The Pharma Innovation Journal 2023; 12(10): 2539-2547

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 2539-2547

www.thepharmajournal.com
Received: 25-08-2023
Accepted: 29-09-2023

Bharti Yadav
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Kiran Dhatterwal
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Rakesh Kr Prajapat
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Garima Vaishnav
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

RS Sain
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Manas Mathur
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Corresponding Author:
Manas Mathur
School of Agriculture, Suresh Gyan Vihar University, Mahal Road Jagatpura, Jaipur, Rajasthan, India

Effect of green synthesis of copper nanoparticles on callus induction and quantification of phenolics in *Sorghum bicolor* (L.) for abiotic stress tolerance

Bharti Yadav, Kiran Dhatterwal, Rakesh Kr Prajapat, Garima Vaishnav, RS Sain and Manas Mathur

Abstract
Callus is an unorganized tissue mass growing on the solid substrate. It is a group of cells derived from competent source tissue that is cultured under *in-vitro* conditions to form an undifferentiated mass of cells. In this study/thesis the Effect of green synthesis of copper nanoparticles on callus induction and quantification of phenolics was observed in *Sorghum bicolor* for abiotic stress tolerance. Studies on micropropagation of callus culture was undertaken. In which explants were inoculated in MS medium fortified with various concentration of 2,4-D, auxin (IAA, NAA and IBA), cytokinin (BAP and Kn), along with that callus induction was also observed using 2,4-D alone. The best callus induction was observed on the medium supplemented with BAP 1.0 mgL^{-1}+ IAA 2.0 mgL^{-1} and BAP 1.0 mgL^{-1}+ IAA 0.1 mgL^{-1} and BAP 1 mgL^{-1}+2 mgL^{-1}NAA respectively and also the growth index of best callus was measured at 2, 4, 6 and 8 weeks. The best callus was sectored into small pieces and further transferred to the different concentration of copper nanoparticles (i.e., 5 mg, 10 mg and 20 mg) and reported novel successful *in-vitro* effects of Cu-NPs on callus of *Sorghum bicolor* to enhance phenolic contents which are important compounds in pharmaceutical industries especially anti-oxidant medicaments. Different concentration and combinations of cytokinins and auxins were again used that were involved in triggering shoot and root generation from callus respectively. The medium with highest concentration of BAP + KN (1.0+0.2); BAP + NAA (1.0+0.2); BAP + KN + NAA (2.0+1.0+0.2) achieved the maximum and multiple shooting. Furthermore, decreasing the concentration of these PGRs also reduced the number of regenerated shoots. Excised shoots from *Sorghum bicolor* were rooted on full strength MS medium with the combination of highest concentration of IBA (3 mg L^{-1}) when given in combination with NAA (0.5 mg L^{-1}) showed maximum roots. In the study the optimal ratio of cytokinins and auxins was taken that promoted the growth of shoots and roots respective of the higher concentration of cytokinins and auxins in the callus.

Keywords: Nanoparticles, PGRs, *Moringa oleifera*, callus, phenols

Introduction
The term "nanotechnology" refers to the branch of technology that operates at the nanoscale and works with parameters and tolerances smaller than 100 nano meters, particularly when it comes to the manipulation of specific atoms and molecules. Research on atomic, molecular, and macromolecular scales that permits the controlled modification and study of structures and devices is also referred to as "nanotechnology." At this scale, things, such "nanoparticles," develop novel properties and functions that differ greatly from those seen at the bulk scale. The name "nanoparticle" is derived from the Greek word "nanos," which meaning "dwarf" or "extremely small." These ultrafine particles have a size range of 10-100 nm. Depending on where the nanoparticles are produced, it may be intracellular or extracellular. Metallic nanoparticles have been created using a range of physical and chemical processes [1]. For the creation of NPs, traditional methods have been utilised for a long time, but studies have shown that green approaches are more effective due to their lower cost, less failure risks, and simplicity of characterisation [2]. Use of plant extracts, fungi and bacteria comes under biological methods [3]. The green method of nanoparticle synthesis is straightforward, effective, and safe for the environment, in contrast to chemical-mediated. Toxic solvents, high pressure, energy conversion, and high temperatures are all part of chemical synthesis. Due to the required laboratory maintenance, microbial synthesis is not industrially practical. [4]. Metal nanoparticles have gained more attention in recent years due to their potential for applications in many fields [5-8].

Copper based nanoparticles are gaining importance due to their applications in catalysis, printed electronics, sensors, *etc.* [9]. Synthesis of copper nanoparticles [10] based on the chemical precipitation method employs harsh reducing agents and organic solvents. The toxicity issue is made worse by the presence of these dangerous substances on the surface of nanoparticles, while environmental problems are brought on by the usage and disposal of toxic solvents. Green and eco-friendly nanoparticle synthesis has gained popularity recently as a solution to these issues. Only a small number of papers, however, describe the environmentally friendly synthesis of copper nanoparticles using reducing agents such as ascorbic acid, plant gums, plant extracts, and microbes. [11]. Different metal nanoparticles, including silver (Ag), copper (Cu), and zinc (Zn) nanoparticles, are preferred for use as antimicrobial agents. Silver is an expensive metal because it costs more to prepare products based on Ag-NPs, while Cu is significantly less expensive and widely accessible. Consequently, it is economical to use copper nanoparticles (Cu-NPs) in a variety of agricultural applications. [12]. Different techniques have been used to create and characterise copper nanoparticles. The attributes of copper nanoparticles are highly dependent on their synthesis processes, and several ways are currently available to manufacture these nanoparticles with controlled size and shape.

