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A PROPER CONCENTRATION OF CARBON BLACK NANOPARTICLES ENHANCES GROWTH OF THE REGENERATED VETIVER GRASS

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Abstract

Carbon black (CB) nanoparticles were used in enhancing efficiency of the plant regeneration process under tissue culture condition. The calli of vetiver grass (*Vetiveria zizanioides* L. Nash) were induced by using axillary bud as the explant. These calli were subjected onto plant regeneration medium, Murashige & Skoog (MS) medium supplemented with 1 mgL^{-1} α-naphthalene acetic acid (NAA), 2 mgL^{-1} 6-benzylaminopurine (BAP) and 2 mgL^{-1} kinetin, adding 0-70 mgL^{-1} concentrations of carbon black (CB) nanoparticles. After six week incubation, vetiver calli developed on the medium supplemented with 40 mgL^{-1} CB nanoparticles showed the rather high frequencies of plant regeneration (93.75%), nevertheless no statistically different with other treatment did. Media containing CB nanoparticles in the range 40-60 mgL^{-1} significantly affected on enhancing the average of shoot number per callus more than other CB concentration did. Especially, the average shoot lengths of plantlets derived from 40 and 50 mgL^{-1} CB media (1.73 and 2.19 cm) are longer than those from non CB or other CB concentration media (0.88-1.05 cm) at the significant level. Adding CB nanoparticles in regeneration medium effects on enhancing electrical conductivity and decreasing the pH value. The concentration of CB nanoparticle effected the ratio of colloidal sizes which was found in the medium. The proper concentration of CB generates the optimum property of the regeneration medium which can promote the average of shoot number per callus, and enhanced shoot elongation of vetiver grass in the regeneration process.

Key words: Vetiver grass, Carbon black, Nanoparticles, Plant regeneration, Plant growth.

Introduction

Nowadays, the applications of nanoparticles in agricultural, industrial and medical products are rising their uses. (Salata, 2004; Stark *et al.,* 2015). Widely utilized nanoparticles in large industrial scale are metal oxide nanoparticles (titanium dioxide, aluminium oxide, cesium dioxide, and zinc oxide), metal nanoparticles (silver and iron), and carbon-based nanoparticles (fullerene, carbon nanotube and carbon black) (Abbas *et al.*, 2013; Anjum *et al.*, 2016). Synthesized nanoparticles with a range of 1-100 nm diameters possess exceptional chemical, electrical, mechanical, and magnetic characteristics that are very different from those of the bulk materials (Monica & Cremonini, 2009). Current researches have indicated that nanoparticles tend to be more bioreactive than larger particles of the same material. Carbon black (CB) nanoparticles are made up of nonmetallic carbon elements and classified as a paracrystalline carbon form (Kang & Wang, 1997). This NPs appears spherical morphology containing the functional surfaces which have a high potential for reacting with organic molecules and also themselves. Therefore, they agglomerate into colloidal clusters in water base solution (Wang *et al.,* 2005).

CB nanoparticles are widely used in various industrial products such as paint, tire, and rubber goods (Bourdon *et al.,* 2012), therefore, these particles are increasing in demand. Lately, biotechnology researchers have been using nanoparticles in agricultural experiments, especially in increasing plant yields. Recently, various kinds of nanoparticles have been successfully used for enhancing growth efficiency and seed germination of several plant species (Milewska-Hendel *et al.,* 2016). ZnO nanoparticles in an optimal concentration were also shown to induce germination of reddish, rape, and corn (Lin & Xing, 2007) and also to increase growth of *Brassica nigra* Seedlings (Zafar *et al.,* 2016). In addition, carbon nanotubes were found to promote growth of cucumber and onion roots (Yang & Watts, 2005). However, only a few experiment concerns about the effects of nanoparticles on calli growth and development. TiO_2 nanoparticles has been shown to dramatically increase in size of barley callus by inducing cell division and expansion without negative effect on callus qualities (Mandeh *et al.*, 2012).

Carbon-based nanoparticles are expected to be less inducing oxidative stress and cytotoxicity than metal oxide nanoparticles are (Yang *et al*., 2009). These nanoparticles have been reported to have both advantage and disadvantage effects on plant physiology (Husen & Siddiqi, 2014; Aslani *et al.*, 2014). Several reports have shown the positive effects of carbon-based nanoparticles (fullerol and carbon nanotube) at a proper concentration on plant growths [Kole *et al.*, 2013; Yuan *et al.*, 2011]. However, there has yet been any report of using CB nanoparticles to enhance plant regeneration efficiency *In vitro*.

