



RESEARCH ARTICLE

Multi-walled carbon nanotubes at 50 µg/mL enhance germination, growth, leaf chlorophyll and seed phosphate and potassium uptake in Indonesian mung bean

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Abstract

A seed priming experiment using Indonesian mung bean cultivar Vina-2 was conducted to evaluate the effects of multi-walled carbon nanotubes (MWCNTs) concentration and seed soaking duration on germination, growth, leaf chlorophyll contents, yield components and phosphorous (P) and potassium (K) uptake in harvested seeds. The MWCNT concentrations were 0, 25, 50 and 75 µg/mL, while the seed-soaking durations were 12, 18 and 24 hr. The results revealed that a concentration of 50 µg/mL of MWCNTs, regardless of the soaking duration, accelerated the length of germinated seeds (19.18 cm) in a week, however the germination percentage (97–100 %) was not statistically significant. Seedlings treated with this concentration exhibited the best germination uniformity index ($T_{75} - T_{25}$) when soaked for 12 or 18 hr. During the post-germination, regardless of soaking duration, it significantly increased seedling length (19.18 cm) after one week; however, the germination percentage (97–100 %) was not significantly affected. Moreover, harvested seeds demonstrated higher P and K contents, irrespective of soaking duration. However, soaking for 24 hr reduced the seed K content. Soaking seeds for 24 hr without MWCNTs treatment (0 µg/mL) resulted in the lowest number of seeds per pod (5.67). In contrast, soaking for 12 or 18 hr under the same control condition produced higher seed numbers (9.67–10.00) and these results were comparable to those of the best treatment. In conclusion, seed priming with 50 µg/mL MWCNTs does not only improve the growth during germination and post-germination in Indonesian mung bean but also enhances the leaf chlorophyll a and b contents as well as seed-P and -K uptake.

Keywords: carbon nanotubes; germination; seed priming; seed soaking; yield

Introduction

Mung bean is one of the three most commonly consumed legume crops in Indonesians often consume after soybeans and peanuts (1). Besides being a source of carbohydrates, mung beans are also a source of protein and minerals (2, 3). Mung bean seeds contain 24.2 % protein, 1.3 % fat and 60.4 % carbohydrates (4). As Indonesia's population increases, people's demand for mung beans also increases. However, a significant decline of the mung bean cultivated area by 25 % was recorded during 2016–17 (5). The average productivity of mung bean in Indonesia in 2019 was 1.079 Mt/ha (6). However, using improved technologies such as certified seeds, fertiliser application and optimum population density, could improve the productivities by 13.88, 25.00 and 3.00 %, which were tested on the Indonesian elite cultivar Vina-1 (1.23 Mt/ha), Sriti (1.35 Mt/ha) and Murai (1.11 Mt/ha) respectively (7). Therefore, the substantial production is required to be filled by increasing productivity. One of them is by agronomic practice.

Carbon nanotubes (CNTs) are an emerging nanotechnology with promising applications in agriculture. One type of carbon nanostructure, namely CNT, is a one-dimensional carbon structure with unique thermal, optical and mechanical properties. Carbon nanotubes can interact with biomolecules due to their nanoscale size at morphological, cellular, genetic and molecular levels. Carbon nanotubes can be classified into two categories, namely single-wall carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) (8). The physical properties of CNTs, such as length, diameter and solubility, are essential parameters that control the penetration rate of nanoparticles in plants (9). Carbon nanotube technology continues to evolve, particularly with respect to its agricultural applications. Increasing plant growth and productivity can be done by treating plant seeds with multi-walled CNTs. However, its use is relatively new and needs to be explored further. Numerous studies have shown that using nanotechnology has a beneficial effect on seed germination. A previous study found that CNTs could penetrate tomato seed coats, thereby accelerating seed germination and seedling growth (10).

The faster germination and growth compared to control is because CNTs speed up the mechanism of water absorption by penetrating the seed coat (11). Other studies found that the use of MWCNT at the concentrations of 50, 100 and 200 µg/mL in agar media increased the germination percentage of barley and soybean by 50 % (12). No toxic effects were observed when MWCNT was applied at the concentrations of 20, 200, 1000 and 2000 mg/L on hydroponic culture (13). The application of MWCNT at the concentrations of 20 and 50 µg/mL improved the elongation of roots and shoot of wheat, maize and white onion. The higher growth and biomass yield were attained when the concentration of MWCNTs-COOH was set at 50 µg/mL (14).