Material and Methods

Plant Material

Seeds of *Sorghum bicolor* var. CSV17 was obtained from the ICAR-Indian Agriculture Research Institute, Pusa, Delhi, India and then stored in darkness at 15 °C before use in the experiments.

Callus induction

As an energy source, MS medium was augmented with 30 g/L sucrose before being brought to final volume and solidified with 0.8% bacteriological grade agar. The seeds were cultured on MS medium with12 different culture medium flasks which were supplemented with different concentrations and combinations of PGRs i.e., 2,4-D (1.0, and 4.0 mg/L), BAP hormones (1.0, 3.0 mg/L), IAA (2.0, 1.0 and 0.1 mg/L), IBA (0.2, 0.3, 3.0 and 4.0 mg/L), NAA(0.2 and 2.0 mg/L), Kinetin (0.01 and 0.1 mg/L), IBA (0.2 and 0.3 mg/L) along with that callus induction was also observed using 2,4-D alone and out of which one was control i.e., hormone free medium. The effects of 2,4-D, KN, IAA, IBA, BAP, and NAA on callus induction were examined using an orthogonal experimental design. With 1 N NaOH or 1 N HCl, the medium's pH was brought down to 5.8 after being provided with the appropriate growth regulators. At intervals of two weeks, all of the preceding cultures were moved to fresh media, and the callus growth index was subsequently computed.

Subculture

The callus was also exposed to various concentrations of copper nanoparticles (control, 5 mg, 10 mg, and 20 mg), and it was retrieved every two weeks for the quantification of phenolic content until it had healed for eight weeks. Later, the greatest phenolic-containing callus was collected and transferred to medium along with the best concentration and combination of auxin and cytokinin for plant regeneration.

Regeneration media

Following treatment with various concentrations of Cu-NPs, high-quality calluses with the highest phenolic content were chosen, and they were fortified with various concentrations and combinations of cytokinins such as BAP (1.0, 1.5, 2.0 mgL^{-1}) and Kn (0.1, 0.2, 0.3, 1.0, 2.0, 3.0, 4.0 mgL^{-1}) and auxins such as NAA (0.1, 0.2, 0.3, 0.5, 2.0, 3.0 mgL^{-1}), IAA (0.5, 1.0, 2.0 mgL^{-1}), IBA (1.0, 2.0, 3.0, 4.0, 5.0 mgL^{-1}). Prior to being transferred to light for two weeks at a temperature of 25±1 °C, all cultures were cultured in the dark for four weeks. Regular observation was conducted, and infected cultures were taken from the shelf and thrown away. Plantlets that had been developed *in vitro* were prepared for field transfer from aseptic culture and were ready for additional growth, hardening, and acclimatisation. Later on, after shooting and rooting they were shifted to the greenhouse.

Green Synthesis of Cu-NPs

Initially Copper sulphate was purchased from Sigma Aldrich and they were reduced biologically using the fresh leaves of *Moringa oleifera*. 0.3 gm leaves were weighed and were grinded properly in the mortar and pestle for 15 to 20 minutes in distilled water. Now these grinded laves were taken in the vials (3-4) and it was centrifuged at 4 ºC, 1000 rpm for 5 minutes. Further supernatant was collected in fresh vial. 0.01 gm, tungsten sulphide was taken in 100 ml autoclaved distilled water in a washed and clean conical flask, which was then kept on magnetic stirrer. Supernatant collected in the vial was added to the flask containing the copper nanoparticle. The mixture was kept overnight and the alteration in the color of the solution was noted down. The variation in the color of mixture is the indicator of reduction of synthesized tungsten sulphide

Characterization of Cu-NPs

Copper nanoparticles synthesis by biological method, were characterized by UV-Vis spectroscopy, and SEM to identify the shape, size and crystalline nature of synthesized particles.

Quantification of Phenolics

Due to its long history of use as a spectrophotometric assay to assess total phenolics in plant materials, the Folin-Ciocalteu method will be utilised in the current study [13]. The total phenolic content was calculated by mixing 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and 2.0 ml of 7.5% sodium carbonate with 0.5 ml of the aqueous extract (0.5 g of callus was macerated in 1 ml of distilled water for 10 minutes, and additional supernatant was collected). The reaction mixture was incubated at 45 °C for 40 min, and the spectrophotometer was used to detect the absorbance at 765 nm. Gallic acid served as the industry standard for phenol.
[14]. The total phenol content was calculated as milligrams of gallic acid equivalents/g extract using the mean of three readings.

