MICROPROPAGATION OF TURMERIC (*Curcuma longa* L.)ON LARGE-SCALE

ABSTRACT

Aim: Turmeric (*Curcuma longa* L.) is a slow-to-reproduce perennial herb that is propagated from ground rhizomes. Despite its well-known medicinal and culinary uses, its supply for propagation is limited due to disease susceptibility and slow rhizome development.

Methodology: To optimize turmeric bud cluster development and determined appropriate concentrations of BAP and NAA for enhancing shoot multiplication and rooting rates.

Results: The results indicated that a combination of 3 mg L^{-1}BAP and 0.5 mg L^{-1}NAA was optimized for turmeric shoot multiplication, while a combination of 1 mg L^{-1}BAP and 0.5 mg L^{-1} NAA was selected as the best concentration for shoot clusters formation of turmeric in vitro. Additionally, increasing NAA concentration led to higher number of roots production, although not to root elongation.

Conclusion: This research contributed to advancing our knowledge of valuable plants in Vietnam, particularly turmeric production on large-scale.

Keywords: *Turmeric, Curcuma longa L.,micropropagation, rhizomes, shoot multiplication, shoot clusters formation, Zingiberaceae.*

UNDER PEER REVIEW

1 INTRODUCTION

Curcuma (*Curcuma longa* L.), a family Zingiberaceae is native to tropical and subtropical areas of India, southern Asia, and southern China. There are about 50 different species of Curcuma in existence today. Many of these, which have ellipsoid tubers, are used in traditional medicine as aromatic stomachics and cholagogues, as well as spices and yellow dye[1]. Turmeric rhizomes have been used to treat dyspepsia and peptic ulcers [2], [3]. Since the species of curcuma contain molecules credited with anti-inflammatory, hypocholesterolemic, choleretic, antimicrobial, insect repellent, antirheumatic, antifibrotic, anti venomous, antiviral, antidiabetic, antihepatotoxic, as well as anticancer properties, the plant is gaining more attention throughout the world as a potential source for new drug(s) to cure a variety of ailments [4]. In addition, turmeric stems and leaves can be utilized as biofertilizers in several nations [5]. With an annual production of roughly 986,690 tons from an area of 194,330 ha, India led the globe in terms of producing, consuming, and exporting turmeric from 2012 to 2013 [6]. The Yearbook of Agricultural Statistics in Bangladesh stated that Bangladesh produces 143,542 tons of turmeric each year, from a cultivation area of 62,746 acres (approximately 25,412 ha). Turmeric has poor multiplication rate of 6 to 10 times, but its rhizome production ranged from 15-20 tons per hectare [7].

The high susceptibility of this crop to leaf spot disease, caused by species of *Taphrina* and *Collectotrichum*, and rhizome rot diseases, caused by Pythium species, result in severe damage and

a lack of healthy planting materials [8]. Additionally, because turmeric is a warm-season crop, it is challenging for rhizomes to survive in cold climates [9]. Rhizomes of *Curcuma longa* only sprout during the monsoon because they have periods of hibernation, which results in a poor rate of propagation. This plant is vulnerable to a number of harms, including insect assault, pathogen infection, and unfavorable environmental conditions. As a result, conventional propagation, conservation, and storage are quite challenging. Shoot multiplication of turmeric in vitro is proved to overcome these obstacles and meet market and planting material demands because it promotes higher levels of substances in biochemical and pharmaceutical production. It also improves multiplication, maintains species variety, and conserves genetic resources.

