J. Trop. Resour. Sustain. Sci. 12 (2024): 61-68
https://doi.org/ 10.47253/jtrss.v12i1.1367

Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

Plantlet regeneration of *Cucumis melo* L. Glamour cv. using different types of cytokinin and explants

Fadzlin Qistina Fauzan[1], Arifullah Mohammed[1]*, Ahmed Mahmood Ibraheem[2], Raimi Mohamed Redwan[1], Suhana Zakaria[1], Dwi Susanto[3] and Mohd Razik Midin[4]

[1]Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[2]Biotechnology Department, College of Applied Science, University of Fallujah, 31002, Fallujah, Anbar, Iraq
[3]Faculty of Agriculture, Universitas Islam Nusantara, 40286 Jawa Barat, Indonesia
[4]Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

Received 07 November 2023
Accepted 31 December 2023
Online 30 June 2024

Keywords:
C. melo, explant culture, benzyl aminopurine, thidiazuron, clone

⊠*Corresponding author:
Dr. Arifullah Mohammed
Faculty of Agro-Based Industry,
Universiti Malaysia Kelantan,
17600 Jeli, Kelantan, Malaysia
Email: aurifullah@umk.edu.my

Abstract

Plantlets or clones' regeneration in Cucurbitaceae species can be performed *in vitro* by applying the tissue culture techniques. Hypocotyls, cotyledons, leaves, cotyledonary nodes and petioles can be used as the explants or starting materials to initiate the regeneration under laboratory condition. BAP (Benzyl aminopurine) and TDZ (Thidiazuron) were among the well-known cytokinin used in tissue culture as plant hormones to regulate the plant growth *in vitro*. The effectiveness of BAP and TDZ was investigated in this study to determine the effect of different types of cytokinin hormone on *C. melo* explants since both hormones might show differences in their activity towards *C. melo* explants. This study found that BAP was superior at all used concentrations compared to TDZ during most of the growing stage of the explants (cotyledons and hypocotyls). Based on this finding, the experiment was repeated by using different types of explants which were nodal explants, petioles and young shoots along with cotyledons and hypocotyls, by using BAP that showed excellent plantlet regeneration. Cotyledon and nodal explants successfully regenerated plantlets with excellent height and significant number of shoots compared to the rest of the explants. The plantlets from cotyledons and nodal explants were regenerated *via* direct regeneration without any formation of callus. The direct shoot regeneration was the preferred method for mass production *via* tissue culture because the chances of somaclonal variation is low. Contrarily, the hypocotyls, petioles and shoot tips mostly developed callus and only several treatments formed very small plantlets.

1. INTRODUCTION

Plant tissue culture involves culturing plant cells, tissues, explants, organs, seeds or protoplasts on a synthetic nutrient media under aseptic and controlled environments of light, temperature, and humidity (Bhatia, 2015). Plant regeneration in Cucurbitaceae species can be conducted *in vitro* by applying the tissue culture techniques. Usually, hypocotyls, cotyledons, leaves, cotyledonary nodes and petioles can be used as the explants or starting materials to initiate the regeneration under laboratory condition (Grozeva *et al.,* 2019). In a study, to avoid the occurrence variation through direct regeneration, explants or nodules were used to regenerate bitter melon (Naitchede *et al.,* 2022). Because it changes the originality of the genes of any type of clonal somatic variation, it is described as genetic variation resulting from the process of tissue culture. In the presence of physical variation, the genetic components of the offspring become different from the original plant material (Košmrlj *et al.,* 2015). Due to its origin that is derived from tissue culture, somaclonal variation is also well-known as tissue culture-induced variation (TCIV) (Debnath & Ghosh, 2022).

To initiate the tissue culture, explants must be selected and prepared, and they can either be collected from *in vivo* growing plants grown in planting area (Naitchede *et al.,* 2022) or *in vitro* growing plant from seed culture in laboratory (Miguel, 2023; Grozeva *et al.,* 2019). As the plants reproduce vegetatively or asexually in tissue culture condition, all offspring regenerated from the single plant are considered as clones. The clones or offsprings is identical to that of all the other offspring and the single parent (Bridgen *et al.,* 2018). During the process of *in vitro* regeneration, clones can be regenerated through organogenesis by growing either shoot or root meristems, as well as through the process of somatic embryogenesis, in which shoot and root meristems form simultaneously. In a short period of time, clones or identical duplicates can be regenerated in a tissue culture system (Debnath, 2018). During the early years, tissue

eISSN Number: 2462-2389

culture techniques were divided into three stages that included the establishment, multiplication, and preparation for reestablishment in soil (acclimatization) (Murashige, 1974). As further studies conducted, the stages were reformulated and divided into five with two additional stages compared to the previous one. The new stages begin with the selection of the plant material, followed by establishment, multiplication, pretransplant or rooting, and transplantation or acclimatization, respectively (Anderson, 1980). Due to the fact that each step has its own set of objectives to achieve, the process of micropropagation was improved by the inclusion of the new stages (Bridgen *et al.,* 2018).

