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A COMPARATIVE APPRAISAL OF ANTIOXIDANT ACTIVITY OF PLANT EXTRACT FOR DONOR AND *IN VITRO* PROPAGATED *MORUS INDICA*

KHAN A[1], KHAN W[2], SAINI N[1], KAUR A[1], PRIYANSHI[1], RAMOLA RC[1], KUMAR A[1] AND GAURAV N[*1]

1: Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001, Uttarakhand, India

2: Department of Pharmacy, College of Pharmaceutical Sciences, Siddhartha Group of Institutions, Dehradun-248001, Uttarakhand, India

***Corresponding Author: Dr. Naveen Gaurav: E Mail: naveensri17@gmail.com; Orcid Id: https://orcid.org/0000-0002-9170-0955**

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ABSTRACT

Mulberries are a deciduous, fast-growing, versatile plant that are prized for their leaves, which increased milk yield of dairy cows and served as the silkworms' primary food supply. The leaves' high nutritional content and medicinal properties, such as its diuretic, hypoglycemic, and hypotensive effects, are mostly ascribed to the flavonoids and antioxidants quercetin and rutin. In this work, the *Morus indica* plant was productively propagated *in vitro*, and the antioxidant activity of the donor and *in vitro*-raised plants was compared with respect to their ability to scavenge free radicals (ABTS), reducing power, and total antioxidant activity. The antioxidant activity of the stem and leaf extract was assessed. With a few modifications, the plant has been grown *in vitro* utilising conventional tissue culture methods. Following the successful *in vitro* replication process and the subsequent ex-vivo plant rearing, the stems and leaves were harvested. After being Soxhlet extracted of these leaves and stems, the extracts were subjected to ABTS radical scavenging activity (50-500 µg/ml) assay. Additionally, the total antioxidant activity and reducing power were calculated and assessed. The total

antioxidant activity, reducing power, and ABTS radical scavenging activity of the donor and in vitro grown plants differed significantly, according to the results. There were significant antioxidant capabilities in the donor plants. On the other hand, plants grown *in vitro* showed greater scavenging abilities, reducing power and total antioxidant activity. These resultsimplied that methods of *in vitro* propagation not only preserved but significantly improved *Morus indica's* antioxidant capacity.

Keywords: Mulberry, *Morus indica,* Antioxidant, In vitro propagation, Total antioxidant activity and Reducing power assay, etc.

INTRODUCTION

The woody perennial plant *Morus indica* L., sometimes referred to as Indian mulberry, is a member of the Moraceae family. The majority of the cultivated types are monoecious and diploid, with fruit set beginning in early September and flowers in August. Considered an economically significant plant, *M. indica* is grown commercially for its edible fruit and leaves, which serve as the main food source for silkworms (*Bombyx mori* L.). This plant's anthocyaninsare used as a natural culinary colouring and fabric tanning agent **[1]**. Additionally, several clinical studies have demonstrated the existence of medicinal substances with hypoglycaemic, hypolipidemic, diuretic, and hypotensive characteristics **[2]**. It has been claimed that the mulberry plant contains a variety of bioactive substances, including isoquercetin, rutin, and quercetin **[3]**. Currently, this plant is undergoing a number of pharmacognostical analyses to investigate its mode of action in various traditional remedies where diverse plant components are the main ingredient. As a result of cross-pollination, Indian mulberry seeds are primarily heterozygous, making cuttings the primary means of multiplication. Nonetheless, a significant worry is the challenge of roots through cuttings **[4]**. Furthermore, the donor plant's genotype, surroundings, and physiological state all affect how well it roots by conventional cutting **[5]**. In the final transplantation stage, less than 40% of patients receive successful outcomes while under intensive care and with the limiting factor standardised. This demonstrates the need for a different, more effective technique for this species' clonal propagation.

The approach of plant tissue culture has proven effective in mass-propagating homogenous Indian mulberry platelets while preserving genetic integrity. Several researchers have reported on the in vitro propagation of *M. indica* using diverse

procedures. 6-Benzylaminopurine (BAP) was thought to be useful in initiating shoots from axillary bud culture in the majority of the reported work **[5, 6]**. One of the most difficult issues, even with several reports of successful micropropagation, is in vitro contamination by bacteria contained in lenticels and scale leaves, especially for *M. indica* explants cultivated in the field **[7]**. An additional obstacle lies in the protracted, multi-phase acclimatisation process, which results in production costs that are not profitable **[7]**. The quality of roots of in vitro plantlets together with the number and status of stomata significantly impacts the fruitful adaptation. Concurrent growth of inflorescence during elongation of the shoot by application of exogenous growth factor is a widespread reproductive attribute in mulberry. Although explant type and age, plant hormones, nutrient composition and physical factors have mutable effects on in vitro flowering course of diverse species, the effect of the type and concentration of sucrose in flowering is principally encouraged by earlier investigators **[8]**. Mulberry under the natural condition is a dioecious plant with male and female flower occurring in different plants. Considering all these facts and details, this present study aimed to successfully propagate the *Morus indica* plant *in vitro* and also to evaluate and compare the antioxidant capacities of ethanol and methanolic extracts of leaves obtained from different *in vitro* cultures and *in vitro*propagated plants of *Morus indica*. ABTS radical scavenging, Total antioxidant activity and reducing power assay were used to assess and compare the antioxidant activity of the donor plant as well as *in vitro* propagate plants.

MATERIALS AND METHODS

Plant material and explants disinfection

We obtained healthy branches of *Morus indica* (MI) from the region of Dehradun, Uttarakhand, India. Axillary buds were positioned 1.6 cm from the proximal and 0.6 cm from the distal cut ends of the 2.5 cm long nodal segments. After washing with a gentle brush and tap water, the explants were shaken for five minutes at 120 rpm in a solution containing two percent tween-20 (Himedia, Mumbai, India). Following that, three rinses in sterile distilled water were performed. Standard procedures were followed to clean and sterilise the explants on the surface. These procedures used 70% ethanol (EtOH), 0.1–0.15 percent mercuric chloride (HgCl2), and 1-3 percent sodium hypochlorite (NaOCl) (Himedia, Mumbai, India). Next, the explants were washed three times in sterile double-distilled water for a length of one minute each. Following that, the culture medium were vertically inoculated inside a standard laminar airflow cabinet and the cut ends were appropriately

trimmed with a sterile knife **[9, 10]**.

Medium composition and culture conditions

The Murashige and Skoog (MS) basal media was used for each culture stage with standard protocol **[11, 12]**.