Multi-wall CNTs can penetrate cell walls and membranes to transmit into the cytoplasm. A previous study found that the growth rate of mustard greens was the highest in treatment with multi-wall CNTs with a concentration of 50 µg/mL (15). At this concentration, the multi-wall CNTs penetrated the seed coat and allowed the absorption of water needed for germination so that it became more effective. Using multi-wall CNTs in legume plants can increase the number of nodules, increasing nitrogenase activity in rhizobium-plant interactions (16). However, further studies are needed to clarify whether synergistic effects occur during germination and post-germination, particularly in yield and nutrient absorption of legumes such as mung bean. Given the limited research, investigations into the use of multi-walled CNTs in agriculture, especially for mung bean seed priming, remain essential. The study aimed to increase the growth of mung bean seeds during germination and subsequent development, as well as the yield components, leaf chlorophyll and the absorption of phosphorous (P) and potassium (K) in harvested seeds and to search the optimum MWCNT concentration and seed soaking duration.

Materials and Methods

Site of study

The research was conducted from March to July 2023 in a greenhouse at the Faculty of Animal and Agriculture Sciences, Universitas Diponegoro, Indonesia. The materials and preparation of the MWCNTs application were obtained and conducted at the Nanotechnology Integrated Laboratory, Universitas Diponegoro. The study consisted of two separate experiments. The first experiment evaluated seed germination using germination test measure. The second one focused on the post-germination, vegetative growth, yield and physiology during subsequent development.

Experimental design

The variety of Indonesian mung bean used was *Vigna radiata* (L.) cultivar Vina-2. The experimental design used 4 × 3 factorial completely randomized design (CRD) with 3 replications with a total of 36 experimental units. Each experimental unit consisted of 10 seeds. The first factor was MWCNT concentration, comprising four concentrations. They were 0 (Z0/control), 25 (Z1), 50 (Z2) and 75 µg/mL (Z3). The second factor was duration of seed soaking, comprising three levels. They were 12 (A1), 18 (A2) and 24 hr (A3). Multi-walled carbon nanotubes (MWCNTs) were dispersed in distilled water and applied on the soaked mung bean seeds. The seeds used had no wrinkles and were uniform size.

The MWCNT were produced at the Nanotechnology Integrated Laboratory, Universitas Diponegoro at a pyrolysis

temperature of 900 °C. A single MWCNT had a diameter of 20–50 nm. The inner tube wall had a diameter of 5–10 nm. The composition of carbon was 99.64 % atoms and 0.36 % iron (Fe). The MWCNTs material has hydroxyl and carboxylic groups resulting from functionalisation with nitric acid on the tube wall (15).

Experiment 1: Germination test

The germination test used a media of rolled paper covered by plastic. The seeds were spread in germination paper sheets with regular space among the seeds, approximately 1 cm from each other. The paper was then rolled up. The germination paper test material filled with mung bean seeds was then placed in a seed germinator, with the air temperature set at 24 °C. At the age of 7 days after germination (DAG), observation on seed viability, seed length and germination uniformity index (GUI) of the germinated seed were carried out. The GUI was calculated based on 75 and 25 % germinations, following the method developed by Jalink and Van Der Schoor (17). The formula is described below.

$$GUI = T_{75} - T_{25}$$

Note:

T_{75} : The time (in days) from sowing to 75 % of the maximum germination

T_{25} : The time (in days) from sowing to 25 % of the maximum germination

Experiment 2: Planting and observation

Treated seeds were planted in polybags. The planting medium consisted of 6 kg rice husks and black mineral soil mixed at a 1 : 2 ratio (v/v). Each polybag measured 35 cm in length and 35 cm in diameter. The polybags were arranged with a square spacing of 20 × 20 cm. Two seedlings were planted per polybag and each experimental unit comprised three polybags. Maintenance and watering were carried out every morning and evening. Fertilizers in the form of urea, SP-36 and potassium chloride (KCl) were applied to the soil at dosages of 54, 108 and 60 kg/ha respectively. Seedling emergence was recorded when the seedlings first appeared above the soil surface. Observation on plant length and number of leaves was carried out at 60 days after planting (DAP). Plants were harvested at 60 DAP. In that period, the colour of the pod was black and dried. The harvested pod were sun-dried for 2–3 days to facilitate a separation of the seeds from the pod.

The dried shoot biomass, yield components, including number of seeds per pod and seed dry weight per plant and harvest index were measured. The dry weight of shoot biomass was calculated after the fresh shoot biomass was oven-dried at 80 °C for 48 hr. Physiological traits were also measured. Seed-P was measured using a spectrophotometer and seed-K was measured using a flame photometer. Chlorophyll a, b and total chlorophyll were measured using the Amon method. Chlorophyll calculation formula is described below.