An alternative and best approach for propagating turmeric is through micropropagation or tissue culture, which has lately been used in various Asian nations [10][7][9]. The first tissue culture trial on turmeric was in 1978 using stem tips of *C. domestica* cultured in MS media supplemented with 6benzylaminopurine (BAP) to propagate shoots reported by Nadgauda et al. The procedure for the quick multiplication and short-term preservation of ginger, *C. domestica*, *C. aeruginosa*, and *C. caesia* was successfully created by Balachandran et al. (1990) [7]. According to his research, 3 mg L^{-1}BAP was the ideal concentration for multiplying shoots across all species. According to Loc et al., (2005) [12], the number of new shoots produced per explant rose dramatically when 0.5 mg L^{-1}NAA was added to medium containing 3 mg L^{-1}BAP. In 1986, Mukhri and Yamaguchi [13] successfully grew shoot, root, and callus of Begonia production utilizing Ring and Nitsch medium [14].
Shoot multiplication of turmeric in vitro is growing more and more popular worldwide yet;It has been studied and produced in large-scale in Vietnam for powder processing to supply for domestic and export. Vietnam has lately emerged as one of the few nations that successfully used nanotechnology to create Nano-Curcumin, a medication derived from turmeric that is used to treat a variety of ailments. There is very little report data about the cultivation of turmeric in Vietnam and the high demands of cleaned seedlings for production, particularly shoot multiplication in vitro. Traditional turmeric propagation has a lot of issues, hence in vitro shoot multiplication is crucial for turmeric. The successful multiplication technology in vitro will help create a large number of seedlings in a short time, uniform quality, healthy and disease-free seedlings. As a result, it is possible to develop areas of this valuable medicinal herb for domestic consume and export. The goal of this study is to ascertain on BAP and NAA affect in vitro micropropagation on large-scale

2 MATERIALS AND METHODS

2.1 Plant materials

Underground rhizomes of *C. longa* L., with larger sizes, heathy mother rhizomes as well as fingerlike lateral offshoots grown in Daklak(native source from high-land of Vietnam) were collected and prepared for the study. The material used is old turmeric root with physiological enough germination. Healthy, disease-free turmeric without any fungi and physical damages on the skins were chosen as initial donors. Turmeric rhizomes were washed thoroughly under running tap water with a small addition of soap to remove all the earthy particles sticking to their surfaces. These rhizomes were

UNDER PEER REVIEW

subsequently soaked into soapy water (1 spoon of soap/liter of water) for 1 hour and washed until they have no soap left to completely remove any remaining dirt. Clean rhizomes were placed on the wet sand for 3 weeks until the shoot sprouts germinate. These shoot sprouts would be used for later experiments

The basic nutrient medium is MS (Murashige and Skoog, 1962) [15], vitamins MW (Morel & Wetmore, 1951) [16], supplemented with 30 g L^{-1}sucrose, 10% coconut water, and 8 g L^{-1}agar, pH wasadjusted to 5.8;and growth regulators BAP (6-benzylaminopurine), NAA (α-naphthalene acetic acid)

Conditions: Media was sterilized in autoclaving at 121ºC, 1 atm. for 20 minutes.Cultures were maintained at a temperature of 26 ± 2 ºC with a 16-hour photoperiod.

2.2 Methods

2.2.1 Shoots sterilization: Sodium hypochlorite solution was prepared by dissolving Hypochlorite-Na powder (Chlorine 70%, Japan) in autoclaved distilled water. After being stirred for 30 minutes, the mixture was filtered through sterilized filter papers twice to eliminate the insoluble residue.

Green and healthy shoot tips (2-3cm) containing a small piece of rhizome were excised from rhizomes using a sharp scalpel and washed again by tap water as well as soapy water. In order to enhance the efficiency of surface sterilization, scale leaves of the rhizome where shoot buds grown, were removed completely. The explants were treated with 0.5% Physan (fungicide-commercial brand) for 30 minutes before being taken to the laminar hood chamber for surface sterilization. After being isolated, shoot tips were immersed in 70% alcohol for 30 seconds, following by Hypochlorite-Na at different concentrations and treatment duration (with an addition of three drops of Tween 20). Finally, explants were treated with 0.1 % (m/v) mercuric chlorine (HgCl2) with three drops of Tween 20 for 10 minutes, then rinsed 3-4 times with autoclaved distilled water to ensure that the materials are free from chemical and ready for inoculation in culture media. The outer layer of explants and the part of the explants which were exposed with chemical and changed color, were trimmed off.

2.2.2 Shoot clusters formation of turmeric in vitro: The formation of bud clusters was examined at four concentration of BAP (1-3-5-7 mg L^{-1})respectively combined with 0.5 mg L^{-1}NAA (Balachandran et al., 1990 [7]; Yusuf et al., 2007 [17]). Parameter evaluation: the number of multi-shoot clusters and number of shoots per cluster. After 30 days, records of data were collected.

2.2.2 Effect of BAP on shoot multiplication in vitro: In vitro shoot multiplication was examined at four BAP (1-3-5-7 mg L^{-1}) respectively with NAA (0.5 mg L^{-1})[7],[17]). Parameter evaluation: the length of shoots and the number of shoots. After 30 days, records of this data were collected.