Shoot multiplication, elongation, Rooting and Acclimatization

To achieve the best effect of plant growth regulators (PGRs) on bud break and subsequent shoot formation, sterilised explants were inoculated in MS culture medium supplemented with 6-benzylaminopurine (BAP; 0.5–2.5 mg L-1), kinetin (Kin; 0.5–2.5 mg L-1), and adenine sulphate (Ads; 10–40 mg L-1) (Himedia, Mumbai, India), either singly or in combination. Following the first thirty days, individual shoots that measured two to three centimetres in height were separated and allowed to develop on MS medium supplemented with 1.5 mg L-1 gibberellic acid **[13, 14]**. After that, the shoots were given another 14 days to incubate. Every culture was routinely checked for indicators of contamination as well as changes in morphology and physiology. To produce a significant number of shoots for further research, shoots were sub-cultured on the same medium.

On MS media supplemented with 0.05 percent (w/v) activated charcoal and 1-naphthaleneacetic acid (NAA, 0.5 mg L-1), indole-3-butyric acid (IBA, 0.5 mg L-1), or indole- 3-butyric acid (IAA, 0.5 mg L-1) (AC), individual shoots with a height of 4-6 cm were grown.

Additionally, a two-phase approach was used to analyse the roots of shoots: First, micro- propagated shoots were cultivated for one to nine days on MS medium with different concentrations of 2,4-D (0.1–2 mg L–1) **[15]**. When the shoots began to exhibit indications of root initials, they were moved to a 1/2 MS hormone-free medium to promote more root growth and proliferation. Over the course of a 21-day culture in 1/2 MS hormone- free media, the qualitative and quantitative characteristics of the proliferating roots were assessed.

After the roots were removed from the culture flasks, the plants were thoroughly cleaned withtap water to get rid of any last bits of agar. After that, the plantlets were placed in circular 500mL garden pots that were filled with a 1:1:2 mixture of autoclaved field soil, sand and vermicompost and covered with polythene bags. Within the culture room, the potted transplanted plants were maintained at 25 ± 2 0C (40 µmol m-2 s-1) with 16-hour photoperiods. Using a dehumidifier (Loba, New Delhi, India), the relative humidity was progressively lowered to 60% over the course of the next six days after being set at 75% for four days. While controlling the

humidity, the polythene bags were taken out of the pots **[16, 17]**. During the first week, the plantlets were irrigated with 1/2-strength MS medium. Before being planted in the field, the plants were developed in the culture chamber for fifteen days. After that, they were transferred to a greenhouse for three weeks to allow for additional growth. Following a four-week period of field planting, the frequency of phenotypic traits andsurvivorship were noted.

Physio-morphological and biochemical study

The donor plant and the in vitro regenerated plants were evaluated for their physio-morphological and biochemical traits. The calculation and procedure outlined by Smart and Bingham, 1974 were used to determine the relative water content **[18]**:

$$RWC\ (\%) = \frac{W - DW}{TW - DW} \times 100$$

where W denotes the sample's fresh weight, TW its turgid weight, and DW its dried weight. The smear impression technique was used to determine the stomatal density (SD) and stomatalindex (SI) after a microscopic count. A thin layer of nail polish was placed on the surface of leaves for microscopic examinations and the smeared leaves was dried in air, and then scrapedoff from the surface.

$$Stomatal\ Density\ (SD) = \frac{Number\ of\ Stomata}{cm^2}$$

$$Stomatal\ Index\ (SI) = \frac{S}{E + S} \times 100$$

where E is the number of epidermal cells per unit area and S is the number of stomata per unitarea. 48 hours of dark incubation at 4 ^{0}C resulted in the extraction of 0.5 g of fresh leaf materialin 25 mL of 80% acetone. Following that, the sample was spectrophotometrically examined at645 and 663 nm **[19]**.

Extraction of plant

One kilogram of fresh *Morus indica* leaves were gathered, dried in the shade, and then ground using a machine to produce a dry sample weighing three hundred grammes. This powdered form was then used for solvent extraction. A Soxhlet extraction method was applied to 300 g of dried sample, with 1000 ml of solvent used for each extraction. For the length of the extraction procedure, the temperature was maintained at 25°C. The extracted components were then concentrated in a rotary evaporator at 45°C and decreased pressure to obtain an extract yield of 7.2%. The following codenames were assigned to the produced extracts.

Table 1: Extract type, plant type and code names of the extracts

Source	Extract Type	Plant Type	Code Name
Morus indica Leaves	Ethanol	Donor Plant	MI-LE
Morus indica Leaves	Methanol	Donor Plant	MI-LM
Morus indica Leaves	Ethanol	*In vitro* propagated plant	MI-LE
Morus indica Leaves	Methanol	*In vitro* propagated plant	MI-LM

Preliminary Phytochemical screening study

Chemical techniques were used to identify the chemical components of the powder extracts using a methodology that has been proposed elsewhere **[20]**.

Antioxidant activity evaluations

ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorizationassay

Water was used to dissolve ABTS to a concentration of 7 mM. The final concentration of 2.45 mM potassium persulfate was added to the ABTS stock solution to create ABTSη. The combination was then left to stand in the dark at room temperature for 12 to 16 hours before being used. To test the samples, phosphate-buffered saline (5 mM; pH 7.4) was added to the ABTS· stock solution until the absorbance at 734 nm reached 0.70. Five minutes after the initial mixing, 1.0 ml of diluted ABTS· was added to 20 µl of sample, and the absorbance reading was obtained **[21**]. The percentage of ABTS· -scavenging for this activity is determined using the formula below:

$$\% \text{ ABTS·-scavenging activity} = [\text{Control absorbance} - \text{Sample absorbance}] / [\text{Controlabsorbance}] \times 100$$

Measurement of total antioxidant activity

Using the thiocyanate technique, the extract's overall antioxidant activity was ascertained **[22]**. Ten millilitres of water were mixed with ten milligrams of extract. The linoleic acid emulsion (2.5 ml) in potassium phosphate buffer (0.04 M, pH 7.0) was mixed with several concentrations of extract (50-250 µg/mL) or standard samples. 17.5g Tween-20, 15.5µl linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0) make up a five millilitre linoleic acid emulsion. However, the 5.0 ml control is made up of 2.5 ml of potassium phosphate buffer(0.04 M, pH 7.0) and 2.5 ml of linoleic acid emulsion. The mixture was kept in the dark and incubated at 37°C in a glass flask. Following three minutes of stirring, the mixture was subjected to a reaction with FeCl2 and thiocyanate at regular intervals during incubation. The absorbance at 500 nm was measured using a spectrophotometer (UV-1601 Shimadzu, Japan) to ascertain the peroxide value. Peroxides were produced during the oxidation of linoleic acid.These substances convert Fe2+ into Fe3+. The

greatest absorbance of SCN− was observed at 500 nm when the latter Fe3+ ions form a compound with it. Consequently, elevated absorbance signifies elevated oxidation of linoleic acid. As blank samples, the solutions devoid of extractor standards were employed. The average of three duplicate analyses is used to determine all total antioxidant activity results. The following formula was used to determine the percentageof lipid peroxidation inhibition:

Inhibition (%) = (A0 – At / A0) × 100

where At represented the absorbance while the sample was present and A0 represented the absorbance of the control response. Every test was run three times, and the mean ± SD values were shown on a graph. Standard antioxidant chemicals such as Gallic acid was utilised.

