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Applications of nanotechnology in improving plant tissue culture media

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Abstract

Plant tissue culture focuses on the development of plant cells or plant parts, bulk propagation, genetic modification, the generation of bioactive compounds, and enhancement. Recently, using nanoparticles has been shown to be effective in removing microbial contaminants from explants, as well as demonstrating NPs' beneficial effects on induction of calluses, genetic transformation, organogenesis, somaclonal variation, somatic embryogenesis, and secondary metabolite synthesis processes that occur in the plants. The goal of this review is to summarize all the existing successes gained via nanotechnology's incorporation into plant tissue culture and to emphasize the benefits of employing nanoparticles in tissue culture. The benefits and drawbacks of employing nanoparticles in culture media are examined and given. Toxicity and safety concerns associated with nanoparticle exposure to plants and the environment have been observed. Finally, consider the future are offered using not only Ag, TiO_2, and ZnO NPs, but also graphene, carbon nanotubes, SiO_2, quantum dots, and dendrimers. Nanomaterials such as nano silver and TiO_2 have the ability to remove microbial contamination from plant tissue culture, The effectiveness of microbial contaminates tissue culture of the plant depends on their dimensions, size, distribution and type. In addition, utilizing Ag NPs in tissue culture media increased growth parameters *in vitro* and increased total phenolic compounds and flavonoids accumulation in MS medium enriched with Au-Ag NPs.

Keywords: Nanomaterials, Nanoparticles, Callus induction, Shoot proliferation, Tissue culture

1. Introduction

One of the most active fields of research in modern materials science is nanotechnology. In plant tissue culture, there are several studies have shown the positive and negative effects of nanoparticles on surface disinfection of explants, callus induction, shoot, induction percentage, number of shoots, callus formation, proliferation, rooting and secondary metabolites.

Several NPs sterilizing agents, including Ag, Al_2O_3, Cuo, Fe_3O_4, AU, MgO, Ni, Si, SiO_2, TiO2 and ZnO, have antimicrobial activity against diverse microbiological contaminations. The inclusions of nanoparticles to *in vitro* culture medium be able to reduce bacterial contamination and improve explant prospective morphogenetic (Kim *et al.*, 2017) [35]. The addition of 1 mg L^{-1} ZnO NPs frequency of shoot formation of stevia rebaudiana (Javed *et al*., 2017) [31]. After 6 weeks of transplant to clay pots containing sand soil, the survival rate of banana plantlets was over 90% and remained stable (Helaly *et al*., 2014) [28]. Meanwhile, increasing lateral roots number and root length of banana shoots invitro were achieved on MS medium contain 150 ppm SiO_2 – NPs after 30 days (EL-kady *et al*., 2017) [14] were achieved containing of silver nanoparticles from 20 to 60 ppm. Silver nanoparticles from 20 to 60 ppm *in vitro* medium increased chlorophyll, carbohydrate and protein contents of plants (Kim *et al.*, 2017) [35]. Nanomaterials' success in plant tissue culture is determined by various factors, including:

1. Input of nanomaterials for surface disinfection of explants.
2. Input of nanomaterials towards callus indication, organogenesis and shoot regeneration.
3. Input of nanomaterials toward shoot growth and proliferation.
4. Input of nanomaterials towards on rooting.
5. Input of nanomaterials towards on growth and development of exvitro.
6. Nonmaterial enhancement of secondary metabolites.

2. Input of nanomaterials for surface disinfection of explants

Even before the propagation mechanism is started, microbial infection might destroy the entire process and efficiency. Removal a microbial contamination from *in vitro* culture of valerian officinal is when single node explants were surface

disinfected with 70% ETOH for 180 minutes, and followed by 100 mg/Lof Ag NP_s for 180 minutes (Abdi *et al*., 2008) [1]. However, the effect of Ag NPs on the medium was found to be the best for sufficiently controlled internal contaminants of olive " Mission" explants treated with 4mg/l Ag NPs (Rostami and shaharvar, 2009) [42]. Apply of Ag NPs *in vitro* culture plant was examined, it observed that used of Ag NPs ate 500 mg/ml under reduced pressure (300 mg of Hg) for 5 min, a biological contamination in plant tissue culture has successfully controlled the microbial contamination (Lee *et al*., 2010) [37]. In addition, inhibited microbial growth was obtained when adding Ag NPs at rate of 50 mg/L to Murashige and Skoog medium (Safavi *et al*., 2011) [44]. Recent studies have shown that surface disinfection of explants with 1.5% NaOcl for l0 min and Ag NPs at rate of 200 mg/L for 15 min significantly reduces microbial contamination in various plants (Fakhrfeshani *et al*., 2012) [18]. The addition of NS and TiO_2 to tissue culture media for removing microorganisms *in vitro* culture and then explants make growth very well (Safavi, 2012) [43]. Mandeh *et al*., 2012 [39] found that the effect of TiO_2 NPs on *Hordeum vulgare* tissue culture, and also reported the bacterial and fungal contamination decreased with 60 mg L^{-1} TiO_2 NPs. Also, Sarmast, *et al*. 2011 [47] reported that internal bacterial contamination rate was reduced from 61.15 to 11.3% when Araucaria excels by addition of 200 mg/L Ag NPs for 180 min to the culture medium.