2.2.3 Effect of NAA on shoot multiplication in vitro: In vitro shoot multiplication was examined at two BAP (3-5 mg L^{-1})combined with NAA (0.3- 0.5-0.7-1.0 mg L^{-1}) respectively [12], [18]. Parameter evaluation: the length of shoots and the number of shoots. After 30 days, records of this data were collected.

2.2.4 Effect of NAA on rooting in vitro: The rooting in vitro was examined with NAA (0.1-0.3-0.5 mg L^{-1}) (Abbas et al.,2011 [19]). Parameter evaluation: number of roots and root length. After 30 days, records of this data were collected.

UNDER PEER REVIEW

2.3 ANOVA Analysis

The experiment was performed four replicates. One replicate was 3 bottle. One bottle contains 3 samples. The data were analyzed by SPSS v.20, one-way ANOVA analysis with Tukey's test at $\alpha = 0.05$

3. RESULTS

3.1 Effects of sodium hypochlorite concentrations and treatment duration on surface sterilization

In the first set of the experiment, the introduction of turmeric's shoot determines the success of all the experiments. However, the establishment of contamination free shoots is a challenging task, which was indicated by the result in which nearly 84% of the total cultures were initially contaminated with fungi and bacteria. Out of the six treatments, treatment with 5% Hypochlorite-Na in 30 minutes, followed by 10 minutes of 0.1% (m/v) HgCl2 showed the highest percentage of survival explants, with 33% of shoots growing normally (Table 1). In detail, the survival rate of explants treating in 40 minutes with 5% and 10% Hypochlorite-Na were lowest at 7% and 13% respectively as compared to the groups in the same concentration. Additionally, while treatment with 5% in 30 minutes could be seen as the optimal concentration and duration for sterilization of turmeric shoot, there was neither statistically significant difference between three treatments applying 10% of Hypochlorite-Na nor two treatments applying 5% of Hypochlorite-Na. However, the standard errors of most of the treatments were quite high which showed the significant impact on the result, except for the treatments using 5% Hypochlorite-Na in 20 and 30 minutes.

Table 1.Effect of Hypochlorite-Na on sterilization.

Hypochlorite-Na (%)	Minutes	Survival (%)
5%	20	10 ± 0.02 b
	30	33 ± 0.00 a
	40	7 ± 0.05 b
10	20	16 ± 0.05 ab
	30	17 ± 0.06 ab
	40	13 ± 0.09 ab

3.2Shoot clusters formation in vitro.

In Table 5 and Figure 1, there was no significant difference in the number of shoots per cluster. However, the number of multi-shoot clusters at concentrations 1, 3 and 5 mg L^{-1} BAP was relatively good compared to other treatments; and the number of multi-shoot clusters was not significant difference (Table 2). This was similar to the medium supplemented with BAP at a concentration of 7 mg L^{-1}. The growth of numerous shoot clusters as well as the number of shoots per cluster were advantageously enhanced by a higher concentration of BAP, even though the results showed no discernible difference between the five treatments.

UNDER PEER REVIEW

Table 2**.** Multi-shoot clusters formation in vitro with different combinations of BAP and NAA concentrations

Medium	concentration NAA (mg L^{-1})	concentrations BAP(mg L^{-1})	Number of shoot per clusters	Number of multi-shoot clusters
MS	0	0	2.95±0.06a	1.65±0.06b
	0.5	1	2.93±0.09a	2.55±0.06a
		3	3.15±0.14a	2.70±0.12a
		5	3.05±0.06a	2.70±0.07a
		7	3.03±0.13a	1.85±0.06b

Different letter designations (a–b) in the same column indicate a significant difference at the 95% confidence level. The values represent mean ± standard deviation

3.3 BAP on shoot multiplication in vitro

The explants were cultured in fresh medium containing BAP hormone at different concentrations for shoot multiplication. In Table 3 and Figure 1, there was a significant difference in the number and length of shoots with different concentrations of BAP. MS medium without two hormones gave the lowest number of shoots. In Figure 3, the number of shoots achieved on the medium with 3 and 5 mg L^{-1}BAP was the highest compared to other treatments. In addition, the length of shoots achieved on the medium supplemented with 1 mg L^{-1}BAP combined with 0.5 mg L^{-1}NAA was the highest.