Statistical analysis

The statistical error of mean was calculated by the following formula:-

$$S.E. = \frac{\sigma}{\sqrt{n}}$$

Where,
σ = standard deviation
n = number of observations

The test of significance (t-test) was calculated by the following formula

$$t = \frac{m_1 - m_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$$

Where,
m_1 = Mean of one set of values.
m_2 = Mean of second set of values.
SEM_1 = Standard error of the first set of values.
SEM_2 = Standard error of the second set of values.

The probability 'p' for obtaining 't' value of at least as great as the calculated one for a given number for the degree of freedom was found in the Fisher' stable.
The p-values were signified according to the following conventions.

$p<0.05$ = Difference was almost significant.
$p<0.01$ = Difference was significant.
$p<0.001$ = Difference was highly significant.

Result

Callus induction from seeds

Table 2 lists the outcomes for callus induction. Media combination MS_{00}, MS_{07}, MS_{08}, MS_{10} MS_{11} showed no response in inducing callus. Media combination MS_{01}, MS_{02}, MS_{03}, MS_{06}, MS_{12} showed poor performance in inducing callus. However, highest callus induction was obtained on the medium MS_{04}, MS_{05} and MS_{09} supplemented with BAP 1.0 mgL^{-1}+ IAA 2.0 mgL^{-1} and BAP 1.0 mgL^{-1}+ IAA 0.1 mgL^{-1} and BAP 1 mgL^{-1}+2 mgL^{-1}NAA respectively.
The probability of survival and proliferation of *Sorghum bicolor* callus tissue on subculturing the callus during the 5th - 6th week was higher in comparison to subculturing after 7th - 8th week, as shown in Fig. 1. Callus growth was represented by a growth index, which was calculated using the following equation and results are shown in Table 1.
Callus growth index = (Final callus fresh weight − Initial callus fresh weight)/Initial callus fresh weigh) × 100
We observed that growth index of callus obtained at 2 weeks was 0.75, at 4 weeks 1.10, at 6 weeks 3.06 and at 8 weeks 2.65 was obtained and maximum growth index was obtained at 6^{th}.

Table 1: Growth index of callus in every two weeks

S. No.	Age of Tissue (In weeks)	Growth Index
1	2	0.75
2	4	1.10
3	6	3.06
4	8	2.65

The absolute media with highest callusing exhibiting friable and embryogenic type callus was sub cultured in fresh media having different concentration of copper nanoparticles and media with MS + BAP 1.0 mgL^{-1}+ IAA 2.0 mgL^{-1} was further used.

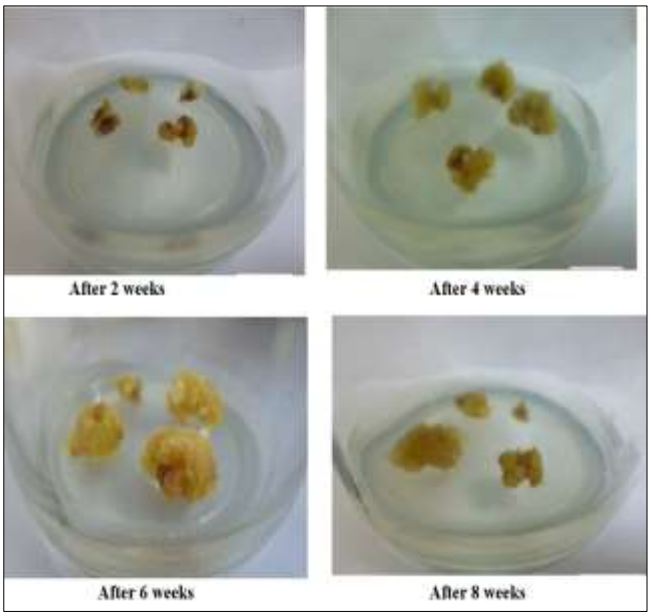

Fig 1: Induction of Callus

Table 2: Result and analysis for callus induction using different combinations of PGRs concentration

Growth Hormones	Modified MS media	Concentration (mgL^{-1})	Seeds Used as explants		
			Biomass	Morphological appearance	Nature of Callus
(i) Without hormone MS	MS_{00}		-	-	-
(ii) Callus induction	MS_{01}	1.0+0.5	C^{+}	BN	FG
MS+2,4-D+KN	MS_{02}	1.0+0.1	C^{++}	BN	FG
MS +BAP+IAA	MS_{03}	4.0+0.01	C^{++}	BN	FG
MS+BAP+IBA	MS_{04}	1.0+2.0	C^{+++}	GN	FG
MS +BAP+NAA	MS_{05}	1.0+0.1	C^{+++}	BN	FG
MS+IAA+IBA MS+2,4-D	MS_{06}	1.0+0.2	C^{++}	BN	FG
	MS_{07}	3.0+3.0	-	-	-
	MS_{08}	3.0+4.0	-	-	-
	MS_{09}	1.0+2.0	C^{+++}	GN	FG
	MS_{10}	1.0+0.2	-	-	-
	MS_{11}	1.0+0.3	-	-	-
	MS_{12}	1.0	C^{+}	BN	FG

C+/C++/C+++ = Amount of callus produced, Abbreviation: FG-=Fragile, GN= Green, BN= Brown, - = Noresponse

Green synthesis of Nanoparticles

When the plant leaf extract was added to the copper sulphate powder, the colour of the mixture changed from light yellow to light green, and then, as seen in Fig. 2 and 3, from brownish to dark brownish, indicating the creation of copper nanoparticles. Additionally, different methodologies were used to characterise nanoparticles.