The concentration of CB particles in the media is considered to affect on the agglomerated CB particles. Various sizes of agglomerated particles effect surface area and contribute the physicochemical properties of media. These media properties may influence on calli development and regeneration. In this study, we investigated the effect of CB nanoparticle concentrations on the properties of the plant regeneration medium. Consequently, the influences of these CB particles in regeneration medium were analyzed for developing of vetiver calli. In addition, qualities of plant cells grown in medium containing different CB concentrations were determined, in order to find a suitable concentration of CB for the plant cell development. These data can be used for estimating the productive concentration for other varieties of plant cells.

Materials and Methods

Preparation of CB colloid stock solution: The manufactured CB nanoparticles are uniformly spherical shape with a diameter in range 50 nm. derived from commercial company (Thai Carbon Black). These nanoparticle were dispersed in deionized water by ultrasonication at 37 kHz frequency for 30 minutes. This solution was used as a stock solution for preparing all plant regeneration media.

Plant media preparation: The basal medium in the both stages of callus induction and plant regeneration was MS medium (Murashige & Skoog 1962). Callus induction medium was supplemented with 2 mgL^{-1} NAA, 1 mgL^{-1} BAP and 20 gL^{-1} sucrose, while plant regeneration medium was supplemented with 1 mgL^{-1} NAA, 2 mgL^{-1} BAP, 2 mgL^{-1} kinetin and 30 gL^{-1} sucrose (Sompornpailin & Khunchuay, 2015). This plant regeneration medium was added with different concentrations of CB nanoparticles (0, 30, 40, 50, 60 and 70 mgL^{-1}). The pH of the CB supplemented medium was adjusted to 5.7 with potassium hydroxide (KOH). All plant culture media were added agar up to make 8 gL^{-1} and autoclaved for 15 minutes at 121ºC.

CB colloidal size distribution and effects on media properties: The distribution of CB colloidal sizes and their effects on the plant regeneration medium were analyzed under liquid condition. The particle size distribution of CB colloid was determined by Delsa Nano Particle Analyzer. The electrical conductivity (EC) and pH of each sterilized medium differently containing CB were measured with a conductivity meter (CON 6/TDS 6 Conductivity/TDS meter) and a pH meter (UB-5, Denver instrument). Three replicates were done for statistical analysis of data.

Effects of CB concentrations on plant regeneration: Aseptic axillary bud of *Vetiveria zizanioides* L. Nash cv. Suratthani was used as explant. In the callus induction stage, axillary buds were cultured on callus induction medium at 25±2ºC under a dark condition for 4 weeks. After that, the calli were transferred onto a fresh medium of the same formula and then cultured under the same condition for another 2 weeks. The same size of proliferating calli was used for the regeneration process. Calli prepared in the previous step were cultured on plant regeneration medium supplemented with different concentrations of CB nanoparticles following the protocol of media preparation. The calli were incubated at 25±2ºC with a 16-h light/8-h dark photoperiod for 6 weeks. The percentage of plant regeneration, number of shoots per callus, the length of shoot and the morphology of the plantlets were recorded at six weeks. The percentage of plant regeneration were calculated using the following equation.

$$\text{Plant regeneration (\%)} = \frac{\text{Number of calli produced plantets}}{\text{Number of total calli}} \times 100$$

Electrolyte leakage of plantlet: After culturing on media containing CB nanoparticles, plantlets were analyzed for the electrolyte leakage. Plantlets from each treatment were collected and cut into 0.5-cm pieces. These pieces were immersed in deionized water at 30ºC. After thirty-minute immersion, the electrical conductivity (EC_i) of the water with the immersed pieces of plantlets was measured with a handheld conductivity meter. Then, after six hours of immersion, the electrical conductivity (EC_f) was measured again. Finally, the water with the immersed pieces of plantlets was boiled at 100ºC for 1 minute and cooled down to room temperature. Then, the electrical conductivity (EC_t) was measured again. These electrical conductivity values were used to calculate the percentage of electrolyte leakage of the plantlets from this following equation:

$$\text{Electrolyte leakage of the (\%)} = \frac{ECf - ECi}{ECt - ECi} \times 100$$

Statistical analysis: The experimental results were subjected to one-way analysis of variance (ANOVA) to determine the effects of different treatments. Means were separated by using Duncan's multiple range test (DMRT) at a probability level of 0.05. All statistics were computed by SPSS software.