Table 3.Effect of BAP on shoot multiplication in vitro with different combinations of BAP and NAA concentrations

Medium	NAA (mg L^{-1})	BAP (mg L^{-1})	Number of shoot per clusters	Number of multi-shootper clusters
MS	0	0	4.85±0.06d	7.22±0.02b
	0.5	1	7.50±0.07b	8.70±0.05a
		3	8.45±0.06a	6.09±0.02d
		5	8.20±0.09a	6.46±0.02c
		7	5.60±0.09c	5.65±0.02e

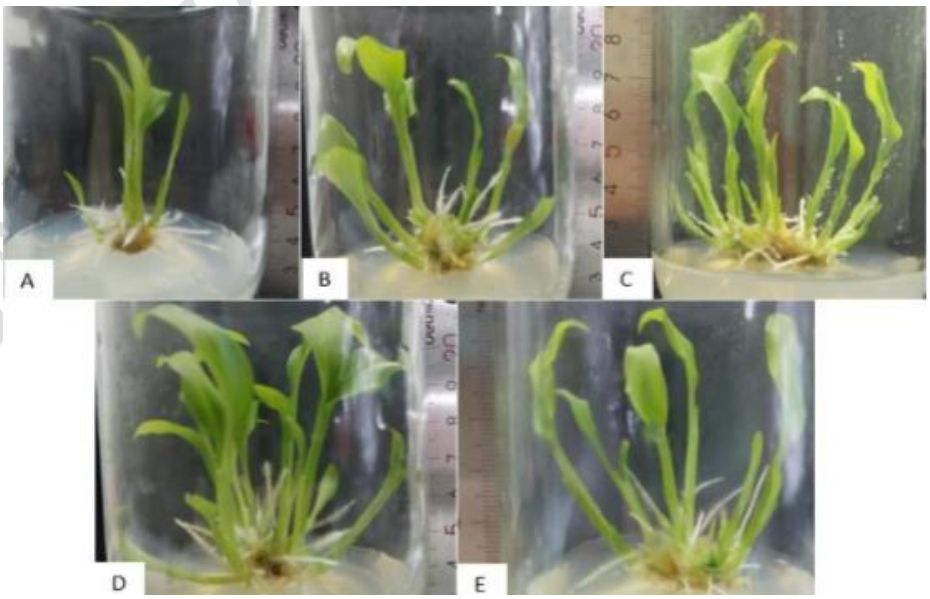

Figure 1. Effect of BAP on shoot multiplication in vitro after 30 days. (A) Without BAP and NAA, (B) BAP (1 mg L^{-1}), (C) BAP (3 mg L^{-1}), (D) BAP (5 mg L^{-1}), (E) BAP (7 mg L^{-1})

UNDER PEER REVIEW

3.4Effect of NAA on shoot multiplication in vitro

BAP with concentrations of 3 mg $L^{-1}$and 5 mg $L^{-1}$was chosen as a stable factor for this experiment based on the findings from the previous experiment [12], [18]. After 30 days, the number of shoots treated with nine different BAP and NAA combinations was reported and listed in Table 4. There was a significant difference in the number and length of shoots in this experiment. The graph demonstrated that explants growing on hormone-free MS media had the lowest capacity for shoot multiplication. Hormone inclusion into the culture media was crucial for shoot multiplication because other hormone treatments also resulted in positive shoot growth. The treatment with 5 mg L^{-1}BAP and 0.3 mg L^{-1}NAA produced the highest rate of shoot multiplication. In Figure 2 and 5, the higher number of shoots from the medium with low NAA concentrations (0.3 and 0.5 mg L^{-1}) and decreased significantly when increasing the concentration of NAA to 0.7 and 1.0 mg L^{-1}. The treatment with the combination of BAP 3 mg $L^{-1}$and NAA 0.7 mg L^{-1}gave the highest shoot length and the combination of BAP 3 mg $L^{-1}$and NAA 0.5 mg L^{-1}gave the lowest shoot length.

Table 4.Effect of NAA on shoot multiplication in vitro with different combinations of BAP and NAA concentrations.