Removal a microbial contamination from *in vitro* culture shoot buds of tobacco and Potato had culture was done with Ag NPs or TiO_2 NPs addition on MS medium (Safavi *et al*., 2011 and Safavi, 2012) [44, 43]. The addition of Zn NPs and ZnO particles fig (1) at different concentrations (40,100 and 200 mg L-1) can eliminate bacterial contamination of banana *in vitro* cultures, (Helaly *et al*., 2014) [28].

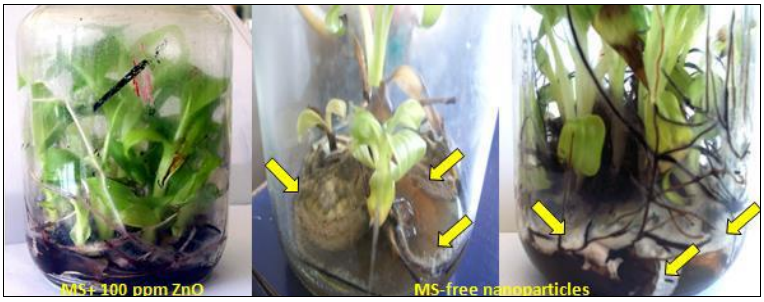

Fig 1: Effect of ZnO nanoparticles for surface disinfect of banana during establishment stage (Helaly *et al*., 2014) [28]

In plant tissue culture, treatment of *Bacopmonnier* explants with 160 mg/L Ag NPs, the bacterial contamination was significantly reduced in callus cultures (Priya *et al*., 2014) [41]. Meanwhile; explants of Rosa hybrid were tested by 200 mg/L silver nanoparticles solution for 20 minutes, which decreased contamination. Also, reducing the rate of bacterial contamination and also phenolic oxidation were observed when the explants were tested through 100 mg/L^{-1}Ag nanoparticles was added on MS medium (Shokri *et al*., 2014) [50]. Contamination-free cultures were simulated by adding 200 mg/L Ag nanoparticles solution and 100 mg/L thymol to the culture media of *Cynodon dectylon* for 60 minutes, (Taghizadeh and solgi, 2014) [55]. The supplemented of 100 mg/ l Ag NPs to the medium sufficiently controlled internal contaminations in leaf explant of *Vitis Vinifera* (Gouran, *et al*. 2014) [23]. Silver (Ag), (Al_2O_3), copper oxide (CuO), iron oxide (Fe_3O_4), gold (Au), magnesium oxide (MgO), nickel (Ni), silicon (Si), SiO_2, Titanium dioxide (TiO_2), and zinc oxide (ZnO) nanoparticles have all been shown to minimize microbial contamination in plants (Beyth *et al*., 2015) [10].

Fig. (2) Shows the effect of silver NPs at rates 1, 5, 10 and 20 mg/L^{-1}, the highest rate of survival was 88.89%, the lowest contamination rate was (11.11%) and zero mortality rate. Meanwhile, the contamination rate was higher (22.22%) when explants were sterilizer on 5 mg/L silver NPs when added with sodium hypochlorite and mercuric chloride and indicated zero rate of mortality (El sharabasy *et al*., 2017) [16].

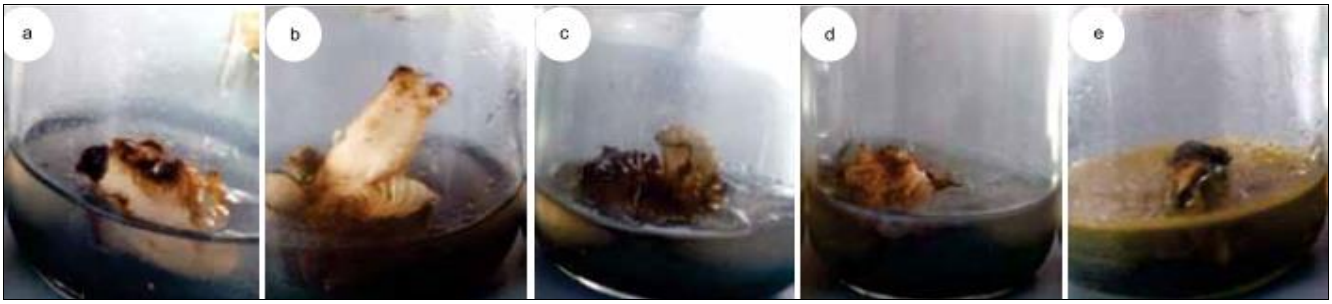

Fig 2: Effect of different concentrations of sterilized date palm cv. Barhee shoot tip explants. (a)Sterilized with sodium hypochlorite, mercuric chloride, 1mg/L Ag NPs. (b) Sterilized with 5 mg/L Ag NPs alone. (c) Sterilized with sodium hypochlorite, mercuric chloride. (d)Sterilized with mercuric chloride, 1 mg/L Ag NPs. (e)Sterilized with sodium hypochlorite, mercuric chloride + Ag NPs 20 mg/L (El sharabasy *et al*., 2017) [16].