Chlorophyll a = 1.07 (OD at 663 nm wavelength) - 0,094 (OD at 644 nm wavelength)

Chlorophyll b = 1.77 (OD at 644 nm wavelength) - 0,28 (OD at 663 nm wavelength)

Chlorophyll total = 0.79 (OD at 663 nm wavelength) - 1,076 (OD at 644 nm wavelength)

(OD: optical density)

Statistical analysis

The data obtained from the factorial experiment were using two-way analysis of variance (ANOVA). The post-hoc test was carried by Duncan multiple ranged test (DMRT) at 5 % significant rate if the Anova results of F-test > F-table. An analytical software of Statistix ver. 9.1, build on 11 January 2013, was used.

Results

Effects on germination in experiment 1

Treatments of MWCNTs concentration, soaking duration and their interaction did not significantly affect the germination percentage (Table 1). It was similar across treatments, ranging from 97–100 %. However, the length of germinated seeds at 7 DAG was affected by MWCNTs. Higher concentration of MWCNTs resulted in higher length of the germinated seeds, but the optimum concentration was achieved at 50 µg/mL. Interestingly, the soaking duration significantly affected the GUI when interacted with MWCNTs concentration. The soaking duration alone did not deliver significant effect. Treatments of a concentration of 50 µg/mL and soaking durations of 12 and 18 hr yielded the most uniform seedling growth. The difference between 25 and 75 % germination was 0.67, indicating that seedlings required less than one day to achieve uniform germination. The growth of the germinated seeds at 7 DAG treated with various MWCNTs concentrations is visually shown in Fig. 1.

Effects on germination period and phenotypic performance in experiment 2

Plant height and number of leaves were significantly affected by MWCNTs concentration (Table 2). However, the effect of soaking duration and their interaction were not. The optimum concentration of 50 µg/mL resulted in the maximum plant length and number of leaves at 60.7 cm and 19 respectively. Fig. 2 presents the growth appearance at varying MWCNT concentrations.

Table 1. Effects of multi-walled carbon nanotubes (MWCNTs) and seed soaking duration on seed viability, length and growth uniformity of the germinated seed at 7 days after germination (DAG) in experiment 1

Treatment	Germination percentage (%)	Length of germinated seeds (cm)	Germination uniformity index (GUI)
MWCNT			
Z0 (0 µg/L)	98 ± 1.92 ^{ns}	15.56 ± 0.19 ^c	1.78 ± 0.67 ^a
Z1 (25 µg/L)	100 ± 0.00	17.02 ± 0.74 ^b	2.11 ± 0.53 ^a
Z2 (50 µg/L)	100 ± 0.00	19.18 ± 0.55 ^a	0.78 ± 0.26 ^b
Z3 (75 µg/L)	97 ± 5.09	17.44 ± 0.53 ^b	1.78 ± 0.67 ^a
Soaking duration			
A1 (12 hr)	98 ± 1.92 ^{ns}	17.42 ± 1.33 ^{ns}	1.42 ± 0.63 ^{ns}
A2 (18 hr)	100 ± 0.00	17.41 ± 1.72	1.33 ± 0.62
A3 (24 hr)	97 ± 4.71	17.07 ± 1.61	2.08 ± 0.79
Interacting factors			
Z0A1	97 ± 5.77 ^{ns}	15.58 ± 0.64 ^{ns}	1.67 ± 0.58 ^a
Z0A2	100 ± 0.00	15.73 ± 0.40	1.33 ± 0.58 ^b
Z0A3	97 ± 5.77	15.36 ± 0.26	2.33 ± 0.58 ^a
Z1A1	100 ± 0.00	17.60 ± 0.78	1.67 ± 0.58 ^a
Z1A2	100 ± 0.00	17.27 ± 0.51	2.00 ± 0.00 ^a
Z1A3	100 ± 0.00	16.19 ± 0.44	2.67 ± 0.58 ^a
Z2A1	100 ± 0.00	18.78 ± 1.08	0.67 ± 0.29 ^d
Z2A2	100 ± 0.00	19.81 ± 0.46	0.67 ± 0.29 ^d
Z2A3	100 ± 0.00	18.94 ± 1.15	1.00 ± 0.00 ^c
Z3A1	97 ± 5.77	17.70 ± 1.87	1.67 ± 0.58 ^a
Z3A2	100 ± 0.00	16.83 ± 2.67	1.33 ± 0.58 ^{bc}
Z3A3	90 ± 11.55	17.80 ± 0.85	2.33 ± 0.58 ^a

n = 10. Means followed by the same alphabetical order are not significantly different by DMRT at 5 %. ns: not significant; ±: standard deviation; MWCNT: multi-walled carbon nanotubes.