Medium	BAP (mg L^{-1})	NAA (mg L^{-1})	Number of shoots per clusters	Length of shoots (cm)
MS	0	0	4.85±0.06g	7.22±0.02f
	3	0.3	8.00±0.09c	8.91±0.04c
		0.5	8.45±0.06b	6.09±0.02i
		0.7	7.60±0.09d	10.05±0.03a
		1.0	6.55±0.06f	7.88±0.02e
	5	0.3	9.40±0.09a	8.62±0.02d
		0.5	8.20±0.09bc	6.46±0.02h
		0.7	6.70±0.07f	6.79±0.01g
		1.0	7.15±0.06e	9.26±0.03b

UNDER PEER REVIEW

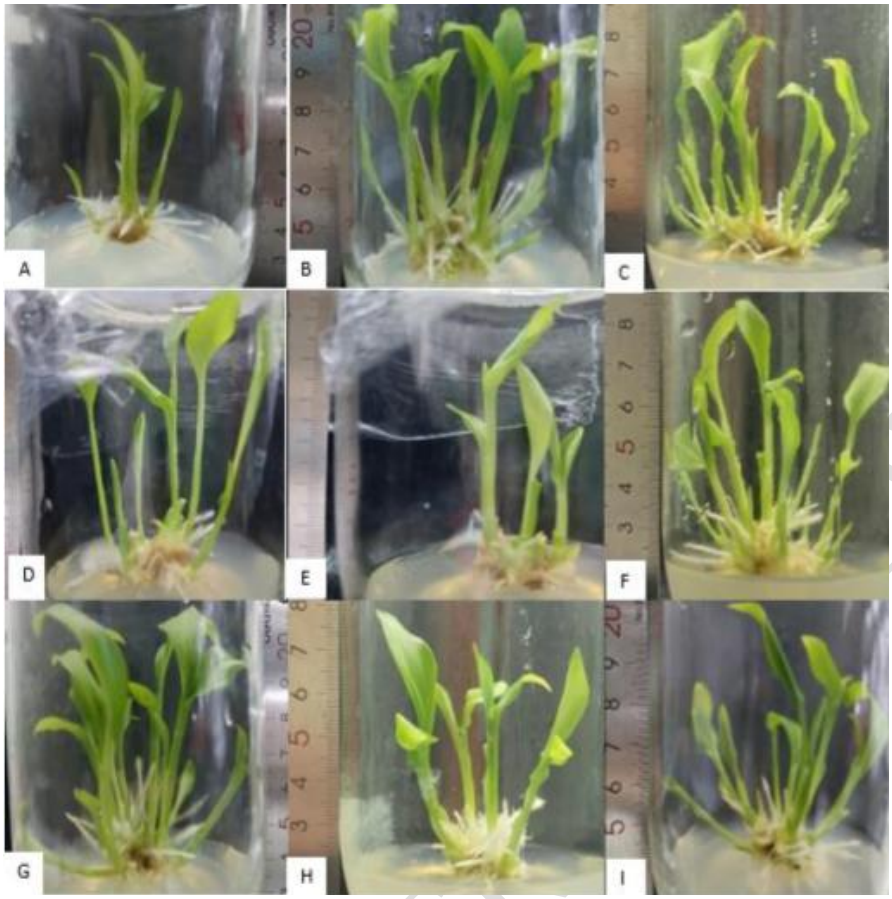

Figure 2.Effect of NAA on shoot multiplication in vitro after 30 days
(A) Without BAP and NAA, (B) NAA (0.3 mg L^{-1}) + BAP (3 mg L^{-1}), (C) NAA (0.5 mg L^{-1}) + BAP (3 mg L^{-1}), (D) NAA (0.7 mg L^{-1}) + BAP (3 mg L^{-1}), (E) NAA (1.0 mg L^{-1}) + BAP (3 mg L^{-1}), (F) NAA (0.3 mg L^{-1}) + BAP (5 mg L^{-1}), (G) NAA (0.5 mg L^{-1}) + BAP (5 mg L^{-1}), (H) NAA (0.7 mg L^{-1}) + BAP (5 mg L^{-1}), (I) NAA (1.0 mg L^{-1}) + BAP (5 mg L^{-1})

3.5 Effect of NAA on rooting in vitro

In Table 5 and Figure 3, there was a significant difference in the number and length of roots with different concentrations of NAA. The results showed that the MS control medium without NAA produced lowest the number of roots. In addition, in MS medium added 0.5 mg L^{-1}NAA, the highest number of roots was 9.05 roots. Root length was significantly affected by increasing NAA concentration. The length of roots with 0.5 mg L^{-1}NAA was the lowest in all four treatments. The highest root length was found when adding 0.1 mg L^{-1}NAA.