Results and Discussion

Effects of CB concentrations on colloidal sizes and media properties: Since the nutrient compositions in the culture media and CB concentration might affect the clustering of CB. Therefore, the sizes of CB colloid in nutrient-filled aqueous medium without agar was determined. The result showed that the colloid sizes of CB in all concentrations of media were classified in the range of 500 nm to 2500 nm (small size particles) and over than 5000 nm (large size particles) in a diameter (Table 1). The ratio of dispersion percentages of small size and large size particles is approximately 40 to 60. The percentage of small size CB colloids is increased when CB concentration elevated up to 50 mgL^{-1}. CB concentrations higher than 50 mgL^{-1} effected on an increasing percentage of large size CB colloids. Concentration of CB nanoparticles influenced on both EC and pH values of liquid media. These results showed in Fig. 1. Regeneration medium, adding with CB in the range 30-50 mgL^{-1} showed the increasing EC level of medium. However, the CB concentration at a level higher than 50 mgL^{-1} did not clearly increase the EC level of medium. Adding CB concentrations at 30, 40 mgL^{-1} to medium effects on decreasing the pH value of the medium from 5.5 to 5.33, 5.27, respectively. Media containing 40 and 50 mgL^{-1} CB have similar pH value (5.25). Media containing 60 and 70 mgL^{-1} CB slightly reduced the pH level (5.23 and 5.21, respectively) after comparing to those containing 40 and 50 mgL^{-1} CB.

The dispersion and stability of CB colloids in a medium depend on the repulsive and attractive forces between the all of the components in the colloidal suspension. The attractive forces can be determined from the surface energy of CB colloids and the ionic strength of the medium. Under the experimental condition, the same ionic strength of the medium was used. Therefore, colloidal size distributions were variable depending on the CB concentration. Carbon atoms in CB are arranged in paracrystalline structures which is short and medium range ordering in lattice (Voyles *et al.*, 2001). This lattice surface of CB has hydrophobic property which causes CB particles easily aggregation into colloids in aqueous solution (Hadana *et al*., 2013). When the energy of CB surface is much higher than other aqueous-based suspension components which CB particles are dispersive as colloid, thereby these particles attract each other and aggregate into clusters (Wang *et al*., 2005). CB particles have high surface area which contains the functional group of chemically-bonded oxygen (i.e., hydroxylic acid, carboxylic acid). In aqueous solution, these functional groups transfer a hydrogen ion to water molecule and hydroxonium ion are formed. Therefore, pH and EC of media were changed when the different concentrations of CB were added.

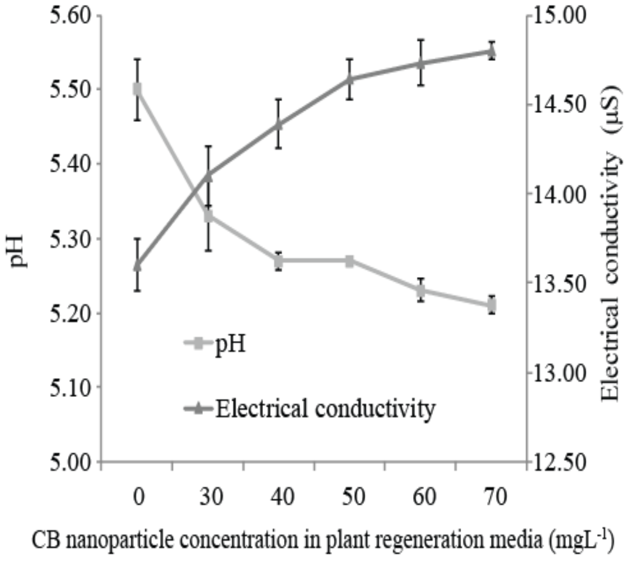

Fig. 1. The pH and electrical conductivity values of the plant regeneration medium supplemented with different concentrations of CB nanoparticles (0, 30, 40, 50, 60 and 70 mgL^{-1}) after autoclaving.

Table. 1. The percentages of small and large sizes of CB colloids at concentration in the range of 30-70 mgL^{-1} distributed in the plant regeneration medium.