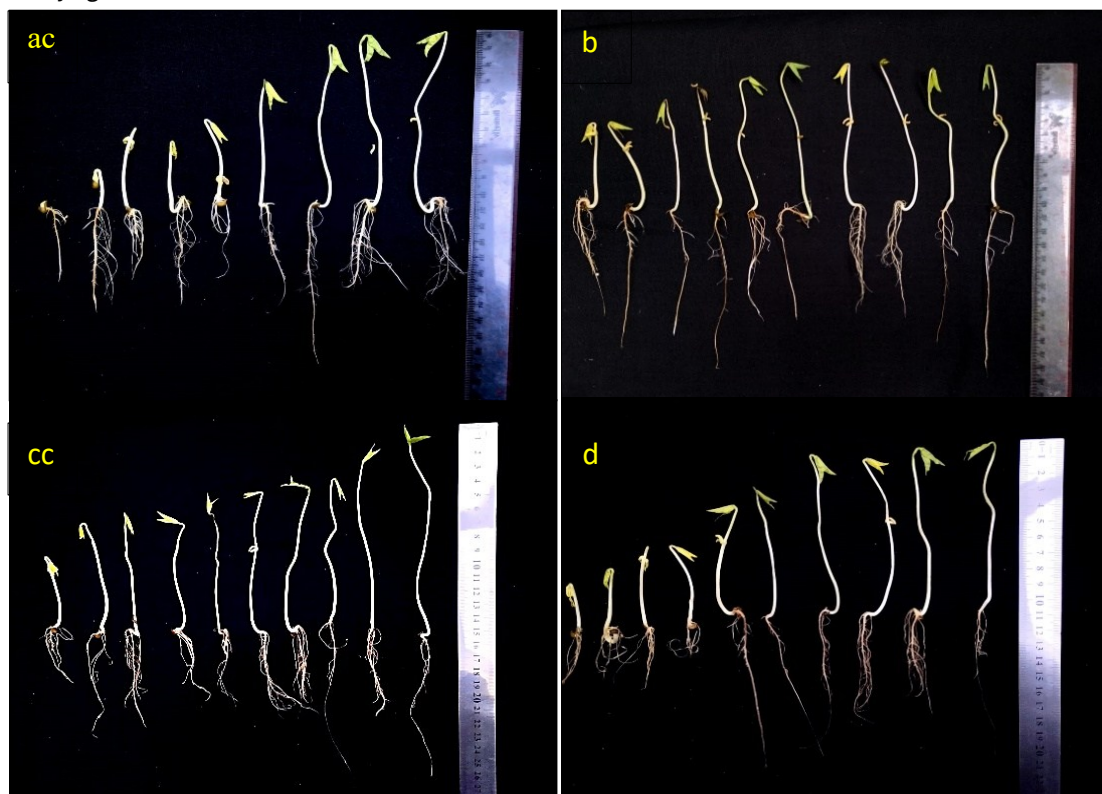


Fig. 1. A growth appearance of the germinated seeds treated with non-functionalised multi-walled carbon nanotubes (MWCNTs) solution (0 µg/mL) (a) and functionalised MWCNT solutions with concentrations of 25 (b), 50 (c) and 75 µg/mL (d) at 7 days after germination (DAG).

Table 2. Effects of multi-walled carbon nanotubes (MWCNTs) and seed soaking duration on yield components in experiment 2

Treatment	Plant height (cm)	Number of leaves
MWCNT		
Z0 (0 µg/mL)	50.44 ± 7.42 ^b	13 ± 1.00 ^c
Z1 (25 µg/mL)	51.14 ± 1.52 ^b	14 ± 0.58 ^b
Z2 (50 µg/mL)	60.67 ± 3.66 ^a	19 ± 3.86 ^a
Z3 (75 µg/mL)	49.63 ± 6.39 ^b	13 ± 1.73 ^c
Soaking duration		
A1 (12 hr)	54.70 ± 2.27 ^{ns}	15 ± 5.25 ^{ns}
A2 (18 hr)	54.21 ± 6.50	14 ± 1.71
A3 (24 hr)	50.00 ± 9.45	14 ± 1.56
Interacting factors		
Z0A1	52.67 ± 5.51 ^{ns}	12 ± 1.73 ^{ns}
Z0A2	56.50 ± 4.50	14 ± 0.00
Z0A3	42.17 ± 4.31	13 ± 1.73
Z1A1	52.82 ± 5.02	14 ± 0.00
Z1A2	50.75 ± 5.41	15 ± 1.73
Z1A3	49.85 ± 6.47	14 ± 3.00
Z2A1	56.50 ± 6.29	23 ± 3.89
Z2A2	62.17 ± 5.39	16 ± 1.73
Z2A3	63.33 ± 7.91	17 ± 1.53
Z3A1	56.83 ± 11.16	12 ± 1.73
Z3A2	47.42 ± 3.38	12 ± 1.73
Z3A3	44.63 ± 7.38	15 ± 1.73