Measurement of reducing power assay

The previously mentioned approach was used to determine the extract's reducing power **[23]**. The various extract concentrations (ranging from 50 to 250µg/mL) were combined with potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%) and phosphate buffer (2.5 ml, 0.2 M, pH 6.6) in 1 millilitre of distilled water. For twenty minutes, the mixture was incubated at 50 ◦C. After adding 2.5 ml of 10% trichloroacetic acid to the mixture, it was centrifuged for 10 minutes at 3000 rpm. The upper layer of solution (2.5 ml) was combined with 2.5 ml of distilled water and 0.5 ml of FeCl3 (0.1%). A spectrophotometer (UV -1601 Shimadzu, Japan) was used to detect the absorbance at 700 nm. Greater reducing power was shown by the reaction mixture's higher absorbance. Standard antioxidant chemicals such as gallic acid were utilised.

Experimental design and statistical analysis

Every fully randomised experiment had 10 replications of each treatment. One replication is equal to one culture container with one explant. The statistical significance of the mean differences between the various treatments was evaluated at $p < 0.05$ using Tukey's multiple range test. The mean ± standard deviation (SD) was used to display the data. Graph-Pad Prism 8 (GraphPad Software, USA, www.graphpad.com) was used for statistical analysis.

RESULTS AND DISCUSSION

Culture initiation, shoot multiplication and Rooting

Despite successful shoot development, latent contamination was still present after three to five weeks, suggesting that subculturing is required to control endophytic fungus. Bud break beganon the seventh day, indicating that the ideal circumstances for shoot initiation were MS medium supplemented with different concentrations of Kin. The maximum shoot length, leaf number, and internode number were measured using 1 mg L-1 Kin. Although BAP is usually the chosenmethod

for mulberry in vitro shooting, Kin has demonstrated efficacy in breaking buds and promoting regeneration. However, treating BAP and Kin together had no negative effects. When sucrose was replaced with table sugar (3%) no appreciable comparable effects were seen. Additionally, for two weeks, microshoot culture in GA3 (1.5 mg L-1) supplemented MS mediaproduced the best elongation, readying them for injection into the rooting medium.

The study found that a variety of factors, such as the amount of activated charcoal (AC) and the type, concentration, and duration of auxin treatment, significantly affected the roots of theremoved mulberry microshoots. A two-phase rooting method using 2,4-D resulted in shorter culture durations and greater root growth as compared to conventional methods. More roots were produced in less time by initially growing microshoots in full-strength MS medium containing 2,4-D and then growing in 1/2 MS hormone-free media.

Acclimatization, Physio-morphological and biochemical study

After being cultivated for 28 days in the rooting phase, the plantlets showed strong root and shoot systems. Subsequently, they were placed in garden pots and covered with polythene bags to ensure a high relative humidity. In the containers was a 1:1:2 autoclaved mixture of sand, field soil and vermicompost. Three phases were conducted, each 48 hours apart, with a 5% decrease in the relative humidity of the culture room in an effort to diminish the death rate. Thenumber of open stomata and stomatal density are two critical factors for in vitro grown plantsto properly acclimatise. When transplanted in field circumstances, in vitro plants with a greaternumber of open stomata withered dramatically. In order to help bring the percentage of open stomata down to roughly 22%, the relative humidity in the culture chamber was gradually reduced. Because of the reduction in stomatal conductance and leaf transpiration, there was reduced water stress and a higher chance of survival. **Figure 1-4** displays a comparison of the physio-morphological and biochemical traits of the donor plant and the plantlets that were grown in vitro. Relative Water Content (RWC) decreased noticeably upon transplanting the in vitro-raised platelets into ex vivo environments. Nevertheless, RWC did eventually get better after becoming adjusted to the new environment. The acetone-extracted chlorophyll levels of the in vitro grown plants were somewhat lower than those of the donor plants. Further study is needed to understand how chlorophyll synthesis works in relation to different nutrient concentrations in the culture medium and under different cultural circumstances in order to improve the production of chlorophyll.

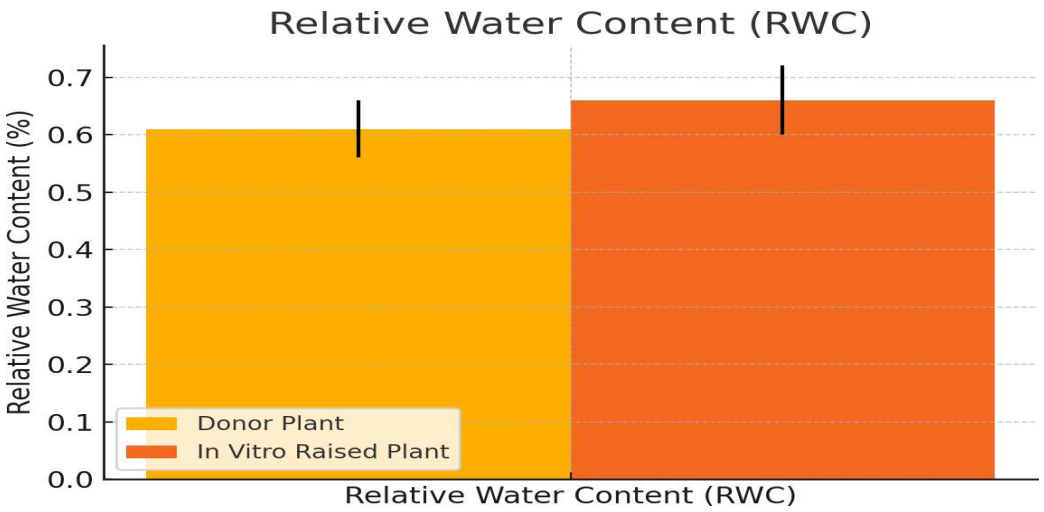

Figure 1: Relative water content (RWC)