Addition of 50-200 mg/L Ag nanoparticles to shoot cultures of vanilla plant significantly inhibited microbial growth, (Spinoso-Castillo *et al*., 2017) [52]. Also, Wang *et al*., 2017 [60] found that added of metal ion and metal oxide having been made available to be real beneficial used for removal of different microbes. Also, free of contamination was

detected when external clean shoot buds were cultured on medium supplemented with nanoparticles Ag NPs, (Mahendran *et al*., 2019) [38]. In addition, study the effect of ZnO Nps antimicrobial contamination of date palm cultured tissues. Is shown in Fig (3), the results showed that lowest contamination ratio was recorded in MS medium added with 100 and 150 mg/L of ZnO nanoparticles when supplementary before and after purification media followed by treatment of 50 mg/L ZnO NPs compared to the control before and after sterilization of media (Awad *et al*., 2020) [8].

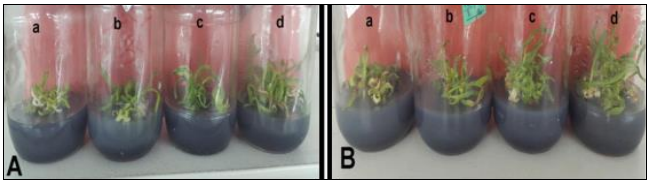

Fig 3: Effect of ZnO (NPs) for surface disinfect of date palm (A) after and (B) before sterilization media (Awad *et al*., 2020) [8].

3. Input of nanomaterials to callus induction, organogenesis and shoot regeneration:

Many trails have encouraged the effect of nanoparticles on callus initiation, shoot regeneration and organogenesis. Effect of Ag NPs was studied on the growth of rape seed (*Brassicanopus* L.) The result showed that adding $AgNO_3$ to callus initiation medium had significant effect on shoot regeneration from the leaves, (Akasaka *et al*., 2005) [4].

In *Capsicum annuum*, the highest increase in the shoot bud differentiation and elongation from the cultured cotyledons was noticed in medium supplemented with higher copper oxide nanoparticles (CuO NPs) level in the medium (Joshi and Kothari, 2007) [32].

In plant tissue culture, Aghdaei *et al*., 2012 [2] shown positive effects of NPs callus induction and shoot regeneration planted on MS medium containing 10 mg/L of Ag NPs, 2.5 mg/L of BAP and 0.1 mg/L of IAA. In addition, Helaly *et al*., (2014) [28] used Zn NPs and ZnO particles at rate of 50,100 and 200 mg/L on organogenic regeneration of banana *in vitro* cultures, the result showed that callus growth was decreased by the increasing of nanoparticles dose. Meanwhile, the effect of TiO_2NPs on *Hordeum volgare* tissue culture. Showed that the callugenesis and the size of callus were increased with 60 mg L^{-1} of TiO_2 NPs (Mandeh *et al*., 2012) [39].

In vitro growths of *Cayratia trifolia* with 0.2 mg/L ZnO nanoparticle, the NPs enhanced the percentage of callus and growth, (Gour *et al*., 2013) [22].

MS medium added with 100 mg/L zinc NPs produced 92% callus induction followed by 200 mg/L and 50 mg/L, as well as ZnO inducting 79.5% and 63.1 calluses, separately for shoot tip explants (Helaly *et al*., 2014) [28]. In addition, Ewais *et al*., 2015 they found that different concentrations, of an aqueous silver (0, 2, 4 and 8 mg/L^{-1}) with MS medium evaluated for callus production in (*solanum nigrum* L.) Also, they reported increased callus formation and callus fresh weight. Meanwhile, callus morphology de formations were found with higher concentrations of nanoparticles. However, the influence of multi walled carbon nanotubes (25-500 mg ml) on leaf explant development and callus induction was studied. The result showed that addition of multi walled carbon nanotubes at concentration from 25 to 50 mg/L^{-1} to callus; induction medium was significantly effective for improving growth. Meanwhile, callus biomass was decreased by the increasing of multi- walled carbon nanotubes at concentration from 100 to 500 mg (Ghorbanpour and Hadian, 2015) [21]. On MS media enriched with 15mg/l of ZnO nano and 3.0g of NaCl, callus development and plant regeneration in tomato plants were maximized. (Alharby *et al*., 2017) [5].

Many investigations have found that plant responses to CNT treatments are similar to those generated by biotic and abiotic stress factors. In general, the interaction of carbon nanotubes (CNTs) and other carbon nanomaterials (CNMs) with complete plants appears to be a highly complex process involving three interconnected components (plant, CNMs, and growth media), Fig (4) showed that the change in one of these components can entirely change the outcome (Zaytseva and Neumann, 2016) [64].