Means followed by the same alphabetical letters are not significantly different by DMRT at 5 %. ns: not significant. ±: Standard deviation; MWCNT: multi-walled carbon nanotubes

Effects on shoot biomass, yield components and harvest index in experiment 2

Factor of MWCNTs concentration alone significantly affected the shoot biomass (Table 3). A concentration of 50 µg/mL produced the highest biomass (5.67 g). The number of seeds per pods was significantly affected by both concentration and soaking duration, as well as by their interaction. Soaking the seeds for 24 hr without MWCNTs treatment resulted in the lowest number of seeds (5.67). Meanwhile, soaking durations of 12 and 18 hr under the same control condition produced higher seed numbers (9.67–10.00) and these results were comparable to those of the best treatments. Seed dry weight and harvest index were not significantly affected. Seed dry weight and harvest index ranged from 5.00–8.05 g/plant and 51.07–63.69 respectively.

Table 3. Effects of multi-walled carbon nanotubes (MWCNTs) and seed soaking duration on shoot biomass, yield components and harvest index at 60 days after planting (DAP) in experiment 2

Treatment	Dried shoot biomass (g)	Number of seeds/pods	Seed dry weight (g/plant)	Harvest index
MWCNT				
Z0 (0 µg/mL)	3.50 ± 0.44 ^c	8.44 ± 2.41 ^b	5.42 ± 0.99 ^{ns}	59.84 ± 3.95 ^{ns}
Z1 (25 µg/mL)	4.72 ± 0.63 ^{ab}	11.00 ± 0.00 ^a	6.72 ± 0.28	59.12 ± 4.02
Z2 (50 µg/mL)	5.67 ± 0.29 ^a	10.33 ± 0.88 ^a	6.63 ± 1.31	53.73 ± 4.73
Z3 (75 µg/mL)	4.17 ± 0.29 ^{bc}	8.78 ± 1.50 ^b	5.72 ± 0.72	57.44 ± 3.68
Soaking duration				
A1 (12 hr)	4.63 ± 0.96 ^{ns}	9.67 ± 0.98 ^{ab}	6.00 ± 0.66 ^{ns}	56.78 ± 5.31 ^{ns}
A2 (18 hr)	4.63 ± 0.75	10.58 ± 0.50 ^a	6.75 ± 0.88	59.26 ± 2.31
A3 (24 hr)	4.29 ± 1.20	8.67 ± 2.60 ^b	5.61 ± 1.17	56.55 ± 5.28
Interacting factors				
Z0A1	3.33 ± 0.29 ^{ns}	9.67 ± 0.58 ^a	5.87 ± 0.60 ^{ns}	63.68 ± 4.12 ^{ns}
Z0A2	4.00 ± 0.87	10.00 ± 1.73 ^a	6.09 ± 1.36	60.05 ± 9.89
Z0A3	3.17 ± 0.29	5.67 ± 0.58 ^c	4.29 ± 1.86	55.78 ± 10.80
Z1A1	5.17 ± 0.76	11.00 ± 0.00 ^a	6.95 ± 0.27	57.51 ± 3.58
Z1A2	5.00 ± 0.50	11.00 ± 0.00 ^a	6.41 ± 0.68	56.15 ± 4.25
Z1A3	4.00 ± 1.32	11.00 ± 0.00 ^a	6.81 ± 0.17	63.69 ± 7.89
Z2A1	5.50 ± 2.18	9.33 ± 1.53 ^{ab}	5.47 ± 0.10	51.07 ± 9.43
Z2A2	5.50 ± 1.00	11.00 ± 0.00 ^a	8.05 ± 1.99	59.19 ± 2.53
Z2A3	6.00 ± 0.87	10.67 ± 0.58 ^a	6.36 ± 1.74	50.93 ± 6.03
Z3A1	4.50 ± 0.87	8.67 ± 2.52 ^{ab}	5.70 ± 2.10	54.85 ± 5.50
Z3A2	4.00 ± 1.32	10.33 ± 0.58 ^a	6.44 ± 1.57	61.65 ± 9.36
Z3A3	4.00 ± 1.32	7.33 ± 3.21 ^b	5.00 ± 1.38	55.82 ± 1.27

Means followed by the same alphabetical letters are not significantly different by DMRT at 5 %. ns: not significant. ±: Standard deviation; MWCNT: multi-walled carbon nanotubes.