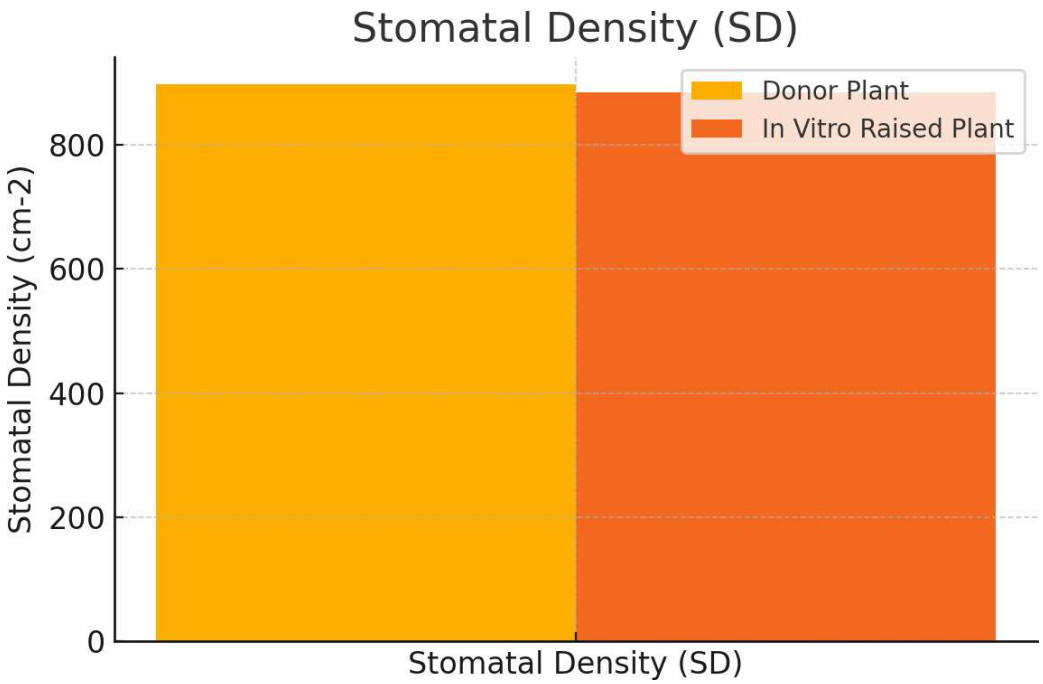

Figure 2: Stomatal density (SD)

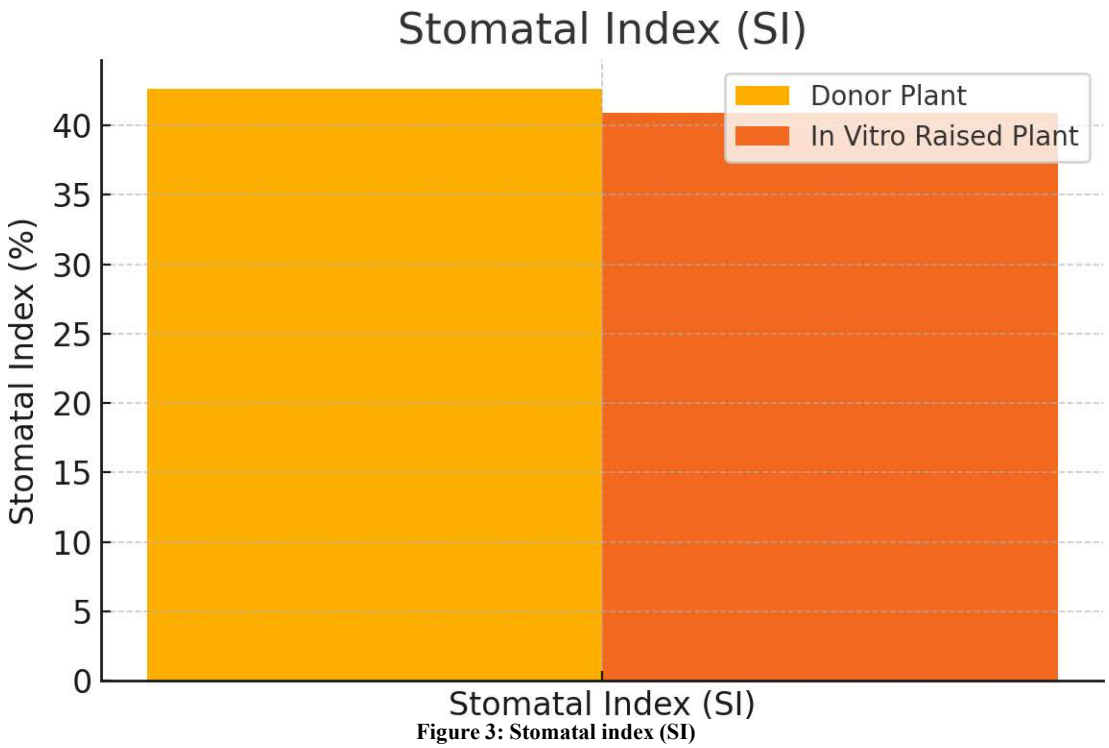

Figure 3: Stomatal index (SI)