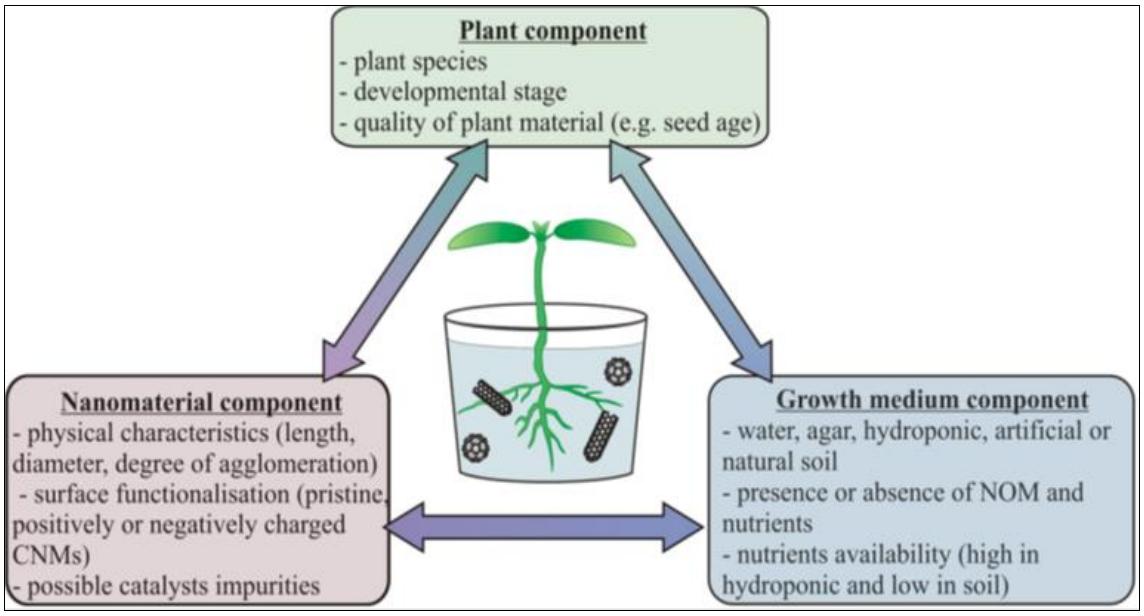

Fig 4: Plant, carbon nonmaterial, and growth medium relationships as a complex system determining nonmaterial influence on plant development (Zaytseva and Neumann, 2016) [64].

Amiri *et al*., (2016) [6] found that different concentrations of Zn sulfate ($ZnSO_4$) including 0, 2.43, 1.86 and 7.29 mg/L that used in MS basal salt medium containing 100 mg/L 2,4. D, 3mg/L zip, 3g/L activated charcoal, 30g/L sucrose, 40 mg/L adenine sulfate and 1 mg / L biotin were evaluated for callus culture development in date palm (*Estamaaran suger*L.), they reported that the best callus induction as callus fresh and dry weight belong to MS medium containing 2.43 mg/L ZnO NPs Meanwhile, higher ZnO NPs concentrations had negative effect on callus formation.

The effect of different concentrations of copper oxide (CuO NPs) on tissue culture of (*Oryza sativa* L.). The obtainable organogenesis was significantly increased at 15-20 mg/L CuO NPs, (Anwaar *et al*., 2016) [7]. On the other hand, Fazal *et al*., (2016) [19] reported that silver and gold nano were tested in MS media at varied concentrations (1:2, 1:3, 2:1, and 3.1) alone or in combination with naphthalene acetic acid (NAA) for callus culture development in (*pruncella vulgaris*L.), they reported that Ag NPs (30 ug/L) improved callus proliferation (110 D %). Meanwhile, Mousavi and Lahouti, (2018) used zinc oxide nanoparticles (ZnO NPs), ZnO bulk particles (BPs.) and relevant metal ions (Zn+) particles at different concentrations (10, 25, 50 and 100 mgL^{-1}) as ZnO on the tissue culture of rapeseed (*Brassica napus* L.). The results showed that callus and shoot regenerations was significantly induced at 10 mg/L^{-1} of ZnO NPs, it can be concluded that optimum concentration of ZnO NPs can be beneficial for inducing callus and hoot regeneration in the plant tissue culture. However, higher concentrations of ZnO NPs in the MS medium of tissue culture plants decreased the development of callus and regeneration of shoots (Khan *et al*., 2019) [34]. Many studies have shown that ZnO nano during application periods increased date palm shoot regeneration (Zayed *et al*., 2020) [63]. In addition, Ahmed, 2021 [3] concluded that higher concentration of 150 mg/L^{-1}ZnO NPs to MS medium of date palm, exhibited a slight growth and a brown color the callus tissue. Also, development of buds was also low after 6 and 12 weeks on the respective medium. The addition of ZnO NPs and SeO NPs to MS medium increased callus extract of *Ziziphus spina Christi*. Changing the color to yellow and red after mixing callus extract with zinc acetate and selenium oxide was detected (Islam *et al*., 2021) [29].