Table 5. Effects of NAA on rooting in vitro

Medium	NAA (mg L^{-1})	Number of roots	Length of roots (cm)
MS	0.0	5.45±0.35d	2.62±0.16c
	0.1	6.35±0.17c	4.52±0.01a
	0.3	7.75±0.27b	3.19±0.06b
	0.5	9.05±0.06a	1.35±0.15d

UNDER PEER REVIEW

Figure 3. Effect of NAA on rooting in vitro after 30 days
(A) Without NAA,(B) NAA (0.1 mg L^{-1}),(C) NAA (0.3 mg L^{-1}),(D) NAA (0.5 mg L^{-1}).

4. DISCUSSION

4.1 Effects of sodium hypochlorite concentrations and treatment duration on surface sterilization

In this research, the combination of fungicide, ethanol 70%, Hypochlorite-Na, mercuric chlorine as well as Tween 20 was tested to ensure that there was as little contamination as possible. At the pre-treatment step, turmeric was treated carefully with soap and Physan which is a fungicide to eliminate as many pathogens as possible. Hypochlorite-Na is an alteration to the traditional bleach (NaOCl) due to its higher content of chlorine, easy to manage the concentration and its stability in preservation [20]. Most bleach solutions on the market are only available as industrial bleach (5%) which was difficult to ensure a definite concentration due to the evaporation or leakage of chemical during transportation or preservation. Meanwhile, Hypochlorite-Na can be purchased in powder form and overcome the above disadvantages. There was little evidence that Hypochlorite-Na was applied for surface sterilization in turmeric, therefore, this study will contribute greatly to the sterilization protocol using this sterilizing agent. Similar to the research of Islam [21], 0.1% mercuric chlorine in combination with Tween 20 was tested in this research to enhance the survival rate of explants after sterilization. It was doubted that thanks to this combination, a number of contaminated-free shoots were successfully established in this study.

From the result, it is noticeable that the highest survival rate (33%) was achieved when the explants were treated with 5% of Hypochlorite-Na in 30 minutes. In contrast, more than 80% of the explants in the other treatments were found to be infected with bacteria and fungi after 3 to 14 days of inoculation. However, once the explants were introduced successfully after a month, contamination was no longer found. The explants started to proliferate and turned green after a week after the establishment. Although the treatment with 5% of Hypochlorite-Na in 30 minutes showed a positive result, there was almost no significant difference from the others. It seems that the longer durations were applied, the more death rate appeared. When explants were exposed to chemical for a longer time, turmeric shoots would be damaged due to the penetration of sterilizing agents into host cells which would cause lethal to initial explants [22],[23],[24]. This should be similar to the increase in the

UNDER PEER REVIEW

concentration of Hypochlorite-Na; however, the use of hypochlorite-Na with two concentrations did not give an acceptable result. A higher percentage of contamination-free explants was observed when the explants were treated with 10% of Hypochlorite-Na in 20 minutes and 40 minutes, at 16% and 13% respectively, while this rate at 5% was only 10% and 7%. Although this study used a combination of many surface sterilizing agents, its result was not as favorable as the other research which used the combination of only three chemicals such as fungicide, alcohol, $HgCl_2$ or $NaHClO_3$, ethanol and 0.1% $HgCl_2$[25], [24].

Most of the contamination in general or surface adhering microbial contaminants (epiphytic) in specific are usually reduced and controlled by suitable surface sterilizing agents [26]. However, the elimination of the endophytic contaminants which are present within the explants is a laborious process, especially bacteria contaminants [27]. The disinfection for these types of contamination not only required an abundance of time and effort, but also should be performed with suitable fungicides or antibiotics. Hence, this could be a reasonable explanation for the undesirable results of this sterilization experiment. During the pre-treatment procedure, putting the rhizomes of turmeric in either wet sand or water could lead to the establishment and penetration of pathogens into the explants. Although soaking the rhizomes in soapy water in 1 hour could reduce the fungi or bacteria to some certain extent, high moisture during the germination created an ideal environment for fungi and bacteria to exist and develop, enhancing the level of difficulty of the challenges for in vitro sterilization later.