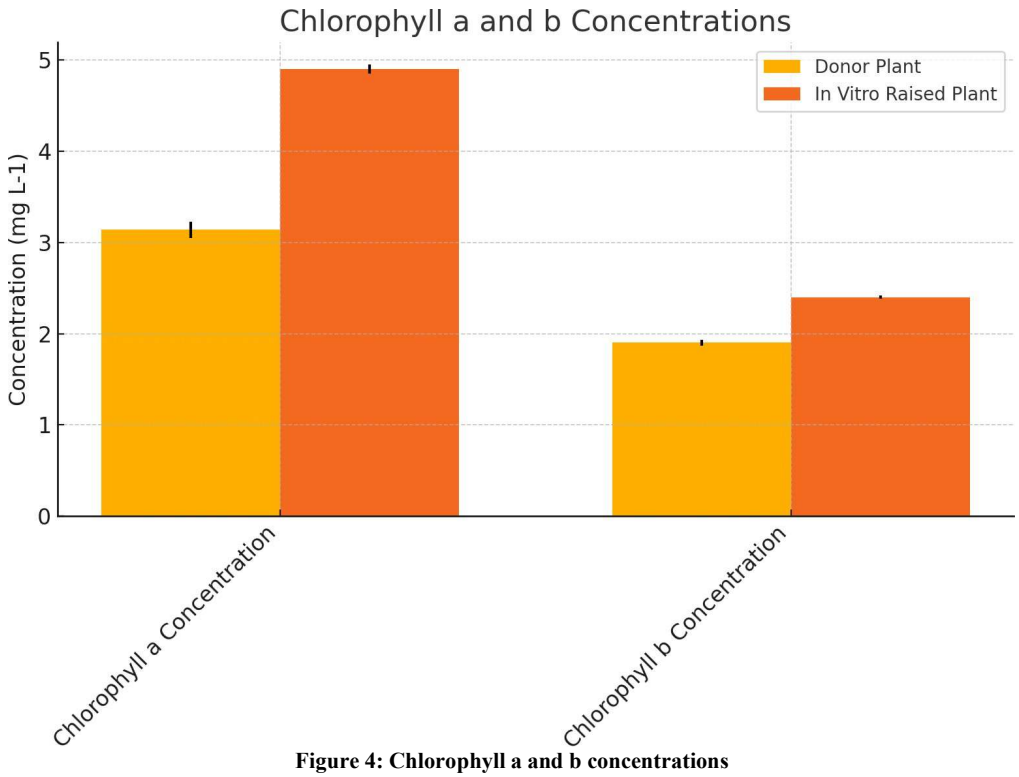

Figure 4: Chlorophyll a and b concentrations

Preliminary Phytochemical Screening

Using various chemical techniques, the powdered leaf extracts were submitted to a preliminary phytochemical screening that revealed the presence of proteins, amino acids, flavanoids, hydrolysable tannins, tannins, phytosterols, and saponins. Similar phytochemical screening revealed the presence of proteins, amino acids, alkaloids, saponins, phytosterols, flavanoids, tannins, and phenolic compounds in the ethanolic and methanolic extract of the powdered leaves of the plant (both donor and in vitro grown) **(Table 2)**. Nevertheless, in contrast to the donor extract, hydrolysable tannins were not found in the ethanol extract of leaves grown in vitro.

Tablet 2: The results of the preliminary pharmaceutical screening for all the extracts

Phytochemical test	MI-LE (Donor)	MI-LM (Donor)	MI-LE (*In vitro* Raised)	MI-LM (*In vitro* Raised)
Alkaloids	+	+	+	+
Carbohydrates	+	+	+	+
Phytosterols	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Phenols	+	+	+	+
Tannins	+	+	-	+
Proteins	+	+	+	+
Terpenoids	+	+	+	+

Antioxidant activity

ABTS (2, 2' – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorizationassay

The ABTS radical decolorization assay estimated the ability of antioxidants to quench ABTS radicals, presenting an suggestion of the antioxidant capability of the investigated extracts. In this study, ethanol (MI-LE) and methanol (MI-LM) extracts of MI leaves from both donor andin vitro raised plants were evaluated together with ascorbic acid and quercetin as standard antioxidants. For the donor plant extracts, the results showed a progressive increase in radical scavenging activity with increasing concentrations. At 50 µg/ml, MI-LE exhibited 35.493±0.579% inhibition, while MI-LM showed 34.693±0.769% inhibition. At higher concentrations of 100, 150, 200, and 250 µg/ml, MI-LE displayed 39.663±0.749%, 75.573±0.339%, 86.653±0.579%, and 94.013±0.689% inhibition, respectively. Similarly, *MI* LM showed 38.883±0.899%, 74.793±0.569%, 85.873±0.689%, and 93.143±0.749% *Inhibition* at these concentrations. Comparatively, ascorbic acid and quercetin exhibited higher ABTS radical scavenging activity, with quercetin reaching a inhibition of 97.9±0.599% at 250 µg/ml.For the in vitro raised plant extracts, a comparable trend was noticed. At 50 µg/ml,

MI-LE showed 37.464±0.65% inhibition, and MI-LM showed 36.664±0.84% inhibition. Atconcentrations of 100, 150, 200, and 250 µg/ml, MI-LE demonstrated 41.634±0.82%, 77.544±0.41%, 88.624±0.65%, and 95.984±0.76% inhibition, respectively. MI-LM showed 40.854±0.97%, 76.764±0.64%, 87.844±0.76%, and 95.114±0.82% inhibition at the same concentrations. Ascorbic acid and quercetin again displayed superior antioxidant activity, withquercetin reaching a inhibition of 97.9±0.599% at 250 µg/ml concentration. In inference, both ethanol and methanol extracts of MI leaves from donor and in vitro raised plants exhibited strong antioxidant activity, as evidenced by the ABTS radical decolorization assay. The in vitro raised plant extracts demonstrated slightly higher scavenging activity than the donor plant extracts. Among the solvents, ethanol extracts generally showed marginally higher antioxidantactivity than methanol extracts.

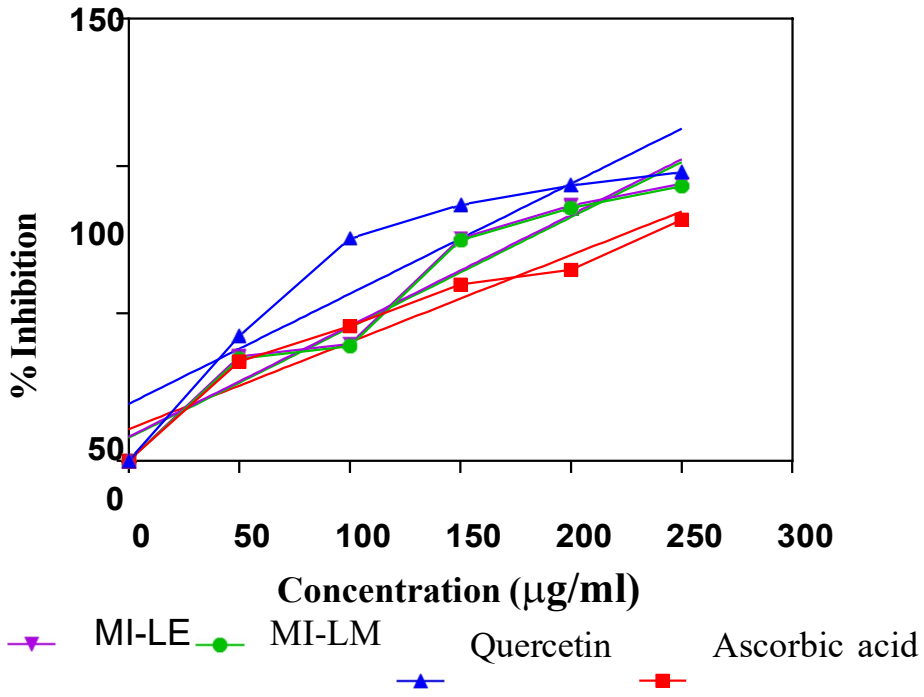

Figure 5: ABTS radical decolorization assay of ethanol and methanol extracts of MI leaves(Donor plant)