The higher shoot growth and bud sprouting percentage of olive explants were obtained on OM medium supplemented with nanoparticles Ag NPs) at low concentrations of 5 mg / L, (Hegazi *et al*., 2021) [27].

4. Input of nanomaterials toward the growth of shoot and proliferation:

Shoot number of *Stevia rebaudiana*, was improved when the stem of explants was cultured on MS medium supplemented by copper oxide nanoparticles (CuO Nps) (Kalpana *et al*., 2009) [33]. However, the maximum number shoots, growth and shoot induction rate of *tecomella undulate* were achieved on the medium containing l0 mg/L Ag Nps, (Aghdaei *et al*., 2012) [2]. In addition, inclusion Ag nano at 50mg/L on MS media significantly increased seedlings growth of *Brassica Juncea*. L (Sharma *et al*., 2012) [49]. In chrysanthemum plants, growth improved was maximized on medium containing 16 mg/L of silver nanoparticles dose (Tung *et al*., 2018) [58]. Similarly, the addition of Ag NPs at 60 mg/L to *tecomella undulate* suspension culture improved the growth characteristics such as number of shoots, shoot length and percentage of produced shoots (Sarmast *et al*., 2015) [46]. Meanwhile, significantly increased shoot length and Fresh weight by 52% and 39%, respectively compared with control of *verbnabi pinanatifida* seedlings was achieved on MS medium supplemented at 5 mg/L^{-1}(Cu So4) NPs (Genady *et al*., 2016) [20]. The inclusion of 1-2 mg/L^{-1} ZnO NPs in to MS medium improved that growth of stem explants (Zafar *et al*., 2016) [62]. In the presence of SiO_2-NPs, the plant height of banana (7.5 cm) was obtained when nodal explant was cultured on MS medium supplemented with 150 ppm SiO_2- NPs. In addition, leaves number increased to 6.67 and 7.00 leaves in addition of 150 ppm SiO_2- NPs (EL-kady *et al*., 2017) [14].

On *stevia rebaudiana*, Javed *et al*., (2017) [31] found that the addition of 1mg/L^{-1} ZnO NPs to the culture medium gave the highest frequency of shoot formation (89.6%). Also, Selivanov *et al*., (2017) [48] indicated that adding Ag NPs at rate of 30 mg/L to the media improved biomass growth of *Arabidopsis thaliana*. In addition, Spinoso-Castillo *et al*., 2017 [52] showed that bacterial contamination was reduced at 50, 100 and 200 mg/l of Argovit. Growth stimulation was observed at 25 and 50 mg/l of Argovit, while significant inhibition was detected at 100 and 200 mg/l of Argovit.

On the other hand, Venkatachalam *et al*., (2017) [59] reported that addition of MS medium containing 2.5 mg/L GFAg NPs 50 mg /L Ad combination increased shoots, Also, addition of 0.5 mg/L GFAg nano + 50 mg/L Ads combinations giver 51.4 % of multiplication shoot 38.5 shoots/culture of *Alternanthera sessilis* L.

Meanwhile, Dang *et al*., (2018) [13] shown in fig. (4) that addition of nano silver (Ag NPs) at 1 ppm in MS medium of banana plantlets increased shoot length (2.45cm), maximum number of shoots (8.40), The highest rate of fresh weight /dry weight (2.24/0.2g) and the most leaves (12.10) These values were 1.5–2 times greater than explants cultured in media without nano silver. Meanwhile, the number of shoots per explant was 1.4-1.6 times higher in the medium added with Ag nanoparticles at 3 or 5 ppm.

Fig 5: Effect of nano silver different concentrations on banana shoots culture (1- control: 0 ppm; 2- SH1: 1 ppm; 3- SH2: 3 ppm; 4- SH3: 5 ppm;5- SH4: 7 ppm (Dang *et al*., 2018) [13]

In addition, Hassan *et al.*, 2019 [26] found that addition of nano silver (Ag Nps) at 5 mg/L^{-1} in MS medium of olive plantlets increased explant development followed by Ag NPs at 10 mg/L^{-1} as compared with the control. However, Awad *et al*., (2020) [8] studied the effect of ZnO NPs on growth promoting of date palm cultured tissues, the data indicated that two-fold of increase multiplication rate of proliferated shoot was observed with ZnO NPs at 150 mg/L^{-1} as compared to the control treatment. The multiplication rate was 46.6% at control and 86.67% 93.34% in ZnO NPs before and after sterilization.

5. Input of nanomaterial's towards on rooting: -

Many researchers have demonstrated that, manipulating nonmaterial in growth medium can have a considerable impact on the quality of various plant species' cultures. Exogenous treatment of nano SiO_2 particles on Changbai larh (*Larix olgensis*) seedlings improved growth and seedling quality, including average seedling height, root collar diameter, main root length, and lateral root number of seedlings, (Bao-Shan *et al*., 2004) [9].

