTs with plasmid construct were used to genetically transform the protoplast, and high concentrations were utilized to genetically alter the callus and leaf explant. The DNA delivery in protoplast and walled plant cells was demonstrated by SWCNTs-based Nano carriers, whereas MWCNTs were only able to alter protoplast due to the cellulose wall's inhibitory effects on their entry into the cells [5], [15].

Carbon nanotubes can move through cellular membranes directly or by endocytosis. By creating electrostatic interactions, arginine functionalization increases the solubility of CNTs and facilitates the adsorption of DNA (GFP-expressing plasmid). Additionally, it has been noted that polyarginine may function as an SV40-like nuclear localization signal (NLS), causing cargo to be transferred to the nucleus of tobacco intact root cells. Western blotting analysis and fluorescence microscopy were used to demonstrate the effectiveness of this gene delivery [5], [15]. Efficient plasmid DNA delivery into intact plants of several non-model plant species (arugula, wheat, and cotton) using functionalized high-aspect-ratio carbon nanotube (CNT) nanoparticles (NPs), resulting in high protein expression levels without transgene integration has been reported [8]. Although, CNTs can bypass different cellular barriers and deliver biomolecules into living cells. However, their use in plants is limited by due to the presence of a cellulosic cell wall. It has been reported that CNT with immobilized cellulase can serve as an efficient DNA delivery system for plant cells [12].

It has been reported that a simple and efficient carbon nanotube-based Nano-carrier delivers multiple plasmids into different tissues of monocotyledonous crops simultaneously [34]. CNT-mediated plasmid DNA delivery into rice has been reported recently, indicating that CNTs facilitate plasmid DNA delivery in rice leaf and embryo tissues, resulting in transient GFP, YFP, and GUS expression [9].

Challenges and future perspectives

Nano-biotechnology, a rapidly expanding area of study has varied applications in the fields of bioengineering, agriculture, and medicine. Due to the rising demand for agricultural products, it is crucial to identify alternatives to current agricultural methods in order to lessen the burden on the environment and the use of pollutants like pesticides, herbicides, insecticides, and fertilizers that are released into the environment as a result of agricultural use. It is imperative that agriculture become more sustainable. In this context, using modest quantities of carbon nanotubes to boost crop development and yield presents a workable alternative. Gene transfer is an important part of genetic engineering-based plant improvement. *In vitro* plant transformation and regeneration presents bottleneck because of the lengthy process of establishing a robust tissue culture protocol. *In planta* transformation, directly leading to transformed plants may bypass tissue culture, but efficient and successful *in planta* delivery and transformation remains a challenge. The use of nanocarriers to transport DNA into plant cells offers an attractive solution and a growing area of research. Carbon nanotubes can enter plant cells in a size, concentration, and solubility dependent way, causing translational and metabolic alterations that are then seen to improve plant growth and yield. CNTs, therefore are a suitable alternative to promote agriculture sustainably by improving plant growth and development as has been reported by numerous studies.

Despite the many advantages of CNT application and use, there are several barriers to the successful and widespread use of CNTs in plant systems. The use of CNTs at high concentrations is known to induce phytotoxicity in plants by promoting oxidative stress and probably changing the genome expression in plants [54], [55]. However, the future potential and benefits of using CNTs for improvement in plant growth and development far outweigh the potential negative impacts. The Therefore, more investigation is needed before they may be used in agricultural crops.