CB concentration in culture medium (mgL^{-1})	The average of colloidal sizes	
	Small size (500-2,500 nm)	Large size (>5,000 nm)
30	41.91 ± 2.29^{c}	58.09 ± 2.29^{a}
40	45.25 ± 5.02^{c}	54.75 ± 5.02^{a}
50	62.53 ± 8.46^{a}	37.47 ± 8.46^{b}
60	56.64 ± 6.21^{b}	43.36 ± 6.21^{b}
70	45.33 ± 4.05^{c}	54.17 ± 4.05^{b}

The difference letters in each column show the significant difference at $p \leq 0.05$

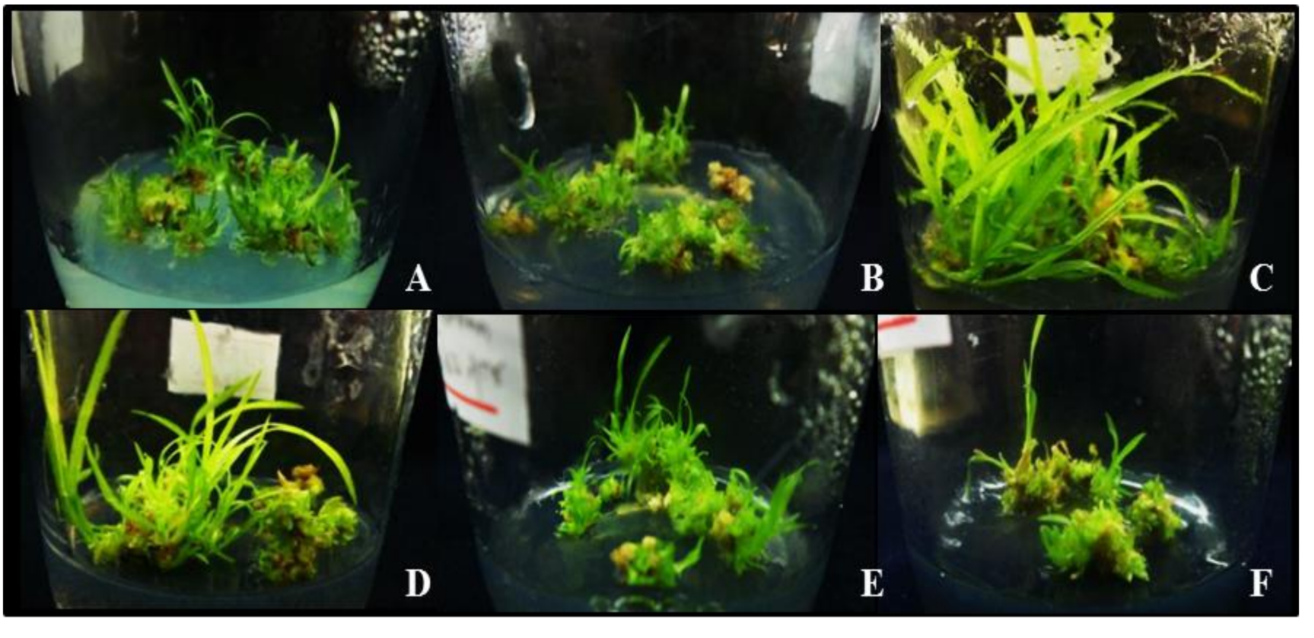

Fig. 2. Morphology of vetiver regeneration on plant regeneration medium added with different concentrations of CB nanoparticles under 16-h light/8-h dark photoperiod for 6 weeks later. Plantlets developed on plant regeneration medium with 0 mg L^{-1} (A), 30 mgL^{-1} (B), 40 mgL^{-1} (C), 50 mgL^{-1} (D), 60 mgL^{-1} (E) and 70 mgL^{-1} CB nanoparticles (F), respectively.

Table 2. The concentrations of CB nanoparticle added in plant regeneration medium effect on vetiver developments.

The concentration of CB nanopartiles (mgL^{-1}) in plant regeneration medium	Plant regeneration (%)	Average shoot no. per callus (shoots)	Average shoot length per callus (cm)
0	87.50 ± 14.4^a	8.20 ± 0.6^b	0.98 ± 0.08^c
30	87.50 ± 14.4^a	8.38 ± 0.8^b	0.88 ± 0.05^c
40	93.75 ± 12.5^a	9.71 ± 0.6^a	2.19 ± 0.09^a
50	87.50 ± 14.4^a	9.50 ± 0.7^a	1.73 ± 0.09^b
60	87.50 ± 14.4^a	9.60 ± 0.3^a	1.05 ± 0.14^c
70	81.25 ± 12.5^a	9.00 ± 0.5^{ab}	0.90 ± 0.19^c

The difference letters in each column show the significant difference at $p \leq 0.05$.