However, different types of nonmaterial multi walled carbon nanotubes (MWCNTS) (Ag, Cu, ZnO and Si) and their corresponding bulk counter parts a root elongation of cucurbitapepo (*zucchini*). Was studied since zinc oxide nanoparticles have no negative effect on the variables under study (Stampoulis *et al.*, 2009) [53]. Also, higher CuO nanoparticles at 9 mg/L-1 level in the medium increased leaves number (8.56), roots (11.00), and root length (6.40 cm) / plantlets of banana plantlets (Hasanin *et al*., 2021) [25].

In plant tissue culture, increasing shoot and root lengths of two tested corp plants (*pahseolus vulgaris* L. and *zeamays* L.) *in vitro* were achieved on medium containing of silver nanoparticles from 20 to 60 ppm (Salama, 2012) [45]. In addition, the positive effect of Ag NPs may be contributed to increased root length in Barley (Gruyer *et al*., 2013) [24].

Helaly *et al*., (2014) [28] found that the best medium for rooting from shoot tip explants of banana was MS media added with Zn NPs at 100 mg/L which caused the maximum rooting (6.57%) average number of roots /shoot and (2.93 cm) maximum average root length followed by treatment consisting of MS media added with 150mg/L nano Zn and ZnO NPs at 100 mg/L and 150mg/L, respectively.

In *Oryza sativa*, growth of the plants was maximized on lower dose of CuO NPs. On the other hand, high concentrations in the medium reduce the plant biomass, root and shoot growth (Da costa, 2016) [12]. In addition, increasing root length 21F over the control of *verbena bipinnatifida* nut seedling was achieved on MS medium containing 5 mg/L^{-1} $CuSo_4$ NPs (Genady *et al*., 2016) [20]. Meanwhile, Zafar *et al.*, (2016) [62] found that the production of thin roots with thick root hairs was achieved on MS medium supplemented zinc oxide (ZnO) nanoparticles at 10 mg/L^{-1} of *Brassica nigra* L. Increasing root formation of brassica nigra plants *in vitro* was achieved on containing MS medium to 1-20 mg/L^{-1}ZnO nanoparticles, however, enhance shoots of Stevia *rebaudiana* was observed when used MS medium supplemented with (0.1 – 1000 mg/L) ZnO NPs, (Zafar *et al*., 2016 [62] and Javed *et al*., 2017) [31].

Increasing lateral roots number (9.33) and root length (15 cm) of banana shoots *in vitro* (fig.6) were achieved on MS medium containing 150 ppm SiO_2 NPs after 30 days (EL-Kady *et al*., 2017) [14].

Fig 6: The effect of nano silicon dioxide on banana "Musa sp" in rooting stage under the treatments described in the method, (EL-Kady *et al*., 2017) [14]

Several studies have positive effect of Ag NPs on rooting of banana plantlets. Shown in fig (7) the highest roots number (7.10) and roots length (7.70 cm) for plantlets of *in vitro* an addition, increase shoot length (2.90 cm) and number of leaves per shoot (4.40) on MS medium added with 3 ppm Ag NPs (Dang, *et al*., 2018) [13].

Fig 7: Effect of nano silver different concentrations of banana plantlets incubated *in vitro* (R0: 0 ppm; R1: 1 ppm; R2: 3 ppm; R3: 5 ppm; R4: 7 ppm), (Dang *et al*., 2018) [13].

In addition, higher increasing in root length of Maize was noticed in the medium supplemented with CuO NPs as compared with the controlled samples (Toqeer *et al*., 2020) [57]. Also, increasing percentage of rooting and number of roots of date palm was recorded when added 50 or 75 mg / L ZnO nanoparticles to MS medium (Ahmed, 2021) [3].

6. Input of nanomaterial's towards on growth development of exvitro: -

Several researches have shown that the effect of nonmaterial towards on growth development of exvitro investigated the effect of silver particles (20 to 60 ppm) on the growth of plantlets (*Phaseolus vulgaris* L and *Zea mays* L), the results showed that lower concentrations of silver nanoparticles had stimulating effect on the growth of plantlets. While, higher concentrations induced an inhibitory effect. Meanwhile, an increase shoot and root lengths and leaf surface were observed when the increased the concentration of silver nanoparticles from 20 to 66 ppm, (Salama, 2012) [45].

In addition, the effect of silver nanoparticles (0, 25, 50, 100, 200 and 400 ppm) on growth and development of *Brassica Juncea* seedlings was studied. The result showed that root and shoot length, Fresh weight and vigor index of seedlings ins positively at affected by using sliver nanoparticles It induced a 183% increase in vigor index of the treated seedlings and 326% increase in root length (Sharma *et al*.,

2012) [49]. However, Helaly *et al*., (2014) [28] found that the survival percent of banana plantlets was about 90% after 6 weeks of transfer to earth in pots containing soil and saved and remained constant.