UV-Visible Spectroscopy

To analyse the structure and optical characteristics of metal nanoparticles, Ultraviolet vis. Absorption is a crucial technique. The solution of colloidal Cu-NPs is distinctively yellow-orange. The copper sulphate solution changed from pale green to brownish once the aforementioned plant extracts were added. The final hue will slowly darken and turn dark brown. The peak for synthetic Cu-NPs was in the region of 490–500 nm, as shown in Fig. 4. Copper nanoparticles' reported absorption bands are in the range of 200–600 nm.

Scanning Electron Microscopy

SEM was used to examine the morphological traits and particle size of biogenic Cu-NPs. The SEM scan showed that the biogenic Cu-NPs had a spherical form and were aggregated, with an average diameter of 80 nm (Fig. 5).

Fig 2: Light green colour before reduction of NPs

Fig 3: Reduction of nanoparticles as shown by change in colour from light green to dark brown

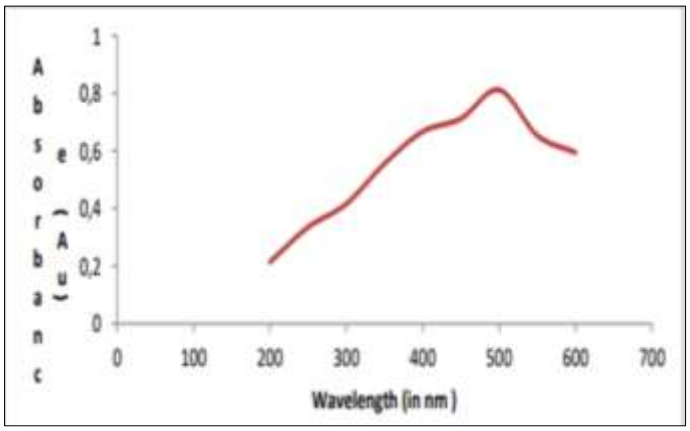

Fig 4: UV Spectra of Copper nanoparticles prepared from *Moringa oleifera*

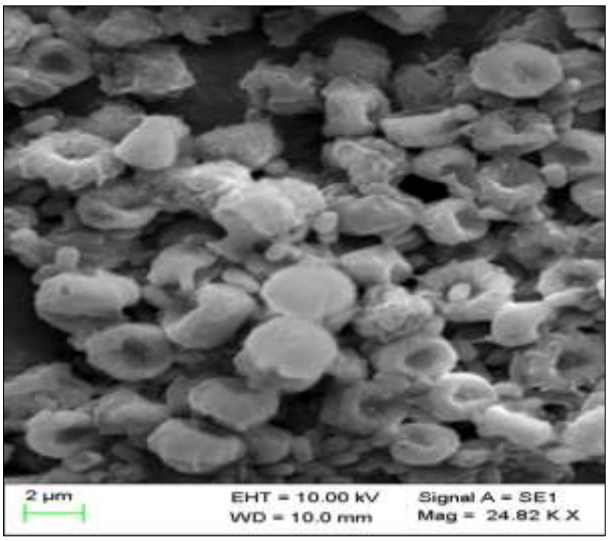

Fig 5: 80nm sized nanoparticle was observed by Scanning Electron micrograph (SEM).

Subculture of Calli

The generated calli from the explant were transferred on MS + BAP 1.0 mg L^{-1}+ IAA 2.0 mg L^{-1}per and supplemented with various concentrations of copper nanoparticles in four flasks: control, 5 mg, 10 mg, and 20 mg. As a result, best regeneration of callus was observed after 45 days on media supplemented with MS + BAP1.0 mg L^{-1} + IAA 2.0 mg L^{-1}+ 10 mgCu-NPs/30 ml. Size and weight were observed of best callus i.e., 1.09cm in diameter and 1.12 gm respectively as variation of weight was also observed on different conc. of Cu-NPs as shown in Fig. 6. At 10 mg of Cu-NPs the highest callus was recorded which was more than control. On the other hand, as demonstrated in Fig. 7, callus significantly decreased when Cu-NPs concentrations were increased over 10 mg. Calli's colour transitioned from off-white to lemon yellow over the course of a month. The calli had a mushy, slimy texture, but they were also progressively larger and less compact. On the surface of a few calli, little spherical formations also developed.