Effects of media containing CB on plant development: Explants were cultivated on a callus induction medium under darkness of tissue culture condition for six weeks. Yellow-creamy calli were then subcultured into several plant regeneration media supplemented with different concentrations of CB. The percentages of plantlets developed from calli on each plant regeneration medium were recorded at 6 weeks and shown in Table 2. Media supplemented with CB nanoparticles at 30, 40, 50, 60, and 70 mgL^{-1} showed slightly higher average percentages of developed green spots than the plain medium with no CB nanoparticles did, but the differences were not statistically significant. Calli cultured on media containing different concentrations of CB nanoparticles were developed into the similar morphologies of green spots (data not showed). These green spots further developed into shoots within 3-4 weeks. At 6 weeks of incubation period, the average percentages of plantlets produced from the regeneration media adding CB nanoparticles were not statistically different from that without adding CB nanoparticles. Even though, calli cultured on the regeneration medium containing 40 mgL^{-1} CB nanoparticles yielded a rather high in the average percentage of plantlets (93.75%). The averages of shoot numbers generated from media supplemented with 40-60 mgL^{-1} CB were in the range of 9.50-9.71 shoots per callus. These averages are significantly higher than those from the media without CB or media supplemented with 30 mgL^{-1} CB, (8.20 and 8.38 shoots, respectively). Moreover, calli on the medium supplementing with 40 mgL^{-1} CB nanoparticles produced the longest shoot length (2.19 cm in average) following with those on the medium supplemented with 50 mgL^{-1} CB did (1.73 cm shoot length in average), as shown in Fig. 2 and Table 2. Plantlet grew up quickly on both media adding 40 and 50 mgL^{-1} CB nanoparticles. Calli grew on medium containing high concentrations of CB (60-70 mgL^{-1}) show that the proliferating plant cell and also growth and development of plantlets were lesser than those on medium containing 40-50 mgL^{-1} CB. In this experiment, even though, the slightly high CB concentration (60-70 mgL^{-1}) in the media seem to reduce growth and development of plant cell when compared to the media with proper CB concentrations (40-50 mgL^{-1}). These plant growth and development data; plant regeneration percentage and average shoot length are the same statistical group as those data from the non CB containing medium. Under this experimental condition, medium containing 40 mgL^{-1} CB nanoparticles is the optimum concentration of CB nanoparticles, which enhances the developmental efficiency of green spot into shoots and also increase the shoot length of vetiver grass (Table 2). These results showed that CB nanoparticles at a suitable concentration is not toxic to vetiver plantlets, similar to the study of red spinach grown in a nano-carbon supplemented medium which showed a non-toxic result (Begum & Fugetsu, 2012).

Although CB nanoparticles did not make a statistically significant difference in plant regeneration frequency, but they could significantly help an increase of shoots per callus. Calli cultured on regeneration media with the optimum concentration of CB produced a high number of shoots presented after 6 weeks of incubation period. CB particles may act like activated charcoal that facilitates long-term embryonic development in a medium, because it removes some growth-stunting compounds in the culture medium (Motoike *et al.,* 2001), or it may act like carbon nanotubes that induce the growth of tobacco cells by stimulating the genes involved in cell division and water transport (Khodakovskaya *et al.,* 2012).

The shoot length of vetiver grown in this study significantly depended on the concentration of CB nanoparticles adding in the plant regeneration media. This plant elongation effect may be attributed to the CB nanoparticles inducing more water absorption, hence more nutrients carried into plant cells, like carbon nanotubes do—carbon nanotubes form new capillaries that increase water uptake in addition to the regular means of water absorption (Tripathi *et al.,* 2011). However, nanoparticles at high concentration may cluster and the big clusters may not be able to penetrate through the cell membrane pores, so that they can not enhance water and mineral absorptions into the cell, similar to the clustering behavior of carbon nanotubes at high concentration (Dai, 2002).

The sizes of CB nanoparticle are smaller than the size of activated charcoal. These colloids of CB nanoparticles contain the porous carbon matrix which undergoes physicochemical properties with the plant nutrition. Therefore, these particles are useful in a control of the nutrient releasing in the agricultural process (Siddiqui *et al.,* 2015). Moreover, the CB surface area contains various kinds and degrees of oxygen complexes depending on the manufacturing conditions (Bradley *et al.*, 1995). The chemically-bonded oxygen, especially carboxylic acids, on a surface of CB have potential to react with ionic bases in the regeneration medium and to form carboxylate salts. A cation can substitute the hydrogen position of the hydroxyl group and produce water and carbon dioxide as the by products. Enhancing level of carbon dioxide in tissue culture condition may increase photosynthetic rate of the regenerated plant. Moreover, carboxylic acids on CB surface are naturally modified to thioesters which known as derivatives of coenzyme A in plant metabolic reactions. Plants grown in media containing appropriated CB concentration may increase metabolic reaction insight plant, therefore significantly enhance their biomass.