**Fig. 2.** Growth appearance of mung bean at 60 days after planting (DAP) treated with non-functionalised (0 µg/mL) (a) and functionalised multi-walled carbon nanotubes (MWCNT) solutions with concentrations of 25 (b), 50 (c) and 75 µg/mL (d).

Effects on leaf chlorophyll, seed-phosphate and potassium uptakes in experiment 2

The effects of MWCNTs concentration and seed soaking duration on leaf chlorophyll a, b and total contents, as well as seed-P and -K contents are shown in Table 4. Both leaf chlorophyll a and b contents were significantly influenced by MWCNTs concentration, but total chlorophyll was not. Application of MWCNTs delivered higher content of chlorophyll a (1.78–1.93 µg/L) than control (1.50 µg/L), while the highest content of chlorophyll b (1.01 µg/L) was achieved by a concentration of 50 µg/mL. Interaction of MWCNTs concentration and soaking duration significantly affected the uptakes of P and K in harvested seeds. A concentration of 50 µg/mL was the most effective treatment for increasing the seed-P content (0.97–1.03 mg/g), irrespective of soaking duration. However, the seed-K content was significantly enhanced when seeds were soaked for 12 and 18 hr (6.93–7.04 mg/g). Soaking for 24 hr reduced its content significantly (6.59 mg/g). Nevertheless, the highest seed-K content was observed at 25 µg/mL with a 24 hr soaking duration (7.56 mg/g), but this result was not statistically different from treatments with 50 µg/mL at 12 and 18 hr.

Discussion

The insignificant effect across MWCNTs concentration, soaking duration and these treatment interaction on germination percentage was likely attributable to the quality of the seeds used. The consistent germination percentage across treatments indicates that seed quality was well-maintained. The supply of energy and moisture content inside the seeds was still quite sufficient to support metabolism and germination.

The effect of MWCNTs concentration was clearly detected on the length of germinated seeds and GUI. An optimum concentration of 50 µg/mL promoted seedling growth and yielded the best performance in germination uniformity. Enhanced growth during the early germination stage subsequently influenced plant performance during the vegetative phase, particularly shoot biomass. The improved shoot biomass was most likely attributable to the improved vegetative performance of the treated plants, particularly in terms of plant height and number of leaves.

The MWCNTs are known to enhance water absorption in seeds by penetrating seed cell walls. MWCNTs can penetrate seed coat, thereby facilitating water uptake (14, 18). A recent study showed that CNTs influence the expression of aquaporin-related genes in soybean (*Glycine max* (L.) Merr.), leading to increased water transport capacity and higher seedling survival rates under water-stress conditions (19). Carbon nanotubes have been shown to act as nanocarriers, delivering essential nutrients directly to germinating seeds, thereby improving early-stage metabolism and energy availability. In addition to water transport, CNTs also modulated seed metabolic activity by increasing ATP production and enhancing enzymatic activities related to starch hydrolysis (20). This resulted in significant increases in seedling biomass and root-stem elongation. The absorbed water facilitates the breakdown of food reserves, which serve as an energy source for seedling growth, enabling rapid and synchronous germination. The use of MWCNTs, interacting with the seed coat, creates nanochannels that facilitate increased water and nutrient uptake, which are essential for the initial seed germination process (21). The water imbibition enhances seed metabolism, resulting in improved germination (22). Water plays a crucial role in the imbibition process, the synthesis of indole-3-

Table 4. Effects of multi-walled carbon nanotubes (MWCNTs) and seed soaking duration on contents of chlorophyll a, b and total as well as contents of seed-phosphorous and potassium in experiment 2