A) Control+5 mg Cu-NPs (B) Control + 10 mg Cu-NPs

(C) Control + 20 mg Cu-NPs

Fig 6: Result of different concentration of Cu-NPs

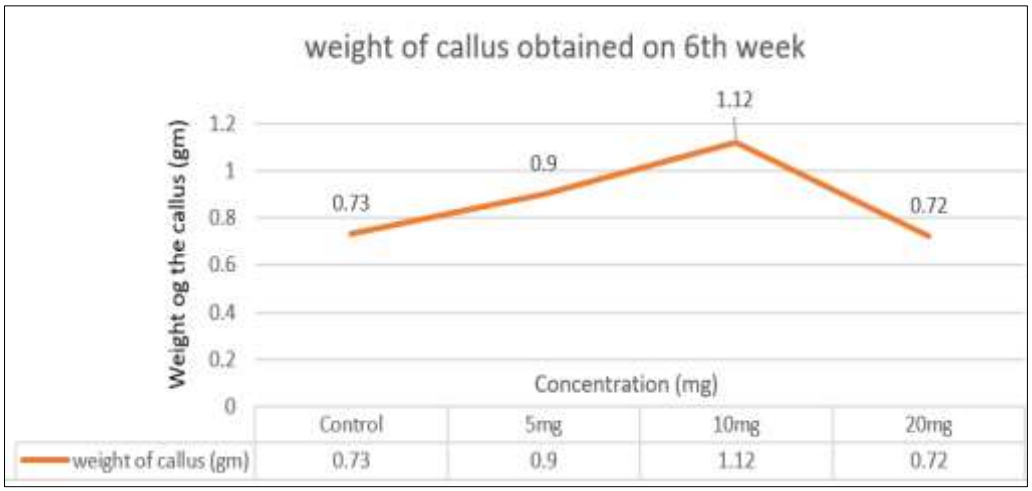

Fig 7: Weight of the callus obtained on subculturing it using different concentration of Cu-NPs on 6th week.

Table 3: Result and analysis for plant regeneration using different combinations of PGRs concentration

Growth Hormones	Modified MS media	Concentration (mgL^{-1})	Biomass
MS + BAP+KN	MS_{01}	1.0+0.1	C^{+}
	MS_{02}	1.5+0.2	C^{++}
	MS_{03}	2.0+0.2	$C^{+msh+++}$
	MS_{04}	2.0+1.0	C^{+msh+}
MS +BAP+NAA	MS_{05}	1.0+0.1	C^{+msh+}
	MS_{06}	1.0+0.2	$C^{+msh+++}$
MS +KN+IAA	MS_{07}	1.0+0.5	C^{+msh}
	MS_{08}	1.0+1.0	-
	MS_{09}	2.0+1.0	C^{+msh+}
	MS_{10}	3.0+1.0	-
	MS_{11}	4.0+2.0	-
MS +BAP+KN+NAA	MS_{12}	1.0+0.1+0.1	C^{+msh+}
	MS_{13}	2.0+1.0+0.2	$C^{+msh+++}$
	MS_{14}	2.0+0.3+0.3	C^{+msh+}
MS +IAA+IBA	MS_{15}	1.0+1.0	C^{+R+}
	MS_{16}	1.0+2.0	C^{+R+}
	MS_{17}	0.5+3.0	C^{+R++}
	MS_{18}	1.0+4.0	C^{+R+}
	MS_{19}	2.0+5.0	C^{+R+}
MS +IBA+NAA	MS_{20}	1.0+2.0	C^{+msh+}
	MS_{21}	2.0+3.0	C^{+R+}
	MS_{22}	3.0+0.5	C^{+R+++}
	MS_{23}	3.0+0.3	C^{+R++}

Regeneration of plants from plated calli on MS Medium which showed maximum phenolic content msh+/ msh++/msh+++ = Multiple shoots, R+/R++/R+++ = Root produced.

Shoot generation

This variety have resulted in increasing and extending the shoot induction for some of the growth regulators while for others it has either shown no response or resulted into poor shoot induction. Green healthy and friable calli which showed best results for callus induction and maximum phenolic content (MS + BAP1.0 mgL^{-1}+ IAA 2.0 mgL^{-1}+ 10 mg Cu-NPs) cultured on fresh shoot regeneration media consisted of MS media supplemented with growth hormones. The medium with highest concentration of BAP + KN (1.0+0.2) MS_{03}; BAP + NAA (1.0+0.2) MS_{06}; BAP + KN + NAA (2.0+1.0+0.2) MS_{13} achieved the maximum and multiple shoot regeneration. Furthermore, decreasing the concentration of these PGRs also reduced the number of regenerated shoots.

Root generation

Excised shoots from *Sorghum bicolor* were rooted on full strength MS medium with the combination of IBA+NAA. The medium with highest concentration of IBA (3 mgL^{-1}) when given in combination with NAA (0.5 mgL^{-1}) i.e., MS_{22} showed maximum roots.

Hardening and Acclimatization

After multiple shooting and rooting in plant regeneration media (Fig. 8) plantlets were successfully acclimatized without growth chamber facility. 100% of the plantlet survival was seen after hardening on garden soil, farmyard and sand (2:1:1) for three weeks (Fig. 9). Hardened plantlets were successfully transferred to botanical evaluation garden and kept under shade in a net house for further growth and development after three weeks (Fig: 10). However, the survival rate decreased from 100 to 80%, respectively after ten weeks of acclimatization.

Fig 8: Regeneration of plants from embryogenic callus of sorghum via callus.