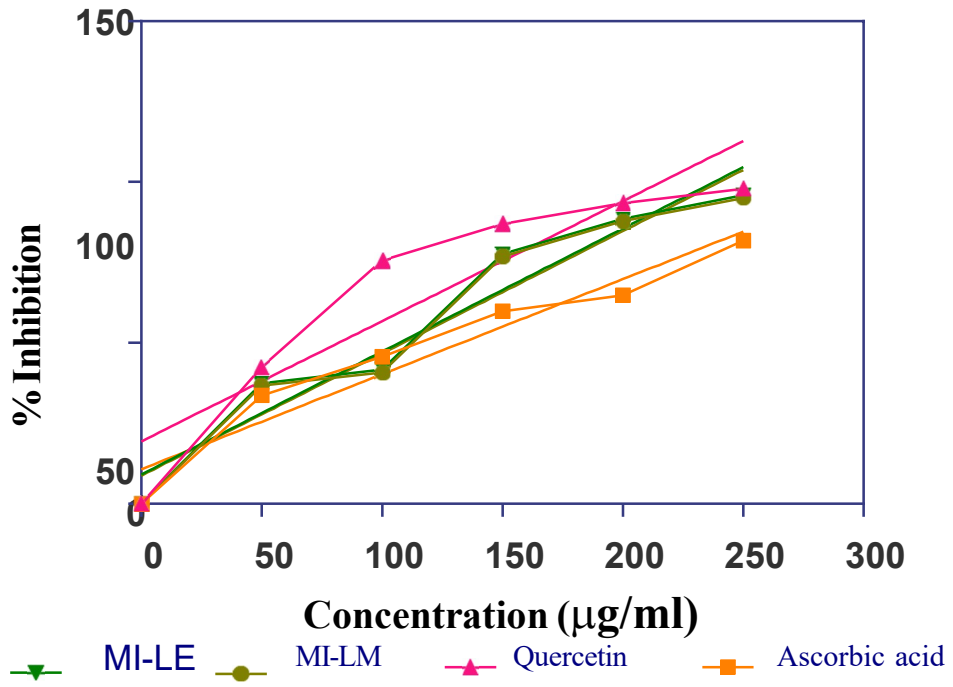

Figure 6: ABTS radical decolorization assay of ethanol and methanol extracts of MI leaves(In vitro raised plant)

Table 3: The calculated IC_{50} values for ethanol and methanol extracts of MI leaves (Donor and *In vitro* Raised Plant) in ABTS radical decolorization assay model

Extracts	IC50 (µg·mL ^{1}) ABTS radical decolorization assay	
	Donor	*In vitro* raised
MI-LE	111.11	107.02
MI-LM	112.81	108.67
Ascorbic acid	132.95	132.95
Quercetin	82.04	82.04

Total antioxidant activity determination in linoleic acid system

The plant extract's overall antioxidant activity was assessed using the thiocyanate technique. At 250 µg/ml, the extract demonstrated strong and efficient antioxidant activity. **Figure 1** illustrates the impact of an extract concentration of 250 µg/ml on the peroxidation of linoleic acid emulsion. The plant extract's antioxidant activity first rose as the incubation period extended, but as the time period continued, it also showed a decline in activity. The quantity of the extract under study demonstrated reduced antioxidant activity in comparison to the 250 µg/ml concentration of α Gallic acid. In the linoleic acid system, the percentage inhibition of peroxidation of the extract (donor plant) was determined to be 61.18±1.12% and 63.63±1.15% for MI-LE and MI-LM, respectively. Additionally, 41.87±1.70% of the 250 µg/ml quantity of gallic acid was observed to be inhibited. In a similar vein, the percentage inhibition of

extract peroxidation in the linoleic acid system for in vitro grown plants was determined to be 62.88±1.12% and 66.84±1.19% for MI-LE and MI-LM, respectively. Additionally, 41.87±1.70% of the 250 µg/ml quantity of gallic acid was observed to be inhibited.

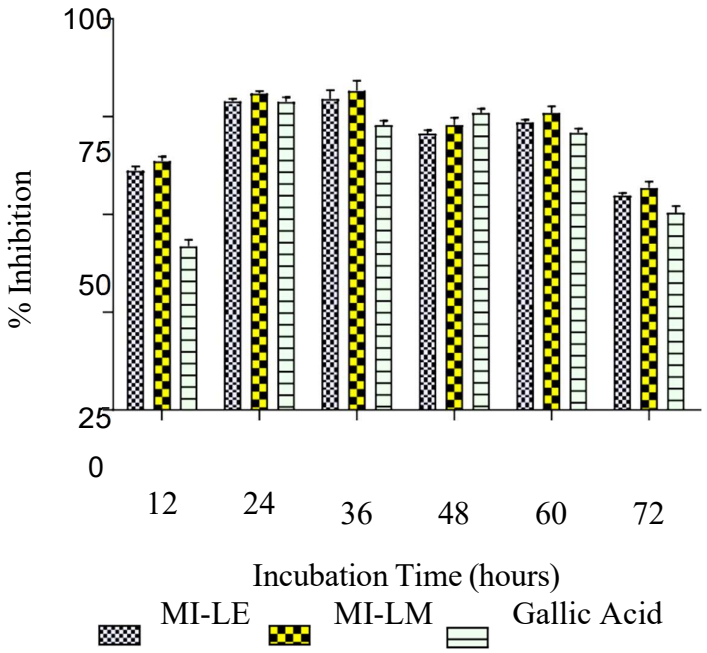

Figure 7: Total Antioxidant activity of ethanol and methanol extracts of MI leaves (DP)