Treatment	Chlorophyll a (µg/L)	Chlorophyll b (µg/L)	Chlorophyll total (µg/L)	Seed-P (mg/g)	Seed-K (mg/g)
MWCNT					
Z0 (0 µg/mL)	1.50 ± 0.20 ^b	0.78 ± 0.23 ^b	0.46 ± 0.08 ^{ns}	0.74 ± 0.05 ^b	5.8 ± 0.08 ^b
Z1 (25 µg/mL)	1.78 ± 0.13 ^a	0.93 ± 0.09 ^{ab}	0.57 ± 0.01	0.73 ± 0.15 ^b	6.6 ± 0.08 ^a
Z2 (50 µg/mL)	1.93 ± 0.09 ^a	1.01 ± 0.15 ^a	0.60 ± 0.04	1.18 ± 0.25 ^a	6.9 ± 0.02 ^a
Z3 (75 µg/mL)	1.88 ± 0.07 ^a	0.88 ± 0.15 ^{ab}	0.59 ± 0.05	0.69 ± 0.02 ^c	5.2 ± 0.03 ^c
Soaking duration					
A1 (12 hr)	1.68 ± 0.31 ^{ns}	0.88 ± 0.20 ^{ns}	0.55 ± 0.04 ^{ns}	0.83 ± 0.16 ^{ns}	5.9 ± 0.08 ^c
A2 (18 hr)	1.83 ± 0.15	0.95 ± 0.19	0.56 ± 0.13	0.78 ± 0.16	6.3 ± 0.07 ^a
A3 (24 hr)	1.82 ± 0.16	0.88 ± 0.13	0.56 ± 0.02	0.89 ± 0.38	6.2 ± 0.11 ^b
Interacting factors					
Z0A1	1.27 ± 0.42 ^{ns}	0.60 ± 0.20 ^{ns}	0.49 ± 0.11 ^{ns}	0.70 ± 0.06 ^{de}	5.11 ± 0.02 ^{fg}
Z0A2	1.65 ± 0.28	1.05 ± 0.17	0.37 ± 0.18	0.72 ± 0.01 ^{de}	6.65 ± 0.03 ^{bc}
Z0A3	1.58 ± 0.26	0.71 ± 0.17	0.52 ± 0.06	0.80 ± 0.06 ^{cd}	5.79 ± 0.02 ^{cde}
Z1A1	1.65 ± 0.65	1.01 ± 0.15	0.57 ± 0.02	0.91 ± 0.12 ^{bc}	6.13 ± 0.03 ^{cd}
Z1A2	1.81 ± 0.05	0.84 ± 0.07	0.58 ± 0.03	0.73 ± 0.17 ^{de}	6.05 ± 0.01 ^{cd}
Z1A3	1.89 ± 0.08	0.94 ± 0.08	0.57 ± 0.01	0.60 ± 0.15 ^e	7.56 ± 0.04 ^a
Z2A1	1.84 ± 0.10	0.86 ± 0.10	0.58 ± 0.08	1.03 ± 0.01 ^a	6.93 ± 0.01 ^{ab}
Z2A2	2.02 ± 0.09	1.16 ± 0.15	0.64 ± 0.28	1.03 ± 0.06 ^a	7.04 ± 0.01 ^{ab}
Z2A3	1.95 ± 0.11	1.00 ± 0.12	0.57 ± 0.01	0.97 ± 0.12 ^{ab}	6.59 ± 0.10 ^{bc}
Z3A1	1.97 ± 0.06	1.03 ± 0.06	0.56 ± 0.01	0.69 ± 0.01 ^{de}	5.28 ± 0.02 ^{efg}
Z3A2	1.83 ± 0.10	0.74 ± 0.28	0.65 ± 0.15	0.66 ± 0.01 ^e	5.56 ± 0.01 ^{def}
Z3A3	1.85 ± 0.11	0.86 ± 0.08	0.57 ± 0.01	0.71 ± 0.02 ^{de}	4.88 ± 0.02 ^g

Means followed by the same alphabetical letters are not significantly different by DMRT at 5 %; MWCNT: multi-walled carbon nanotubes; P: phosphorous; K: potassium; ns: not significant. ±: standard deviation.

acetic acid (IAA), gibberic acid (GA) and cytokinin hormones, enzyme hydrolysis, tissue hydration, the transport of hydrated molecules to the growing point and assimilation for respiration. During germination, absorbed water softens the seed coat and promotes endosperm and embryo development. Any impediment to water absorption will disrupt these processes, resulting in delayed germination and reduced seedling growth (22). Low seed viability is associated with suboptimal energy metabolism caused by seed cell damage (23). Seed metabolism becomes suboptimal when cellular damage occurs. Our findings agreed with ones reported previously (15). They identify that MWCNTs at 50 µg/mL is the optimal concentration which enables penetration into seed cell walls and root systems, thereby enhancing biological activity during germination. Improved water uptake at this concentration may further sustain synthesis of plant phytohormones such as IAA, GA and cytokinin. These hormones are central to stress adaptation. For instance, GA promotes seedling elongation under limited resources (24), while cytokinins delay senescence under drought or salinity stress (25). This concentration therefore creates favourable conditions where seed vigour and stress resilience converge, enabling seedlings to establish successfully even under adverse environmental conditions.