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LITERATURE CITED

1. Abdalla S, Al-Marzouki F, Al-Ghamdi AA, Abdel-Daiem A. 2015. Different technical applications of carbon nanotubes. *Nanoscale Research Letters* 10(1): 1-10.
2. Al-Rekaby LS. 2018. Influence of multiwalled carbon nanotubes and biostimulators on growth and content of bioactive constituents of karkade (*Hibiscus sabdariffa* L.). *Journal of Botany* 1: 1-11.
3. Annu A, Bhattacharya B, Singh PK, Shukla PK, Rhee HW. 2017. Carbon nanotube using spray pyrolysis: Recent scenario. *Journal of Alloys and Compounds* 691: 970-982.
4. Ates M, Eker AA, Eker B. 2017. Carbon nanotube-based nanocomposites and their applications. *Journal of Adhesion Science and Technology* 31(18): 1977-1997.
5. Burlaka OM, Pirko YV, Yemets AI, Blume YB. 2015. Plant genetic transformation using carbon nanotubes for DNA delivery. *Cytology and Genetics* 49(6): 349-357.
6. Cañas JE, Long M, Nations S, Vadan R, Dai L, Luo M, Ambikapathi R, Lee EH, Olszyk D. 2008. Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and Chemistry: An International Journal* 27(9): 1922-1931.
7. Cano AM, Kohl K, Deleon S, Payton P, Irin F, Saed M, Shah SA, Green MJ, Cañas-Carrell JE. 2016. Determination of uptake, accumulation, and stress effects in corn (*Zea mays* L.) grown in single-wall carbon nanotube contaminated soil. *Chemosphere* 152: 117-122.
8. Demirer GS, Zhang H, Goh NS, González-Grandío E, Landry MP. 2019. Carbon nanotube–mediated DNA delivery without transgene integration in intact plants. *Nature Protocols* 14(10): 2954-2971.
9. Dunbar T, Tsakirpaloglou N, Septiningsih EM, Thomson MJ. 2022. Carbon nanotube-mediated plasmid DNA delivery in rice leaves and seeds. *International Journal of Molecular Sciences* 23(8): 4081.
10. Fathi Z, Nejad RAK, Mahmoodzadeh H, Satari TN. 2017. Investigating of a wide range of concentrations of multi-walled carbon nanotubes on germination and growth of castor seeds (*Ricinus communis* L.). *Journal of Plant Protection Research*.
11. Flores D, Chaves JS, Chacón R, Schmidt A. 2013. A novel technique using SWCNTs to enhanced development and root growth of fig plants (*Ficus carica*). *In*: Tech*nical Proceedings of the NSTI Nanotechnology Conference and Expo (NSTI-Nanotech'*13. 3: 167-170.
12. Fouad M, Kaji N, Jabasini M, Tokeshi M, Baba Y. 2008. Nanotechnology meets plant biotechnology: Carbon nanotubes deliver DNA and incorporate into the plant cell structure. *In*: *12th International Conference on Miniaturized Systems for Chemistry and Life Sciences*, San Diego, California, USA. pp 12-16.
13. Ghodake G, Seo YD, Park D, Lee DS. 2010. Phytotoxicity of carbon nanotubes assessed by *Brassica juncea* and *Phaseolus*