In stage zero, the plant material is selected for the micropropagation. The donor plant is examined thoroughly in this stage to ensure that it is the accurate plant, healthy and disease-free, has clean surface, and at the right growth rate. Selecting the healthy donor plant is essential as the plant infected with the disease will only multiply the pathogens along with the regenerants produced. Besides, it is preferable if donor plant with clean surfaces is chosen so that the establishment will not require a difficult sterilization process (Bridgen *et al.,* 2018). The selection of a plant at the right growth rate also is important because it is common for younger or immature parts of a donor plant to respond better in tissue culture as compared to older or more mature parts of the plant (Nguyen *et al.,* 2019).

The explants selected that passed stage zero would serve as the materials for stage one. In stage one, explants undergo a sterilization process to decontaminate the explants without introducing any damage to them. Surface sterilization of explants is usually done by sterilizing with sodium hypochlorite (NaOCl) or washing them with water to remove contamination (Bridgen *et al.,* 2018). Because surface sterilization using NaOCl and its toxic properties can be harmful to the plant, the concentration of NaOCl must be changed, and these harmful effects from NaOCl can cause side effects and infections in plant tissues (Fitri et al., 2020). Based on the type of plant and the excised parts, sterilization methods are chosen, whether they are superficial, mild or harsh. For example, in soft tissues, it is preferable to use a moderate sterilization method, and for hard excised plants, a harsh sterilization method is used (Bridgen et al., 2018).

After the surface sterilization process, the explanted plants that survived in the culture medium will be transferred to the multiplication stage, which is the second stage. At this stage, to help stimulate growth, plant growth regulators are used. Cytokinins such as 6-(γ,γ-dimethylallylamino) purine (2iP), kinetin, zeatin and 6-benzylaminopurine (BAP), and for bud proliferation are usually added to culture media (Bridgen et al., 2018). To induce shoots at the best rate, optimization can be performed to determine the optimal concentration of cytokinin. In the third stage, to induce root formation, explants that have succeeded in stimulating shoots are transferred, where auxin is used instead of cytokinin (Chandra et al., 2010). Auxins such as indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), and indole-3-butyric acid (IBA), are used in the new culture medium for rooting (Bridgen et al., 2018).

A high ratio of cytokinin to auxin or auxin to cytokinin promotes shoot and root regeneration respectively, and to enhance callus induction a moderate ratio of auxin and cytokinin can be used (Skoog & Miller, 1957).

Entire plants that successfully develop from bud and root formation can proceed to the fourth stage, which is the acclimatization stage. Regenerated plantlets must develop leaf cuticles that consist of hydrophobic epicuticular wax before being acclimatized to provide protection against water loss during acclimatization. Lower humidity of the growing environment should be introduced in a gradual manner to aid the plant to adapt to the less humid condition and to induce leaf cuticle formation (Chandra et al., 2010). The plants resulting from the tissue culture technique also has a less efficient photosynthetic system such as low chlorophyll content and slow responding stomata due to the condition of the tissue culture plants that received constant sugar supply from the culture media. To acclimatize the plants, zero sugar should be added, as this will trigger the plants to initiate the process of photosynthesis on their own. Besides, the plant should be placed in the shade before being gradually exposed to full sunlight for adaptation. During the duration in the tissue culture environment, cultured plants were exposed to low light intensity; since full sun exposure can affect the acclimatized plants in terms of light and water stress (Bridgen et al., 2018).

2. MATERIALS AND METHODS

Culture medium was prepared using 4.33 g of Murashige and Skoog (MS) Basal Medium powder (Brand: Sigma Aldrich), 3% sucrose, 0.55% gelrite (Brand: Duchefa) for 1 litre medium preparation. pH of the medium was fixed at 5.6-5.8. The prepared Murashige and Skoog (MS) medium was autoclaved at 121℃ for 15 minutes. The BAP hormone was added to the medium before autoclaving, while the TDZ hormone was added to the medium after autoclaving by filter sterilization at 0.2µm pore size under sterile conditions. The seeds of *C. melo* L. Glamour cv. The surface was sterilized with 5.25% NaOCl (commercial bleach) for 1 h and rinsed 3 times with sterile distilled water and according to the method from Grozeva et al. (2019). The seeds were dried on sterile filter paper before inoculation on the prepared culture medium. To prevent any external sources of contamination, surface sterilization was carried out under aseptic conditions.

eISSN Number: 2462-2389

The best cytokinin and the best explants that can provide the best regeneration of *C. melo* Glamor cv plants. Originated from Reticulum var. The investigation has been doneThe seeds were inoculated onto MS medium with different concentrations of BAP and TDZ for germination (0.5, 1.0 and 1.5 mg/L BAP and 0.5, 1.0 and 1.5 mg/L TDZ). Plants and cotyledons generated from seeds were grown on MS0 for 2 weeks before being transferred to different MS media (0.5, 1.0, and 1.5 mg/L BAP and 0.5, 1.0, and 1.5 mg/L TDZ), similar to the previous media but with constant addition of 0.5 mg/L IAA. Most of the methods performed were based on the protocol established by Grozeva et al. (2019) with several modifications. The efficacy of BAP and TDZ was analyzed in the first experiment to study the effect of different types of cytokinin on *C. melo* explants as both hormones may show differences in their activity (Bridgen et al., 2018) toward *C. melo* explants. The experiments were repeated using more explant species including cotyledons, hypocotyls, nodule plants, petioles and shoots using the cytokinin BAP which showed excellent plant regeneration. One-way analysis of variance (ANOVA) was used to test overall differences between the means of different groups, followed by post-hoc DMRT (Duncan's multiple range test) analysis to identify specific groups that differed significantly from each other.