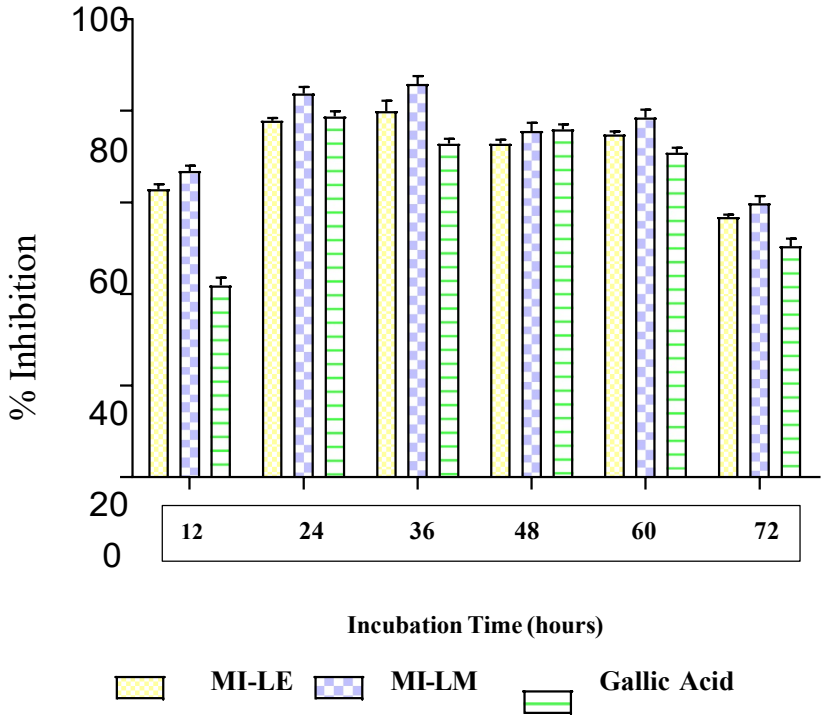

Figure 8: Total Antioxidant activity of ethanol and methanol extracts of MI leaves (In vitroraised plant)

Effect on reducing power assay

Figure 9 and 10 illustrates the extract's reducing power in relation to gallic acid. Using the Oyaizu method, the Fe3+-Fe2+ transition in the presence of extract samples was examined in the reductive ability assessment **[23]**. With an increase in extract concentration, Both the extract's reducing power rose. The extracts had a stronger reducing power than gallic acid at all concentrations examined. The sequence was followed by the extract's and standard compounds' reducing powers: MI-LM > MI-LE > Gallic acid. In this analysis, ethanol (MI- LE) and methanol (MI-LM) extracts of MI leaves from both donor and in vitro raised plants were compared with gallic acid as a standard. The results indicated that both the donor and in vitro raised plant extracts exhibited increasing reducing power with increasing concentration, demonstrating a dose-dependent antioxidant activity. Notably, the in vitro raised plant extracts showed slightly higher reducing power than the donor plant extracts at all concentrations.

Additionally, ethanol extracts (MI-LE) consistently displayed marginally higher reducing power than methanol extracts (MI-LM) for both donor and in vitro raised plants, indicating that ethanol might be a more effective solvent for extracting antioxidant compounds from MI leaves. Despite these findings, gallic acid exhibited significantly higher reducing power compared to both ethanol and methanol extracts, serving as a robust positive control and benchmark for antioxidant activity. Overall, the study highlights that both ethanol and methanol extracts of MI leaves possess considerable antioxidant properties, with in vitro raised plant extracts and ethanol extracts showing superior efficacy.

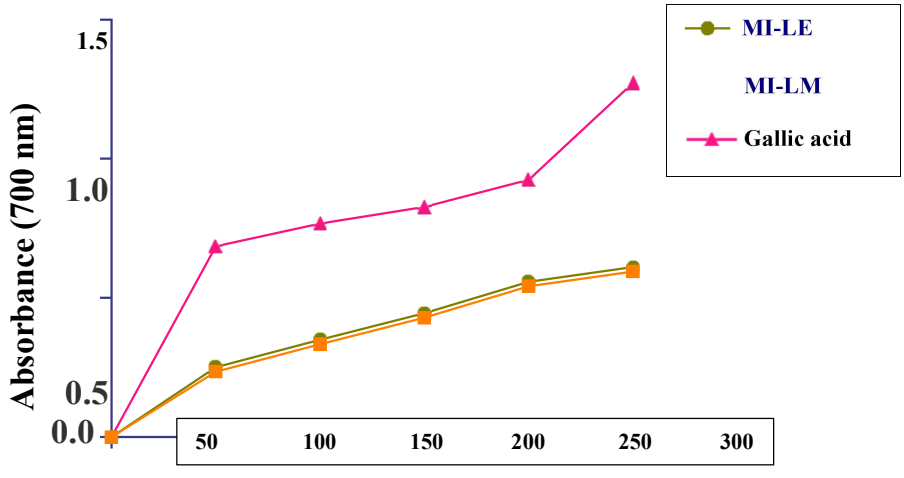

Figure 9: Reducing power assay of ethanol and methanol extracts of MI leaves (DonorPlant)

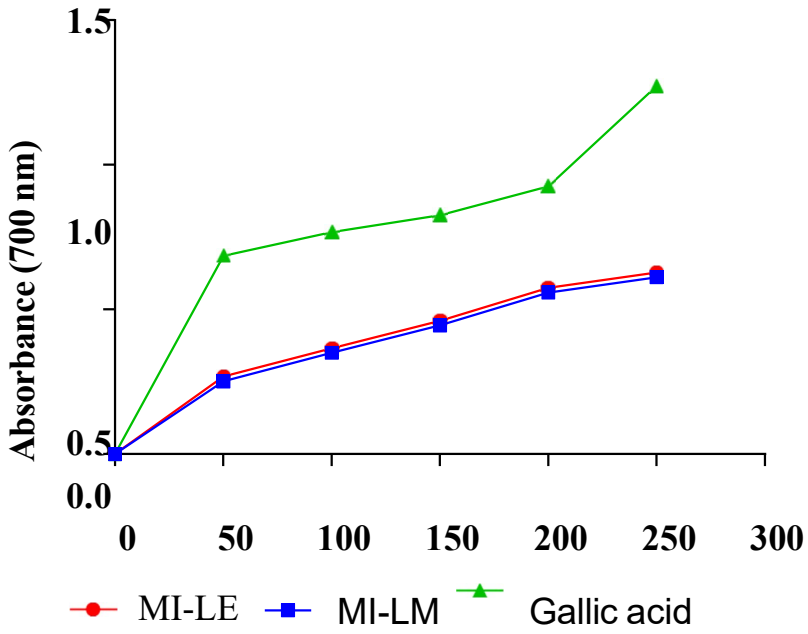

Figure 10: Reducing power assay of ethanol and methanol extracts of MI leaves (*In vitro* Raised Plant)