One reasonable explanation for the inconsistency of clean explants in six treatments. Apart from what has been mentioned, the number of explants in one time of sterilization also affected to the contamination rate of turmeric. To be specific, the more samples treated in a bottle in the sterilization process, the lower the survival explants were. In addition, there were almost no browning explants recorded in all experiments, which indicated that no toxicity was caused by sterilization protocols.

4.2Shoot clusters formation of turmeric in vitro.

It has been reported in the literature that BAP with NAA had the potential to promote cell division, induce shoot proliferation, and induce axillary shoots in turmeric [28], [29], [30], [17]. To promote the growth of numerous shoots, the right cytokinin concentration and kinds were essential [31], [32]. Many studies indicated that MS medium supplemented with 3 mg L^{-1}BAP produced the most numerous shoots of all species [7], [17]. In other words, there was no discernible difference between the five treatments when many shoot clusters were induced. However, 1, 3, and 5 mg L^{-1}BAP in combination with 0.5 mg L^{-1}NAA was the ideal concentration of BAP for the growth of multiple shoot clusters and shoot numbers per cluster. This result was closely similar to the findings of Nasirujjaman[29], who claimed that the medium contained 4.0 mg. L-1 BAP and 0.5 mg L^{-1}NAA had the optimum ability to promote the development of turmeric shoots. However, the expense increased with the hormone concentration. As a result, the optimal dose for the shoot clusters formation of turmeric was 1 mg L^{-1}BAP.

However, the percentage of somaclonal variance rose when PGRs were added to the medium. PGR, such as BAP has been linked to the induction of somaclonalvariation. The impact of PGRs on

UNDER PEER REVIEW

somaclonal variation has been the subject of numerous reports; some focus on auxin and others on cytokinin. Cytokinin disrupts the cell cycle to cause somaclonal variation [33]. From Table 5, MS is the best medium for turmeric to avoid mutations.

Numerous studies showed when the quantity of numerous shoot clusters increased, so did the number of young shoots [28], [29], [30]). It followed that the treatment with a greater capacity for producing multiple shoot clusters also facilitated the proliferation of shoots per cluster. However, it seemed that raising the BAP concentration had a greater impact on the number of shoots, but it had no significant effect on shoot cluster formation.

4.3 Effect of BAP on shoot multiplication in vitro

A complex interplay of hormones, nutrients, and environmental cues regulated the growth of shoots. Many plant growth and developmental processes were regulated by the interaction of the phytohormones auxin and cytokinin [34]. Axillary bud development was coordinated by the antagonistic interactions between auxin and cytokinin. A wide range of plant growth and development features was related to cytokinins. Cytokinin induced cell division, reduced shoot apical dominance and stimulated lateral bud development led to the formation of multiple shoots. When the cytokinin concentration was high, it inhibited the elongation of cells and increased the number of shoots. Auxin stimulated cell elongation, cell division, and root formation. When auxin concentration was high, it released ethylene and inhibited shoot growth[43].

Numerous studies on the cultivation of plants belonging to the Zingiberaceae family (turmeric, galangal) indicated that the use of BAP provided good shoot regeneration. In addition, studies on the culture of turmeric plants indicated that the shoot multiplication was better when BAP-enriched MS base medium was used in combination with NAA. According to studies on *Etlingera elatior* (Zingiberaceae) [31] the MS medium with 2.2 mg L^{-1}BAP had the highest number of shoot multipliers at 3.67 shoots/explants.Rahman [30] studied on MS basal medium supplemented with 2.0 mg L^{-1}BAP produced the highest number of shoots for *C. longa* L in vitro.

The best concentration of MS medium with BAP for shoot growth was found to be 2.0 mg L^{-1}[35], [36]. Mohamed [37] study ginger (*Zingiber officinale* Rosco) plants used the MS medium with BAP (4.5 mg L^{-1}) to grow the maximum shoots in vitro. The maximum shoot growth coefficients were reached when three *Curcuma longa*L. cultivars such as Faisarabad, Kasur, and Bannung were grown on a medium enriched with 4.0 and 5.0 mg L^{-1}BAP, according to research by Naz et al.[18] Although the results showed no significant difference between the medium with BAP 3 mg $L^{-1}$and BAP 5 mg L^{-1}, but in terms of cost or many other factors, the concentration of 3 mg.L-1 BAP is preferred. Furthermore, the shoot length in these two treatments was relatively low, which was suitable for in vitro shoot multiplication purposes. Although the shoot length in 1 mg L^{-1}BAP was the best, the number of regenerative shoots was not high, so it was not suitable for shoot multiplication purposes. However, from Table 6, MS is the best medium for turmeric to avoid mutations.