Also, Soliman *et al*., (2015) [51] found that increasing in growth parameters either under saline or normal conditions of moringa peregrine plants sprayed with hogland solution containing ZnO NPs.

In addition, Dang *et al*., (2018) [13] studies the effect of nano silver on growth and development of ex vitro banana as shown in Fig (7). The plantlets were watered two times/ day by nano silver solution with different concentrations (0, 5, 10 and 15 ppm) and watered are time/week. The data indicated that the highest values of shoot length (4.8 cm), number of leaves per shoot (5.20 cm), number of roots (4.60 cm), root length (4.87 cm) and fresh weight (3.07) were observed when the plantlets water with solution containing 5 ppm Ag NP_s.

Fig 8: Effect of different concentrations of nano silver solution after 30 days at nursery garden Banana plants (N0: 0 ppm; N1: 5 ppm; N2: 10 ppm; N3: 15 ppm) (Dang *et al.*, 2018) [13].

7. Nanomaterial enhancement of secondary metabolites:

Several nanomaterials such as Ag NPs, ZnO NPs and Tio_2 have found application in enhancement of secondary metabolites. Ag NPs have a significant protein and carbohydrate and decreased the total phenol contents catalase and peroxidease activities of Ba *copamannieri* (Krishnaraj *et al*., 2012). However, Salama, (2012) [45] Found that increasing chlorophyll, carbohydrate and protein contents of the two tested crop plants (*phaseeolus vulgaris* L and *zea mays*L.) *in vitro* were achieved medium containing of silver nano particles from20 to 60 ppm.

In addition, hydrogen peroxide malondialdehyde (MDA) and proline contents were decreased through activating antioxidant enzymes of *Brassica Juncea* seedlings when explants were cultured on MS medium with 50 mg/L Ag NPs (Sharma *et al*., 2012) [49]. However, Poborilova *et al*., (2013) [40] found that increased the phenolic content were obtained with medium contains AL_2O_3 NPs (10-100 ug mL) tobacco cell suspension cultures the accumulation of phenolic in the cells was does and exposure time dependent. On the other hand, the increasing the content of artemisinin in hairy root cultures of *Artemeisa anua* was obtained on medium with 900 mg/L^{-1} Ag NPs for 3 days, (Zhang *et al*., 2013) [65]. In addition, Syu *et al*., (2014) [54] found that the highest levels of anthocyanin accumulations were obtained on MS medium supplemented with 25 and 50 mg/L Ag NPs. Also, increased the explants were obtained with increasing nano Zn and nano ZnO. High dose of nanoparticles (20 mg/L) caused about 8-fold increases in proline concentration. The increase was lo wet under nano Zn and the highest under nano ZnO, (Helaly *et al*., 2014) [28]. While, Chamani *et al*., (2015) [11] reported the effect of zinc Oxide NPs in *Liliumle debourii Bioss*, the maximum phenolic content of plantlets was obtained in the medium containing 75 mg/L^{-1} zinc Oxide NPs. Meanwhile, increased anthocyanin content with HA at 100 mg/L^{-1}, the highest Flavonoid content measured at 270,300 and 330 nm wave lengths was observed in plantlets treated with zinc oxide NPs at25 mg/L^{-1} combined with HA at 100 mg/L^{-1} respectively.

However, the highest content of phenolics, flavonoids, rosmaric acid and café acid was obtained on B5 a medium with 100 or 250 ug m/L multi – walled carbon nano tubes in *stevia khuzestainca* leaf cultures Ghorbanpour and Hadian, (2015) [21]. In *prunella vulgaris* the maximum accumulation of total phenolic compounds and flavonoids in callus cultures of *prunella vulgaris* was obtained on MS medium supplemented with Au-Ag NPs (1:3), (Fazal *et al*., 2016) [19]. On the other hand, Genady *et al*., (2016) [20] reported that addition of 5uM $CuSo_4$ NPs to *verbena bipinnatifida* cultures significantly increased the total phenolics in the seedling of *verbena bipinnatifide* was enhanced. The addition, of Cu and CuO NPs to *Menthe alongifolia* cultural increase the essential oil content by 2.226 and 2.19% respectively, Talankan *et al*., (2016) [56].

In plant tissue culture, the maximum total antioxidant capacity, total reducing power, total flavonoid content and total phenolic content were obtained in the medium containing 1 mg/L^{-1} zinc oxide NP_s of *Trigonella foenum-graecum*L explant, Jasim *et al*., (2017) [30]. Meanwhile, El-kady *et al*., (2017) [14] found that after 30 days of culturing on rotting media in MS a species shoots treated with 50,100 and 150 ppm SiO_2 NPs, total chlorophyll content was 15.76,16.83 and 22.57 CCI as compared with the control treatment without Sio_2 NPs (9.57 CCI). On the other hand, total phenol content of leaf decreased at 100 and 150 ppm concentrations and increased at 50 pm compared with the control.