High concentration of CB could not enhance plant biomass because these chemically-bonded oxygens might react with the positive charge of metal elements such as iron and zinc. These elements are co-factor of several enzymes, consequently reducing biosynthesis of plant metabolites. For these reasons, media containing CB at a higher level than an appropriate concentration tend to show the deteriorated effects to the culturing plant, since number of functional groups on the surface area of CB is high and also affects the characteristics of chemical reaction.

Analysis of cell membrane integrity: Electrolyte leakages of vetiver leaf tissues were used to indicate an integrity of plant cell membrane. Six-week-old plantlets developed in regeneration media supplemented with or without CB nanoparticles at different concentrations were analyzed. The electrolyte leakage results are presented in Fig. 3. It was found that vetiver leaves grown in the plant regeneration medium without CB nanoparticles exhibited a percentage of electrolyte leakage (42.33%) higher than leaves grown in media adding CB nanoparticles at all concentration did. The percentages of electrolyte leakage were significantly reduced when CB nanoparticles at concentration in the range of 30-70 mgL^{-1} were added into the medium. The plantlets developed on media adding 40 mgL^{-1} CB nanoparticles gave the lowest percentage of electrolyte leakage. This result is concordant with the growth and development of plantlet data. Moderate high concentrations of CB nanoparticles (in the range of 50-70 mgL^{-1}) in the plant regeneration medium slightly increased cell membrane injury over the optimal concentration of 40 mgL^{-1}. However, these concentrations in the media inflicted less level of electrolyte leakages than non CB or 30 mgL^{-1} CB containing media.

Electrolyte leakage of plant cell was measured for evaluating cell membrane integrity which was damaged from various abiotic stresses including nanoparticles. Electrolyte leakage value normally indicated a oxidative stress of plant physiology. For example, the red spinach cells leaked more electrolytes when more carbon nanotube particles were added in media at the relative high concentration, in the range of 125-1000 mgL^{-1} and caused adverse effects on their plants (Begum & Fugetsu, 2012). In our experiment, these electrolyte leakage results are different from those studies. All plants grown on media with CB nanoparticles were presented a lower level of electrolyte leakage from cell than that on media without CB. Our experiment used low to moderate concentrations of CB that was found not only to be non-toxic to vetiver cells, but also enhance shoot growth and development. Perhaps, the plant cell underwent self-protection from undesired foreign particles by responding with a defensive mechanism like cell wall thickening (Husen & Siddiqi, 2014). Therefore, electrolyte leakages of plant cell grown in CB containing medium are lesser than non CB medium. However, higher than the proper concentrations of CB nanoparticles in media affected the producing compact plantlets with shorter shoots.

The chemical characteristics of CB surfaces can act as the generators for reactive oxygen species (ROSs) in cellular systems (Hussain *et al.*, 2009; Jacobson *et al.*, 2008). High concentration of CB contains a high surface area. It may generate high level of ROSs which have a potential to elevate cellular oxidative stress higher than a proper concentration of CB. Therefore, unappropriated CB concentrations may directly effect on the plant nutrient composition and harmful cellular components via mediating ROSs. These ROSs are well known as the cascade signals which regulate cellular mechanisms at DNA level. (Mroz *et al.*, 2007; Mroz *et al.*, 2008).

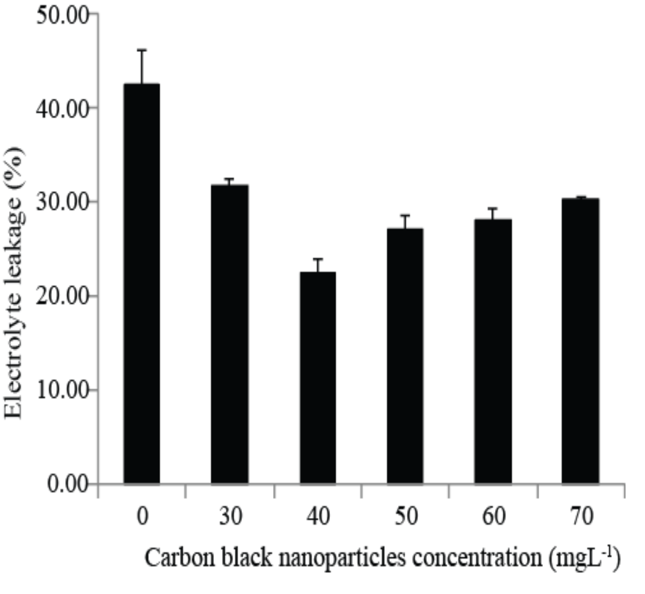

Fig. 3. The electrolyte leakage percentages of plantlets were grown on plant regeneration medium without or with CB nanoparticles at 6 weeks.