Higher MWCNT concentrations do not necessarily result in improved plant performance. A reduction in growth speed occurred when MWCNTs was applied at higher concentration (75 µg/mL). Soaking mung bean seeds in seed priming experiment requires careful consideration of both duration and MWCNTs concentration, as inappropriate combinations may lead to seed rot, thereby reducing viability. The possible cause might have been due to nanotoxicity, which inhibits nutrient transport and impedes seedling growth. Previous findings reported that MWCNTs at 75 µg/mL induces nanotoxic effects in plants (26). Higher MWCNT concentrations may increase water absorption by facilitating the penetration of nanotubes into the seed coat. Seeds are prone to rot when they absorb excessive amounts of water (27). Appropriate concentration of MWCNTs is required to enhance plant growth and biomass (28), as it facilitates nutrient absorption required by the plants. However, the present study did not confirm the presence of MWCNTs residues in plants post-harvest, owing to limited equipment and research funding. Future studies should investigate this matter further.

The potential of MWCNTs as nanotransporters lies in their ability to facilitate nutrient uptake through root cell walls and their subsequent translocation to other plant tissues, thereby enhancing productivity (18). A previous study reported that MWCNTs can double flower and fruit production compared to controls, primarily due to improved nutrient uptake (29). Adequate nutrient uptake ensures sufficient macro- and micronutrient availability. The critical role of P lies in seed and fruit formation besides stimulating root growth and accelerating flowering and ripening (30). Our findings agree with those reported in early results (29, 30). Specifically, a concentration of 50 µg/mL MWCNTs, as reported in our study, enhanced the uptake of P in seeds, yielding the highest seed-P content, irrespective of soaking duration. In addition, combination of this concentration with soaking duration of 12 and 18 hr also increased the uptake of seed-K (Table 4). P and K are essential macronutrients required by plants, although the demand for P is generally lower than that for K, particularly in starchy crops (31). The enhanced growth observed in plants treated with 50 µg/mL MWCNTs was likely attributable to improved nutrient absorption,

particularly P and K from the medium. This increased uptake subsequently promoted higher leaf chlorophyll a and b contents, as well as greater shoot biomass. However, neither MWCNTs concentration nor the soaking duration significantly influenced the harvest index although the shoot biomass was affected by MWCNTs concentration. Our results were in contrast with early findings who reported that MWCNTs promote plant growth, with nutrient absorption directly influencing the harvest index (28). Harvest index is a critical agricultural metric that represents the ratio of harvested economic yield (e.g., grain) to the total aboveground biomass (total dry matter) (32). The similar effects from the concentrations of 50 and 25 µg/mL on shoot biomass and number of seeds per pod as well as the intervention effect from the soaking duration (18 hr) on number of seeds per pod might have contributed to the inconclusive effect of the 50 µg/mL MWCNTs concentration on the harvest index (Table 3).

Conclusion

The application of multi-walled carbon nanotubes (MWCNTs) at a concentration of 50 µg/mL, regardless of soaking duration, during seed priming in Indonesian mungbean Vina-2 does not only accelerate seedling growth and germination uniformity during germination but also enhances vegetative growth and contents of leaf chlorophyll a and b, as well as the uptakes of seed-phosphate (P) and potassium (K). However, soaking the seeds for 24 hr reduces the seed-K content. Unfortunately, the yield components such as number of seeds per pod, seed weight and harvest index are not significantly affected. These findings highlight the potential of MWCNTs as a promising nanotechnology-based intervention to improve crop establishment. The observed benefits underscore their relevance for sustainable agriculture, particularly in enhancing early vigour, growth, photosynthetic-related factors and the uptakes of P and K in harvested seeds. Future research should focus on testing the efficacy of MWCNTs in diverse crop species and under field conditions and assessing their role in improving seed viability, growth and yield components in lower-quality or stress-prone seed lots.

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Authors' contributions

Z was responsible for conducting the study, performing the statistical analysis, verifying the data and preparing the manuscript, including the tables and figures. SB supervised the study and provided guidance on the manuscript content. AS and DS advised on the experimental design and assisted with the application of nanotubes during the experiment. AFI and EF contributed to the manuscript by advising on its content, formatting it according to the journal guidelines, editing the language and improving the overall flow of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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