Fig 9: Multiple shoots induced on regeneration medium, Plantlets transfer to small pots for hardening

Fig 10: Regenerated plants at the screen house

Quantification of phenolics from *in-vitro* culture

Gallic acid was used as a standard phenol (14). The mean of three readings was used and the total phenol content was expressed as µg/mg DW as shown in Table no. 4.

Regarding the total phenols content of callus, treatment with the application of 10 mg L^{-1} of Cu NPs generated an increment in callus induction in relation to the control, while treatment with 5 mg L^{-1} of Cu NPs showed a decrement in callus induction (Fig. 11).

Table 4: Total phenolic content obtained using spectrophotometer (at λ =765nm)

MO-Cu-NPs (mg) +(BAP+IAA) mgL^{-1}	Phenolic content (µg/mg DW)
5+ MS + 1.0 + 2.0	2.98±0.05
10+ MS + 1.0 + 2.0	3.52±0.057
20+ MS + 1.0 + 2.0	3.07±0.052
Control	2.19±0.049

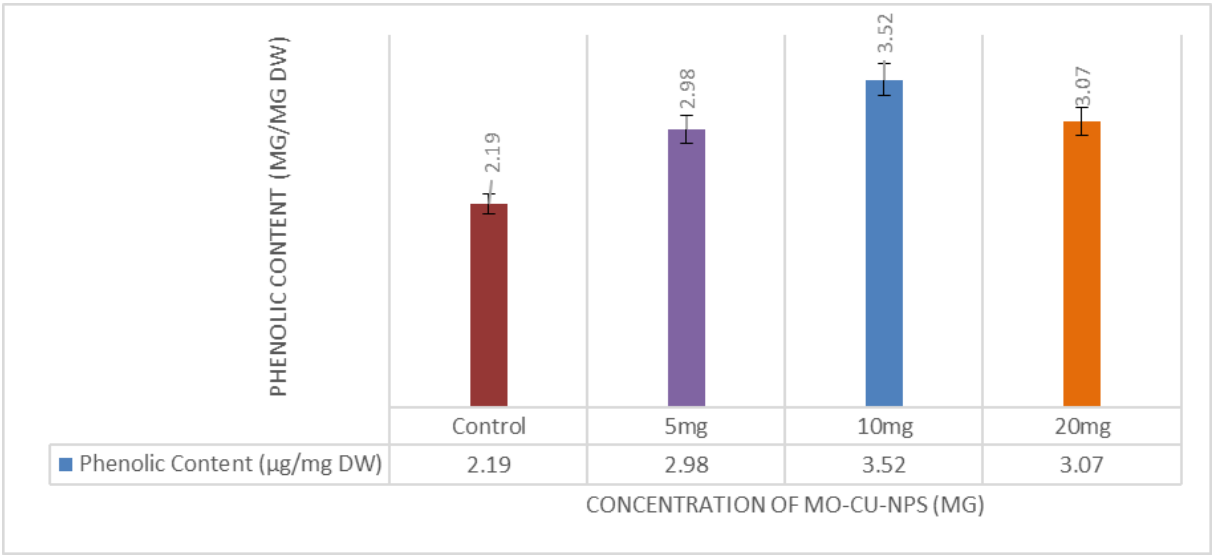

Fig 11: Phenolic content obtained from different concentrations of MO-Cu-NPs in callus media

Discussion

Media plays important role, the addition or deletion of certain growth regulators results into differentiation and/or regeneration vary considerably; usually high concentration of 2,4-D promotes callus formation rather than differentiation [15] and different types of organogenesis could be obtained by varying the concentrations of auxins and cytokinins in the medium [16]. Rice callus derived from the roots using Linsmaier and Skoog's medium (1965) with 10 ppm 2,4-D, when transferred to the same medium without auxins, redifferentiation was observed to form both shoots and roots and subsequently whole plant. Later, it has been proposed proposed that increasing concentration of IBA or NAA usually gives rise to short thick branches [17]. It has been demonstrated the seasonal response in rooting of branch cuttings which later on shown to be a function of cambial activity and auxin production in many woody species [18].The type and concentration of auxins and cytokinins, and their