CONCLUSION

Our work has significantly enhanced the process of micropropagation for *Morus indica*, whichnow presented high prospects for commercial propagation. The combined survival and qualitymanagement of *Morus indica* in vitro culture by effective rooting and improvement reported in this work is noteworthy. It is anticipated to have significant commercial potential and pave the way for additional study on the growth of the plant. Both the donor plant extracts and the in vitro produced plant extracts showed a strong and considerable antioxidant efficacy when evaluating the antioxidant activity in terms of the ABTS radical scavenging, total antioxidant activity and the reducing power assay assessment. Nonetheless, it was discovered that the leaf extracts of the *Morus indica* plant grown in vitro exhibited greater and superior antioxidant activity. All things considered, our findings represent a significant advancement in the techniques for micropropagating *Morus indica* and offer valuable data for more research andpractical use.

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Declaration of interest

The authors declare no conflict of interest in this manuscript.

Add-on information authors' contribution

Develop thought work, Conducted the research, experiment and data analyses,

Prepared and written the initial draft.

Research content

The research content of manuscript is original and has not been published elsewhere.

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All authors agree to publish the paper in **International Journal of Biology, Pharmacy and Allied Sciences**.

Conflict of interest

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REFERENCE

[1] Matus, J. T., Loyola, R., Vega, A., Peña-Neira, A., Bordeu, E., Arce-Johnson, P., & Alcalde, J. A. Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of Vitis vinifera. *Journal of Experimental Botany*, *60*(3) (2009) 853-867. https://doi.org/10.1093/jxb/ern336

[2] Andallu, B., Suryakantham, V., Lakshmi Srikanthi, B., & Reddy, G. K. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. *Clin Chim Acta 314*(1-2) (2001) 47-53. https://doi.org/10.1016/s0009-8981(01)00632-5

[3] Zhishen, J., Mengcheng, T., & Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry 64*(4) (1999) 555-559. https://doi.org/https://doi.org/10.1016/S0308-8146(98)00102-2

[4] Anis, M., Faisal, M., & Singh, S. K. Micropropagation of mulberry (Morus alba L.) through in vitro culture of shoot tip and nodal explants. *Plant Tissue Cult*., *13*(1) (2003) 47-51.

[5] Taha, H., Ghazy, U. M., Gabr, A. M. M., El-Kazzaz, A. A. A., Ahmed, E. A. M. M., & Haggag, K. M. Optimization of in vitro culture conditions affecting propagation of mulberry plant. *Bulletin of the National Research Centre 44* (2020) 1-9.

[6] Zhang, D., Xu, X., Zhang, Z., Jiang, G., Feng, L., Duan, X., & Jiang, Y. 6- Benzylaminopurine improves the quality of harvested litchi fruit. *Postharvest Biology and Technology 143* (2018) 137-142.

[7] Novero, A. U., & Jamiri, F. Plant

regeneration through direct shoot formation from sago palm (*Metroxylon sagu Rottb*) leaf explants. *Asian J Biotech 4* (2012) 92-99.

[8] Kanchanapoom, K., Jingjit, S., & Kanchanapoom, K. In vitro flowering of shoots regenerated from cultured nodal explants. *Notulae Botanicae Horti Agrobotanici Cluj- Napoca 39*(1) (2011) 84-87.

[9] da Silva, J. A. T., Winarto, B., Dobránszki, J., Cardoso, J. C., & Zeng, S. Tissue disinfection for preparation of culture. *Folia Horticulturae 28*(1) (2016) 57-75.

[10] Teixeira da Silva, J. A., Kulus, D., Zhang, X., Zeng, S., Ma, G., & Piqueras, A. Disinfection of explants for saffron (*Crocus sativus*) tissue culture. *Environmental & Experimental Biology 14*(4) (2016) 183-198.

[11] Asmono, S. L., & Sjamsijah, N. The effect of murashige and skoog (MS) modifiedmedium and several types of auxins on the growth of stevia (*Stevia rebaudiana bertoni*) in vitro .N. IOP. *Conf. Ser.:Earth Env. Science.* (2021) 672.

[12] Trigiano, R. N., & Gray, D. J. *Plant Tissue Culture, Development, and Biotechnology*. CRC Press (2016). https://books.google.co.in/books?id=I0LOBQAAQBAJ

[13] Peddaboina, V., Thamidala, C., & Karampuri, S. In vitro shoot multiplication and plant regeneration in four Capsicum species using thidiazuron. *Scientia horticulturae 107*(2) (2006) 117-122.

[14] Wochok, Z. S., & Sluis, C. J. Gibberellic acid promotes Atriplex shoot multiplication and elongation. *Plant Science Letters 17*(3) (1980) 363-369.

[15] De Klerk, G.-J. Rooting of microcuttings: theory and practice. *In Vitro Cellular & Developmental Biology-Plant 38*(5) (2002) 415-422.

[16] Bag, N., Palni, L. M. S., & Nandi, S. K. An efficient method for acclimatization. *Current Science 117*(2) (2019) 288-293.

[17] Zapata, E. V., Morales, G. S., Lauzardo, A. N. H., Bonfil, B. M., Tapia, G. T., Sánchez, A. D. J., Valle, M. V. D., & Aparicio, A. J. In vitro regeneration and acclimatization of plants of turmeric (*Curcuma longa* L.) in a hydroponic system. *Biotecnol. Apl 20* (2003) 25-31.

[18] Smart, R. E., & Bingham, G. E. Rapid estimates of relative water content. *Plant physiology 53*(2) (1974) 258-260.

[19] Paul, V., Sharma, L., Pandey, R., & Meena, R. C. Measurements of stomatal density and stomatal index on leaf/plant surfaces. *Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on—Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants*., 27 (2017).

[20] Harborne, J. B. *Phytochemical methods: A guide to Modern Techniques of Analysis*. Chapman and Hall Publishers (1973).

[21] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine 72* (1998) 1231-1237.

[22] Mitsuda, H., Yuasumoto, K., & Iwami, K. Antioxidation action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo*, *19* (1996) 210-214.

[23] Oyaizu, M. Studies on product of browning reaction prepared from glucose amine. *JpnJ Nutr 44* (1986) 307-315.