Conclusion

An addition of the CB nanoparticles effected on pH and EC properties of the regeneration medium and also affected the culturing plant cell. In this experiment, plantlets generated from media with CB nanoparticles presented lower electrolyte leakage than those from the non CB medium. This result indicated that the CB treated plants did not face with drastic stress conditions. Low to moderate concentrations of CB were found to be non-toxic to the plantlets regenerated *via* calli under tissue culture condition. The proper concentration of CB nanoparticles (40 mgL^{-1}) in regeneration medium was exhibited a long shoot and high quality of plantlets after a long period culturing. These non toxic CB concentration can be applied in tissue culture medium of other grass or dicotyledonus plant species for enhancing growth and development.

References

Abbas, K., F. Simonelli, U. Holzwarth, I. Cydzik, A. Bulgheroni, N. Gibson and J. Kozempel. 2013. Feasibility study of production of radioactive carbon black or carbon nanotubes in cyclotron facilities for nanobioscience applications. *Appl. Radiat. Isotopes*, 73: 44-48.

Anjum, S., B.H. Abbasi and Z.K. Shinwari. 2016. Plant-mediated green synthesis of silver nanoparticles for biomedical applications: Challenges and Opportunities. *Pak. J. Bot.*, 48: 1731-1760.

Aslani, F., S. Bagheri, N.M. Julkapli, A.S. Juraimi, F.S.G. Hashemi and A. Baghdadi. 2014. Effects of engineered nanomaterials on plants growth: An overview. *Sci. World J.*, 2014: 1-28.

Begum, P. and B. Fugetsu. 2012. Phytotoxicity of multi-walled carbon nanotubes on red spinach (*Amaranthus tricolor* L) and the role of ascorbic acid as an antioxidant. *J. Hazard. Mater.*, 243: 212-222.

Bourdon, J.A., A.T. Saber, S. Halappanavar, P.A. Jackson, D.M. Wu, K.S. Hougaard, N.R. Jacobsen, A. Williams, U. Vogel, H. Wallin and C.L. Yauk 2012. Carbon black nanoparticle intratracheal installation results in large and sustained changes in the expression of miR-135b in mouse lung. *Environ. Mol. Mutagen.*, 53: 462-468.

Bradley, R.H., I. Sutherland and E. Sheng. 1995. Relationship between carbon black surface chemistry and energy. *J. Chem. Soc., Faraday Trans.*, 91: 3201-3207.

Dai, H. 2002. Carbon nanotubes: synthesis, integration, and properties. *Acc. Chem. Res.*, 35: 1035-1044.

Hanada, Y., S. Masuda, M. Iijima and H. Kamiya 2013. Analysis of dispersion and aggregation behavior of carbon black particles in aqueous suspension by colloid probe AFM method. *Adv. Powder Technol.*, 24: 844-851.

Husen, A. and K.S. Siddiqi 2014. Carbon and fullerene nanomaterials in plant system. *J. Nanobiotechnol.*, 12: 16.

Hussain, S., S. Boland, A. Baeza-Squiban, R. Hamel, L.C.J. Thomassen, J.A. Martens, M.A. Billon-Galland, J. Fleury-Feith, F. Moisan, J.C. Pairon and F. Marano. 2009. Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: Role of particle surface area and internalized amount. *Toxicology*, 260: 142-149.

Jacobsen, N.R., G. Pojana, P. White, P. Moller, C.A. Cohn, K.S. Korsholm, U. Vogel, A. Marcomini, S. Loft and H. Wallin 2008. Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C(60) fullerenes in the FE1-Mutatrade mark Mouse lung epithelial cells. *Environ. Mol. Mutagen*., 49: 476-487.

Kang, Z.C. and Z.L. Wang. 1997. Chemical activities of graphitic carbon spheres. *J. Mol. Catal. A: Chem.*, 118: 215-222.