relative ratio in the culture medium controls the biosynthesis and accumulation of secondary metabolites. The accumulation of phenolics was stimulated in the presence of low auxin levels, especially NAA [19, 20] or 2,4-D [21]. However, increasing the auxin concentration either stimulated [22] or inhibited phenolic production [23-24]. Various physical and chemical factors affect the growth of the plant tissue cultures and in order to achieve rapidly proliferating undifferentiated callus mass from highly organized multicellular systems, use of optimum combination and concentration of plant growth hormones (i.e., auxins and cytokinins) is very essential [25-33]. It has been reported formation of multiple shoots along with little callusing when MS media was supplemented with BAP (1 mgL^{-1}) and rooting in IAA (2 mgL^{-1}) It has been reported that little callusing when media was supplemented with BAP: NAA (1 mgL^{-1}: 0.2 mgL^{-1}) [34-35]. Plant growth and developmental processes require the action of phytohormones including auxins and cytokinins [36]. Callus formation from explants can be observed by adjusting the concentration of PGRs in the medium, [37] when callus induction treatments composed of both 2,4-D and BAP yielded higher CIP than auxin alone treated explants. The similar results were also supported [38] and suggested that auxins may regulate the level of cytokinin and metabolism of phenolics according to treatment dose. Also, the callus induced from auxin 2,4-D alone yielded lower CIP, however, a combination of both auxin and cytokinin i.e., MS_{04}, MS_{05} and MS_{09}, resulted into higher CIP which was in agreement and suggesting that the presence of cytokinin is also generally required for callus induction [39]. Highest callus induction was obtained on the medium MS_{04}, MS_{05} and MS_{09} supplemented with BAP1.0 mg/L + IAA 2.0 mg/L and BAP 1.0 mg/L + IAA 0.1 mg/L and BAP 1 mg/L +2 mg/L NAA respectively. The similar response for maximum callus induction and quality of callus induction was reported who used 2 mg L-1 IAA and 1 mg L-1 BAP and 1 mg L^{-1} BAP and 0.1 mgL^{-1} IAA respectively [40]. The main advantage of using the green route for the production of Cu-NPs is stabilization [41], while chemical production of Cu-NPs makes them oxidize and settle down after 24 h, along with large-size Cu-NPs production [42]. Plant extract concentration was increased as it fastens the reduction of Cu^{+2}resulting in the decreased size of Cu-NPs and results were supported [43].

Conclusion

The present research work was carried out to analyse the effect of green synthesized Cu-NPs and the various combinations of different concentration of PGRs to obtain the best result for callus induction as well as for the plant regeneration. The green synthesis pathway is preferred because it yields non-toxic material and reduces the production of wasteful products. The synthesis of copper nanoparticles using an extract of *M. oleifera* leaves was successful. SEM and UV-Vis absorption to determine the surface morphology of copper nanoparticles were used for the characterization of nanoparticles. It was observed that the copper Nanoparticles were having vital role in synthesis of phenolic compounds even though the complete role of phenolic acids in plants is still unknown but it is confirmed that they have diverse utility including a role in nutrient uptake, structural components, enzyme activity, protein synthesis, photosynthesis, and allelopathy. By using genotype and subculturing of callus, a simple and highly effective method for both the successful shoot and root formation was obtained.

References

1. Valodkar M, Jadeja RN, Thounaojam MC, Devkar RV, Thakore S. Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Materials Chemistry and Physics. 2011;128(1-2):83-89.
2. Abdelghany TM, Al-Rajhi AM, Al-Abboud MA, Alawlaqi MM, Ganash Magdah A, *et al*. Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review. Bio Nano Science. 2018;8:5-16.
3. Shobha G, Moses V, Ananda S. Biological synthesis of copper nanoparticles and its impact. Int. J pharm. Sci. Invent. 2014;3(8):6-28.
4. Mary MG. Green synthesis of copper nanoparticles using *Eclipta prostrata* leaves extract and their impact on seed germination and seedling growth of *Sorghum vulgare*. Galaxy International Interdisciplinary Research Journal. 2021;9(9):168-186.
5. Narayanan R, El-Sayed MA. Catalysis with transition metal nanoparticles in colloidal solution: nanoparticle shape dependence and stability. The Journal of Physical Chemistry B. 2005;109(26):12663-12676.
6. Perelaer J, Smith PJ, Mager D, Soltman D, Volkman SK, Subramanian V, *et al*. Printed electronics: the challenges involved in printing devices, interconnects, and contacts based on inorganic materials. Journal of Materials Chemistry. 2010;20(39):8446-8453.
7. Gao J, Gu H, Xu B. Multifunctional magnetic nanoparticles: design, synthesis, and biomedical applications. Accounts of chemical research. 2009;42(8):1097-1107.
8. Wang J. Nanomaterial-based electrochemical biosensors. Analyst. 2005;130(4):421-426.
9. Jeong S, Song HC, Lee WW, Lee SS, Choi Y, Son W, *et al*. Stable aqueous based Cu nanoparticle ink for printing well-defined highly conductive features on a plastic substrate. Langmuir. 2011;27(6):3144-3149.
10. Salavati-Niasari M, Davar F. Synthesis of copper and copper (I) oxide nanoparticles by thermal decomposition of a new precursor. Materials Letters. 2009;63(3-4):441-443.
11. Padil VV, Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. International Journal of nanomedicine; c2013. p. 889-898.
12. Ingle AP, Duran N, Rai M. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. Applied microbiology and Biotechnology. 2014;98:1001-1009.
13. Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of separation science. 2007;30(18):3268-3295.
14. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology. 1999;299:152-178.
15. Fujii T. Callus formation in wheat anthers. Wheat Inf. Serv. Kyoto. Univ. 1970;31:1-4.
16. Skoog F. Chemical regulation of growth and organ