In addition, when the concentration exceeded the threshold, the plant growth regulators turned into inhibitors [38]. In particular, the number of shoots was diminished if the concentration of BAP hormones exceeded this threshold. The result showed the number of shoots increased as BAP

UNDER PEER REVIEW

concentrations were increased from 0.0 to 5.0 mg L^{-1}. The number of shoots tended to decline when BAP concentrations were increased to 7.0 mg L^{-1}. Although the number of shoots increased as the BAP concentration increased. However, at the concentration of 7 mg L^{-1}BAP, it exceeded the allowable threshold of turmeric, so BAP became an inhibitor causing the number and length of shoots to decrease significantly.

4.4 Effect of NAA on shoot multiplication in vitro

It was known that the use of BAP in combination with NAA increased the shoot multiplication rate and promoted shoot elongation [21], [12], [39], [30]). Using varied concentrations of NAA, 3 and 5 mg L^{-1}BAP were chosen as the optimal cytokinin concentration for shoot proliferation based on the findings of the previous experiment [37], [12]. According to Loc et al.12], the number of new shoots produced per explant rose dramatically when 0.5 mg L^{-1}NAA was added to MS medium containing 3 mg L^{-1}BAP. That was consistent with the results of this experiment when 3 mg L^{-1}BAP and 0.5 mg L^{-1}NAA had the second highest number of shoots with 8.45 shoots. However, the inclusion of NAA produced the highest length results (BAP 3 mg $L^{-1}$and NAA 0.7 mg L^{-1}) but the number of shoots was relatively low when the shoots were multiplied.

The number of shoots decreased as the NAA concentration increased. Auxin released ethylene at high auxin concentrations, which prevented shoot growth [38], [43]. Therefore, a higher number of shoots was produced by modest doses of 0.3 or 0.5 mg L^{-1}NAA. However, from Table 7, MS is the best medium for turmeric to avoid mutations.

4.5 Effect of NAA on rooting in vitro

In plants, auxins played a role in the development of lateral roots, the regulation of xylem development, and promoted cambium growth [40]. Auxins encouraged the adventitious root production, and its function in regulating the formation of roots, as well as their length and number. Moreover, the role of auxin was more prominent during root induction. In general, the production of roots in plants happened in two stages; the first was auxin-sensitive, whereas the second was not. Therefore, culture medium with enhanced concentration of auxins, encouraged root formation for turmeric in vitro.

Auxin was regarded as a crucial regulator of lateral root development, gravitropism, and root growth [41]. Auxin impacted on root development depended on both its polar translocation and concentration [42]. On an MS medium containing 2.0 mg L^{-1}NAA, ginger shoots successfully developed roots[12]. Abbas et al.[19] reported that an MS medium with 1.0 mg L^{-1}NAA produced the best rooting results for ginger (*Zingiber officinale* Rosco). It showed that the high NAA concentration resulted in a high rooting rate. Similarly, the results obtained in this experiment showed that at the concentration of 0.5 mg L^{-1}NAA there was the highest number of roots, however the roots at this concentration did not grow in length. Overall, all four treatments with increasing NAA concentrations were proportional to the number of roots. The treatments containing NAA increased gradually, the root length tended to decrease.

5. CONCLUSION

UNDER PEER REVIEW

In conclusion, this study found that the number of shoots per cluster at different BAP concentrations was not significantly different. Therefore, considering the cost factor, the concentration of 1 mg L^{-1}BAP was selected as the best concentration for clusters formation of turmeric shoot in vitro. However, with regards to shoot multiplication, 3 and 5 mg L^{-1}BAP were found to produce a significantly higher number of shoots at a relatively shorter length, which was suitable for the purpose of shoot multiplication of turmeric in vitro. Price-wise, 3 mg L^{-1}BAP was preferred for the multiplication of turmeric buds. A combination of 5 mg L^{-1}BAP and 0.3 mg L^{-1}NAA showed the highest number of shoots. This showed that NAA also had a certain effect on shoot multiplication. Rooting experiments with different concentrations of NAA were found to result in a difference in the number of roots. Increasing concentration of NAA resulted in a high number of roots, specifically at 0.5mg L^{-1}NAA concentration had the highest number of roots.

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