mungo. *Journal of Nanoelectronics and Optoelectronics* 5(2): 157-160.
14. Ghorbanpour M, Hadian J. 2015. Multi-walled carbon nanotubes stimulate callus induction, secondary metabolites biosynthesis and antioxidant capacity in medicinal plant Satureja khuzestanica grown in vitro. *Carbon* 94: 749-759.
15. Golestanipour A, Nikkhah M, Aalami A, Hosseinkhani S. 2018. Gene delivery to tobacco root cells with single-walled carbon nanotubes and cell-penetrating fusogenic peptides. *Molecular Biotechnology* 60: 863-878.
16. Gulotty R, Castellino M, Jagdale P, Tagliaferro A, Balandin AA. 2013. Effects of functionalization on thermal properties of single-wall and multi-wall carbon nanotube–polymer nanocomposites. *ACS Nano* 7(6): 5114-5121.
17. Harris PJ, Harris PJF. 2009. *Carbon Nanotube Science: Synthesis, Properties and Applications*. Cambridge university press.
18. Hatami M, Hadian J, Ghorbanpour M. 2017. Mechanisms underlying toxicity and stimulatory role of single-walled carbon nanotubes in *Hyoscyamus niger* during drought stress simulated by polyethylene glycol. *Journal of Hazardous Materials* 324: 306-320.
19. Hofmann S, Ducati C, Robertson J, Kleinsorge B. 2003. Low-temperature growth of carbon nanotubes by plasma-enhanced chemical vapor deposition. *Applied Physics Letters* 83(1): 135-137.
20. Iijima S. 1991. Helical microtubules of graphitic carbon. *Nature* 354(6348): 56-58.
21. Jiang Y, Hua Z, Zhao Y, Liu Q, Wang F, Zhang Q. 2014. The effect of carbon nanotubes on rice seed germination and root growth. *In*: *Proceedings of the 2012 International Conference on Applied Biotechnology* (ICAB 2012). Springer Berlin Heidelberg. 2: 1207-1212.
22. Joshi A, Kaur S, Dharamvir K, Nayyar H, Verma G. 2018. Multi-walled carbon nanotubes applied through seed-priming influence early germination, root hair, growth and yield of bread wheat (*Triticum aestivum* L.). *Journal of the Science of Food and Agriculture* 98(8): 3148-3160.
23. Khodakovskaya MV, De Silva K, Biris AS, Dervishi E, Villagarcia H. 2012. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano* 6(3): 2128-2135.
24. Khodakovskaya MV, Kim BS, Kim JN, Alimohammadi M, Dervishi E, Mustafa T, Cernigla CE. 2013. Carbon nanotubes as plant growth regulators: Effects on tomato growth, reproductive system, and soil microbial community. *Small* 9(1): 115-123.
25. Kim HH, Kim HJ. 2006. The preparation of carbon nanotubes by dc arc discharge using a carbon cathode coated with catalyst. *Materials Science and Engineering*: B 130(1/3): 73-80.
26. Kothari-Chhajer A, Krishna K, Mukherjee AK, Rattanpal T, Dhirayan G, Tanwar A, Monika AM, Aggarwal A, Wadhawan S. 2018. Growth stimulation in wheat and brinjal by impact of water-soluble carbon nanotubes. *DU Jr. Underg Res. Innova.* 4(1): 14-25.
27. Lahiani MH, Dervishi E, Chen J, Nima Z, Gaume A, Biris AS, Khodakovskaya MV. 2013. Impact of carbon nanotube exposure to seeds of valuable crops. *ACS Applied Materials and Interfaces* 5(16): 7965-7973.
28. Lahiani MH, Nima ZA, Villagarcia H, Biris AS, Khodakovskaya MV. 2017. Assessment of effects of the long-term exposure of agricultural crops to carbon nanotubes. *Journal of Agricultural and Food Chemistry* 66(26): 6654-6662.