formation in plant tissue cultured *in vitro*. In Symp. Soc. Exp. Biol. 1957;11:118-131.
17. Lane WD. Regeneration of pear plants from shoot meristem-tips. Plant Science Letters. 1979;16(2-3):337-342.
18. Kawase M. Centrifugation, rhizocaline and rooting in *Salix alba* L. Physiologia Plantarum. 1964;(4):855-65.
19. King P. Utilization of of 2, 4-D by steady state cell cultures of *Acerpseudo platones.* J Exp. Biol. 1976;27:1053-1072.
20. De-Eknamkul W, Ellis BE. Effects of auxins and cytokinins on growth and rosmarinic acid formation in cell suspension cultures of *Anchusa officinalis*. Plant cell reports; c1985. p. 50-53.
21. Sugano N, Iwata R, Nishi A. Formation of phenolic acid in carrot cells in suspension cultures. Phytochemistry. 1975;14(5-6):1205-1207.
22. Adventitius A. Effect of Auxin and Methyl Jasmonate on Total Phenolic Content and Total Flavonoid Content in Adventitious Roots of *Justicia gendarussa*. Malaysian Journal of Analytical Sciences. 2020;24(6):1002-1012.
23. Ibrahim RK, Edgar D. Phenolic synthesis in *Perilla* cell suspension cultures. Phytochemistry. 1976;15(1):129-31.
24. Sahai OP, Shuler ML. Environmental parameters influencing phenolics production by batch cultures of *Nicotiana tabacum*. Biotechnology and bioengineering. 1984;26(2):111-120.
25. Mantell SH, Smith H. Cultural factors that influence secondary metabolite accumulations in plant cell and tissue cultures. In: Plant Biotechnology. Mantell SH and Smith H (Eds). Springer Verlag. Berlin. Germany; c1983. p. 75-108.
26. Lazzeri PA, Hildebrand DF, Collins GB. Soybean somatic embryogenesis: effects of hormones and culture manipulations. Plant Cell, Tissue and Organ Culture. 1987;10:197-208.
27. Redway FA, Vasil V, Lu D, Vasil IK. Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 1990;79:609-617.
28. Fennell S, Bohorova N, Van Ginkel M, Crossa J, Hoisington D. Plant regeneration from immature embryos of 48 elite CIMMYT bread wheats. Theoretical and Applied genetics. 1996;92:163-169.
29. Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. *In vitro* Cellular & Developmental Biology-Plant. 1996;32:272-289.
30. Özgen M, Türet M, Altınok S, Sancak CE. Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) genotypes. Plant cell reports. 1998;18:331-335.
31. Leonardi C, Ruggeri A, Malfa LS. Hormone effects on *in vitro* proliferation and rooting of *Grevillea* explants. Scientia Horticulturae. 2001;90(3-4):335-341.
32. Roy A, Ghosh S, Chaudhuri M, Saha PK. Effect of different plant hormones on callus induction in *Gymnema sylvestris* R. Br (Asclepiadaceae). African Journal of Biotechnology; c2008, 7(13).
33. Lee EJ, Hahn EJ, Paek KY. Effects of chemical and physical environments on cell culture of *Gymnema sylvestre*. Acta. Hort. 2008;786:273-278.
34. Islam S, Jahan MAA, Khatun R. *In-vitro* regeneration and multiplication of year-round fruit beraing *Moringa oleifera* Lam. J Biol. Sci. 2005;5(2):145-148.
35. Suqiong X, Cuo L, Qigao C, Xialo L, Qiao H. Tissue culture and tetraploidinduction of Drum sticks. J. Trop. Sub. Trop. Bot. 2007;15(2):141-146.
36. Bajguz A, Piotrowska A. Conjugates of auxin and cytokinin. Phytochemistry. 2009;70(8):957-69.
37. Pierik RLM. *In-vitro* culture of higher plants, Martinus Nijhoff Publishers, Dordrecht, Netherlands. 1987;19(2):344.
38. Nordström A, Tarkowski P, Tarkowska D, Norbaek R, Åstot C, Dolezal K, *et al*. Auxin regulation of cytokinin biosynthesis in Arabidopsis thaliana: a factor of potential importance for auxin–cytokinin-regulated development. Proceedings of the National Academy of Sciences. 2004;101(21):8039-8044.
39. Aitchison PA, Macleod AJ, Yeoman MM. In: Plant Tissue and Cell Culture. (Ed.): Street, H.E. University California Press, Berkeley; c1977. p. 267-306.
40. Jatoi SA, Sajid GM, Quraishi A, Munir M. Callogenetic and morphogenetic response of leaf explants of *in vitro* grown F1 tomato hybrids to different levels of plant growth regulators. Pak. J Plant Sci. 1995;1(2):281-287.
41. Varshney R, Bhadauria S, Gaur MS, Pasricha R. Characterization of copper nanoparticles synthesized by a novel microbiological method. Jom. 2010 Dec;62:102-104.
42. Jayandran M, Haneefa MM, Balasubramanian V. Green synthesis of copper nanoparticles using natural reducer and stabilizer and an evaluation of antimicrobial activity. Journal of chemical and pharmaceutical research. 2015;7(2):251-259.
43. Shende S, Ingle AP, Gade A, Rai M. Green synthesis of copper nanoparticles by *Citrus medica* Linn. (Idilimbu) juice and its antimicrobial activity. World Journal of Microbiology and Biotechnology. 2015;31:865-873.