On the other hand, supplementation of silver NPs in MS medium for regeneration of *vanilla planifolia*. The maximum phenolic content, antioxidant capacity and lipid peroxidation were obtained in the medium containing with 25 and 50 mg/L, (Spinoso-Castillo *et al*., 2017) [52]. In addition, (Dang *et al*., 2018) [13] Found that total chlorophyll (1.24 mg g^{-1}) was more than control (0.69 mg g^{-1}) when explants were cultured on MS media supplemented with 7 ppm Ag NPs. Total chlorophyll content was 3 folds. Higher than those explants culture on medium with nano silver when the explants were culture on MS medium supplemented with 3 ppm Ag NPs on vitro banana.

The high concentration of ZnO NPs (150 mg/L^{-1}) caused a significant induction in carbohydrates production treated and reached to 8.7 and 8.35 mg g^{-1}fwbefor and after sterilization, in addition, total Soluble protein an accumulation increased at 150 mg/L^{-1}NPs, two folds' increase in protein an accumulation was observed in an above-mentioned treatment compared to control. However, free amino acid content of cultured date palm tissues under NPs application, free amino acid level was 1.20 mg g^{-1} FWin control treatment and reached 2.46 and 2.12 mg g^{-1}fresh weight in ZnO NPs ate 150 mg/L before and after autoclaving (Awad *et al*., 2020) [8].

El Mahdi and Elazab, (2020) [15] reported that high ZnO NPs concentrations (100mg/l) increased the totalchlorophyll

content in leaves on *Pome granate*. In addition, in citrus maximized, higher concentrations of ZnO Nps inhibited leaf chlorophyll accumulation, (Xiao *et al*., 2019) [61].

The highest significant accumulation of chlorophyll a,b and total chlorophyll content were noticed when date palm was cultured on MS medium contained with 75 mg / L ZnO NPs alone (Ahmed, 2021) [3]. Also, the highest amount of chlorophyll was noticed in medium supplemented with 9 mg / L^{-1}st@cuo NPs of Banana plantlets (Hasanin *et al*., 2021) [25].

8. Future prospects

It can problem for tissue cultured of nano material because they can be a risk for humans consuming these in the products. NPs have proved their favorable roles in the first phase, through callus induction, shoot proliferation, shoot elongation, rooting and secondary metabolite production. Nanotechnology is an interdisciplinary subject with a wide range of applications. The incorporation of nanomaterials in to the culture medium had good potential for removing microorganism and survival percentage several investigations have shown that NPs treatments can several harm explant and have a negative impact on their regeneration ability. the toxicity of nanomaterials aerial depends up on the concentration and surface characteristics. while, including NPs into the culture media removes microbial pollutants and inhibitor of ethylene action or its production using Ag No_3 has shown stimulatory effects on callus morphology and alter DNA and protein profiles because nanoparticles play central role in photosynthesis antioxidant and reactions, metabolic respiration and hormones biosynthesis and perception. the positive effects of nanoparticles a callus production and growth parameters during micropropagation stage can be explain is an essential nutrient in plant growth regulators such as IAA, NAA, IBA, BAP, GA_3 and acts as cofactor in many metallo proteins and cell wall metabolism. the effect of nanomaterials on secondary metabolite formation in several key plant species as well as the chemical and physiological charactrics increased with nanoparticles because different NPs plays important role in the activation of several enzymes like peroxides, catalase and nitrate reductase activity which also Favors regeneration through effecting important physiological and biochemical characteristics.

9. Conclusion

Plant nanobiotechnology is quickly gaining traction as a hot issue with huge promise for plant growth. More focused study is required to clarify and simplify the process so that just the beneficial aspects are utilized while the negative consequences are avoided. It's also uncertain how nanoparticles affect callus induction, organogenesis, shoot multiplication, shoot elongation, and roots. In the presence of NPs, the effects of cultures on callus proliferation and shoot multiplication must also be carefully explored. Furthermore, a few studies have shown that the presence of NPs in culture conditions increases soma clonal variation; consequently, long-term exposure of plant cultures to NPs may have a negative effect on morphogenesis. Increased bioactive component content can be achieved by adding NPs to cell, shoot, and root cultures, perhaps owing to the formation of reactive oxygen species (ROS), activation of antioxidant enzymes, and regulation of certain genes; however, the mechanism remains unclear.

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11. Declarations

Author contribution statement: All the authors contributed in the research; sayed Hassan collected the data, and prepared the state; Nagwa Zaied planed the work and write the State of arte.

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12. Additional information

No additional information is available for this state arte.

13. List of abbreviations

BAP. = 6-benzylaminopurine.
GA_3= Gibberellic acid.
IAA= Indole acetic acid
IBA= Indol-3-butyric acid
MS Murashige& Skoog
µL=Micro liter(s)
mg/l= milligram/litter
Mm=Millimeter
2,4-D=2,4-dichlorophenoxy
OM=Olive medium
Chl= chlorophyll
CNTs = carbon nanotubes
NPs= nanoparticles
NMs= nonmaterial's
MDA=malondialdehyde

14. References

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