29. Lara-Romero J, Campos-García J, Dasgupta-Schubert N, Borjas-García S, Tiwari DK, Paraguay-Delgado F, Jiménez-Sandoval, S, Alonso-Nuñez G, Gómez-Romero M, Lindig-Cisneros R, De la Cruz HR. 2017. Biological effects of carbon nanotubes generated in forest wildfire ecosystems rich in resinous trees on native plants. *Peer Journal* 5: p.e3658.
30. Lebedkin S, Schweiss P, Renker B, Malik S, Hennrich F, Neumaier M, Stoermer C, Kappes MM. 2002. Single-wall carbon nanotubes with diameters approaching 6 nm obtained by laser vaporization. *Carbon* 40(3): 417-423.
31. Lee CJ, Lyu SC, Kim HW, Park CY, Yang CW. 2002. Large-scale production of aligned carbon nanotubes by the vapor phase growth method. *Chemical Physics Letters* 359(1/2): 109-114.
32. Li X, Zhou Z, Lu D, Dong X, Xu M, Wei L, Zhang Y. 2014. The effect of pristine carbon-based nanomaterial on the growth of green gram sprouts and pH of water. *Nanoscale Research Letters* 9: 1-6.
33. Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, Fang X. 2009. Carbon nanotubes as molecular transporters for walled plant cells. *Nano Letters* 9(3): 1007-1010.
34. Liu X, Kong W, Xiao N, Huang L, Zhang Y, Chen W, Mo B, Yu Y. 2023. A simple and efficient carbon nanotube-based nanocarriers simultaneously delivers multiple plasmids into diverse mature tissues of monocotyledonous crops. *Science China Life Sciences* 66: 1701-1704.
35. Martínez-Ballesta M, Zapata L, Chalbi N, Carvajal M. 2016. Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. *Journal of Nanobiotechnology* 14(1): 1-14.
36. Maurel C. 2007. Plant aquaporins: novel functions and regulation properties. *FEBS Letters* 581(12): 2227-2236.
37. McGehee DL, Lahiani MH, Irin F, Green MJ, Khodakovskaya MV. 2017. Multiwalled carbon nanotubes dramatically affect the fruit metabolome of exposed tomato plants. *ACS Applied Materials and Interfaces* 9(38): 32430-32435.
38. Miralles P, Johnson E, Church TL, Harris AT. 2012. Multiwalled carbon nanotubes in alfalfa and wheat: toxicology and uptake. *Journal of the Royal Society Interface* 9(77): 3514-3527.
39. Mondal A, Basu R, Das S, Nandy P. 2011. Beneficial role of carbon nanotubes on mustard plant growth: an agricultural prospect. *Journal of Nanoparticle Research* 13: 4519-4528.
40. Morla S, Rao CR, Chakrapani R. 2011. Factors affecting seed germination and seedling growth of tomato plants cultured in vitro conditions. *Journal of Chemical, Biological and Physical Sciences* 1(2): 328.
41. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. 2010. Nanoparticulate material delivery to plants. *Plant Science* 179(3): 154-163.
42. Nalwade AR, Neharkar SB. 2013. Carbon nanotubes enhance the growth and yield of hybrid Bt cotton Var. ACH-177-2. *International Journal of Advanced Science and Technology* 3: 840-846.
43. Oloumi H, Mousavi EA, Mohammadinejad R. 2014. Multi-walled carbon nanotubes enhance Cd2+ and Pb2+ uptake by canola seedlings. Multi-walled carbon nanotubes enhance Cd2+ and Pb2+ uptake by canola seedlings. *Agrochimica* LVIII(2): 91-102.
44. Oloumi H, Mousavi EA, Nejad RM. 2018. Multi-wall carbon nanotubes effects on plant seedlings growth and cadmium/lead