Khodakovskaya, M.V., K. de Silva, A.S. Biris, E. Dervishi and H. Villagarcia. 2012. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano*., 6: 2128-2135.

Kole, C., P. Kole, K.M. Randunu, P. Choudhary, R. Podila, P.C. Ke, A.M. Rao and R.K. Marcus 2013. Nanobiotechnology can boost crop production and quality: First evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC Biotechnol.*, 13.

Lin, D.H. and B.S. Xing 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environ. Pollut.*, 150: 243-250.

Mandeh, M., M. Omidi and M. Rahaie 2012. *In vitro* ınfluences of TiO_2 nanoparticles on barley (*Hordeum vulgare* L.) tissue culture. *Biol. Trace Elem. Res*., 150: 376-380.

Milewska-Hendel, A., R. Gawecki, M. Zubko, D. Stróż and E. Kurczyńska. 2016. Diverse influence of nanoparticles on plant growth with a particular emphasis on crop plants. *Acta. Agrobot.*, 69: 1694.

Monica, R.C. and R. Cremonini 2009. Nanoparticles and higher plants. C*aryologia*, 62: 161-165.

Motoike, S.Y., R.M. Skirvin, M.A. Norton and A.G. Otterbacher. 2001. Somatic embryogenesis and long term maintenance of embryogenic lines from fox grapes. *Plant Cell Tiss. Org. Culture*, 66: 121-131.

Mroz, R.M., R.P.F. Schins, H. Li, E.M. Drost, W. Macnee and K. Donaldson 2007. Nanoparticle carbon black driven DNA damage induces growth arrest and AP-1 and NF kappa B DNA binding in lung epithelial A549 cell line. *J. Physiol. Pharmacol.*, 58: 461-470.

Mroz, R.M., R.P.F. Schins, H. Li, L.A. Jimenez, E.M. Drost, A. Holownia, W. MacNee and K. Donaldson. 2008. Nanoparticle-driven DNA damage mimics irradiation-related carcinogenesis pathways. *Eur. Respir. J.*, 31: 241-251.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497.

Salata, O.V. 2004. Applications of nanoparticles in biology and medicine. *J. Nanobiotech*., 2: 3.

Siddiqui, M.H., M.H. Al-Whaibi, F. Mohammad and M.Y. Al-Khaishany. 2015. Role of nanoparticles in plants. In Nanotechnology and Plant Sciences: Nanoparticles and their ımpact on plants, (Eds.): Siddiqui, M.H., M.H. Al-Whaibi and F. Mohammad. 19-35: Springer International Publishing.

Sompornpailin, K. and C. Khunchuay. 2015. Synergistic effects of BAP and kinetin media additives on regeneration of vetiver grass (*Vetiveria zizanioides* L. Nash) *Aust. J. Crop Sci.*, 10: 726-731.

Stark, W.J., P.R. Stoessel, W. Wohlleben and A. Hafner. 2015. Industrial applications of nanoparticles. *Chem. Soc Rev*., 21(44): 5793-805.

Tripathi, S., S.K. Sonkar and S. Sarkar 2011. Growth stimulation of gram (*Cicer arietinum*) plant by water soluble carbon nanotubes. *Nanoscale*, 3: 1176-1181.

Voyles, P.M., N. Zotov, S.M. Nakhmanson, D.A. Drabold, J.M. Gibson, M.M.J. Treacy and P. Keblinski. 2001. Structure and physical properties of paracrystalline atomistic models of amorphous silicon. *J. Appl. Phys.*, 90: 4437-4451.

Wang, C., K.C. Tam and C.B. Tan. 2005. Dissolution and swelling behaviors of random and cross-linked methacrylic acid-ethyl acrylate copolymers. *Langmuir*, 21: 4191-4199.

Yang, H., C. Liu, D. Yang, H. Zhang and Z. Xi. 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. *J. Appl. Toxicol.* 29: 69-78.

Yang, L. and D.J. Watts. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol. Lett.*, 158: 122-132.

Yuan, H.G., S.L. Hu, P. Huang, H. Song, K. Wang, J. Ruan, R. He and D.X. Cui 2011. Single walled carbon nanotubes exhibit dual-phase regulation to exposed *Arabidopsis* mesophyll cells. *Nanoscale Res. Lett*., 6.

Zafar, H., A. Ali, J.S. Ali, I.U. Haq and M. Zia. 2016. Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and antioxidative response. *Front Plant Sci*., 20(7): 535.

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