uptake in vitro. *Russian Journal of Plant Physiology* 65: 260-268.
45. Pandey K, Lahiani MH, Hicks VK, Hudson MK, Green MJ, Khodakovskaya M. 2018. Effects of carbon-based nanomaterials on seed germination, biomass accumulation and salt stress response of bioenergy crops. *PloS One* 13(8): e0202274.
46. Patel DK, Kim HB, Dutta SD, Ganguly K, Lim KT. 2020. Carbon nanotubes-based nanomaterials and their agricultural and biotechnological applications. *Materials* 13(7): 1679.
47. Rao CN, Müller A, Cheetham K. 2004. *The Chemistry of Nanomaterials: Synthesis, Properties and Applications*. Weinheim: Wiley-VCH Verlag, GmbH and Co., KGaA.
48. Safdar M, Kim W, Park S, Gwon Y, Kim YO, Kim J. 2022. Engineering plants with carbon nanotubes: a sustainable agriculture approach. *Journal of Nanobiotechnology* 20(1): 1-30.
49. Scott N, Chen H. 2012. Nanoscale science and engineering for agriculture and food systems. *Industrial Biotechnology* 8(6): 340-343.
50. Serag MF, Kaji N, Gaillard C, Okamoto Y, Terasaka K, Jabasini M, Tokeshi M, Mizukami H, Bianco A, Baba Y. 2011. Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. *ACS Nano* 5(1): 493-499.
51. Smirnova EA, Gusev AA, Zaitseva ON, Lazareva EM, Onishchenko GE, Kuznetsova EV, Tkachev AG, Feofanov AV, Kirpichnikov MP. 2011. Multi-walled carbon nanotubes penetrate into plant cells and affect the growth of *Onobrychis arenaria* seedlings. *Acta Naturae* (англоязычная версия) 31(8): 99-106.
52. Smith BW, Luzzi DE. 2004. *Carbon Nanotubes*. Introduction to Nanoscale Science and Technology. pp 137-182.
53. Srivastava A, Rao DP. 2014. Enhancement of seed germination and plant growth of wheat, maize, peanut and garlic using multiwalled carbon nanotubes. *Eur. Chem. Bulletin* 3(5): 502-504.
54. Tan XM, Fugetsu B. 2007. Multi-walled carbon nanotubes interact with cultured rice cells: evidence of a self-defense response. *Journal of Biomedical Nanotechnology* 3(3): 285-288.
55. Tan XM, Lin C, Fugetsu B. 2009. Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells. *Carbon* 47(15): 3479-3487.
56. Terrones M. 2003. Science and technology of the twenty-first century: synthesis, properties, and applications of carbon nanotubes. *Annual Review of Materials Research* 33(1): 419-501.
57.Tiwari DK, Dasgupta-Schubert N, Villaseñor Cendejas LM, Villegas J, Carreto Montoya L, Borjas García SE. 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.
58. Tripathi S, Sonkar SK, Sarkar S. 2011. Growth stimulation of gram (*Cicer arietinum*) plant by water soluble carbon nanotubes. *Nanoscale* 3(3): 1176-1181.
59. Villagarcia H, Dervishi E, de Silva K, Biris AS, Khodakovskaya MV. 2012. Bioresponse to nanotubes: Surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants (Small 15/2012). *Small* 8(15): 2327-2327.
60. Wang X, Han H, Liu X, Gu X, Chen K, Lu D. 2012. Multi-walled carbon nanotubes can enhance root elongation of wheat (*Triticum aestivum*) plants. *Journal of Nanoparticle Research* 14: 1-10.
61. Yadav S, Jat SK, Bhattacharya J, Sharma MK. 2023. Nanotechnology mediated gene transfer in plants: a novel approach. *In*: *Nanotechnology in Agriculture and Agroecosystems*. Elsevier. pp 141-168.
62. Yan S, Zhang H, Huang Y, Tan J, Wang P, Wang Y, Hou H, Huang J, Li L. 2016. Single-wall and multi-wall carbon nanotubes promote rice root growth by eliciting the similar molecular pathways and epigenetic regulation. *Iet Nanobiotechnology* 10(4): 222-229.
63. Yan S, Zhao L, Li H, Zhang Q, Tan J, Huang M, He S, Li L. 2013. Single-walled carbon nanotubes selectively influence maize root tissue development accompanied by the change in the related gene expression. *Journal of Hazardous Materials* 246: 110-118.
64. Yatim NM, Shaaban A, Dimin MF, Yusof F, Abd Razak J. 2018. Effect of functionalised and non-functionalised carbon nanotubes-urea fertilizer on the growth of paddy. *Tropical Life Sciences Research* 29(1): 17.
65. Yousefi S, Kartoolinejad D, Naghdi R. 2017. Effects of priming with multi-walled carbon nanotubes on seed physiological characteristics of Hopbush (*Dodonaea viscosa* L.) under drought stress. *International Journal of Environmental Studies* 74(4): 528-539.
66. Yuan H, Hu S, Huang P, Song H, Wang K, Ruan J, He R, Cui D. 2011. Single walled carbon nanotubes exhibit dual-phase regulation to exposed Arabidopsis mesophyll cells. *Nanoscale Res Letters* 6: 1-9.
67. Yuan Z, Zhang Z, Wang X, Li L, Cai K, Han H. 2017. Novel impacts of functionalized multi-walled carbon nanotubes in plants: promotion of nodulation and nitrogenase activity in the rhizobium-legume system. *Nanoscale* 9(28): 9921-9937.
68. Zhai G, Gutowski SM, Walters KS, Yan B, Schnoor JL. 2015. Charge, size, and cellular selectivity for multiwall carbon nanotubes by maize and soybean. *Environmental Science and Technology* 49(12): 7380-7390.