3. RESULT AND DISCUSSION

Regeneration of *C. melo* plants in vitro from different types of explants was initiated by planting sterile seeds in culture medium. Plantlets regenerated from sown seeds were used as sources for transplanted plants in subsequent experiments. Plant samples from natural propagation (non-tissue culture) were initially used as sources of explants for cultures. However, the samples were contaminated with major contamination, thus, they were not proceeded to the next stage of the culture.

As the seeds were cultured in Murashige & Skoog (MS) medium, it was determined that the seedcoat should be removed to improve the percentage of germination. During the seed germination of the *C. melo*, it was observed that the seeds germinated in MS medium containing different concentrations of thidiazuron (TDZ) showed faster seedling growth compared to the seeds germinated in MS media containing benzyl aminopurine (BAP). A study by Fatima et al. (2015) reported that the TDZ helped to promote the morphogenic response of the treated plants. Similar responses were observed in this study after seed germination, as the seeds with TDZ treatment developed apparent morphology of cotyledons and hypocotyls compared to those with BAP treatment. Among the TDZ treatment, 0.5 mg/L (1D) showed the best morphological response, followed by 1.0 mg/L (1E) and 1.5 mg/L (1F) TDZ in the first experiment. For BAP, 1.0 mg/L (1B) demonstrated a good morphological response followed by 0.5 mg/L (1A) and 1.5 mg/L (1C) respectively (Table 1 and Figure 1). Explants were excised from regenerated seedlings of the seed cultures, and used as starting materials for the micropropagation in culture media. The first experiment was conducted using cotyledons and hypocotyls while the second experiment involved more explants including cotyledons, hypocotyls, nodal explants, petioles and shoots.

Table 1: Seedling growth in media containing different hormone treatment after five days.

Hormones	Seedling	Length of cotyledon (mm)	Length of hypocotyl (mm)
0.5 mg/L BAP	1A	10	8
1.0 mg/L BAP	1B	13	12
1.5 mg/L BAP	1C	12	10
0.5 mg/L TDZ	1D	23	19
1.0 mg/L TDZ	1E	14	13
1.5 mg/L TDZ	1F	21	12

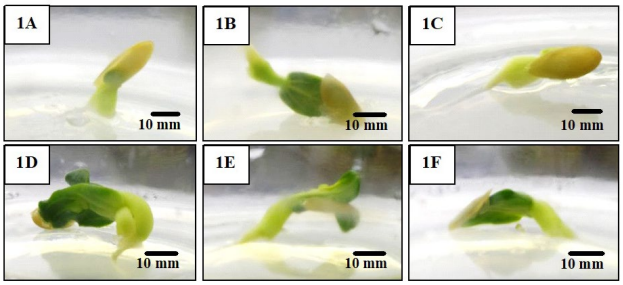

Figure 1: Seed culture after five days incubation

Adverse responses were detected after the cotyledons and hypocotyls were excised from the seedlings and cultured on MS0 containing zero hormone, where the cotyledons and hypocotyls (previously) from the MS media with BAP treatments showed better regeneration compared to the explants from MS media containing TDZ. It was also observed that different types of explants showed different callus formations. Similar observations were observed in a study where some cotyledons displayed white and friable callus while the hypocotyls regenerated watery callus (Grozeva et al., 2019). Most of the explants from MS media with BAP treatments regenerated healthy calli and some developed shoots and roots *via* direct regeneration. The regeneration of plants *via* callus stage was a good observation as it serves as the pre-condition for the production of the genetically stable plant materials (Nunez-Palenius et al., 2008). However, the presence of somaclonal variation might also arise from the cultures since the indirect culture through callus was prone to tissue culture induced variation (TCIV) (Debnath, 2018). The addition of BAP

eISSN Number: 2462-2389

during early age of seedling influenced the growth of the explants even after they were removed from the media. The explants from the MS media containing TDZ showed declining response compared to its first activity. The regeneration of both cotyledons and hypocotyls previously treated with TDZ slowed down compared to the cotyledons and hypocotyls treated with BAP. During this stage, the regeneration of explants displayed that the impact of the hormones introduced earlier into the medium persisted even after the cultures were sub-cultured on to MS0.

The next sub-culture was conducted after two-weeks explants culture in MS0. The cotyledons and hypocotyls from the MS0 were sub-cultured into their original media as in the first experiment containing either the BAP or TDZ but with constant addition of 0.5 mg/L indole-acetic acid (IAA). After three weeks, it was observed that the MS media containing BAP treatment was still displaying better explant regeneration compared to TDZ. Within three weeks, the cotyledon from 1.0 mg/L BAP + 0.5 mg/L IAA successfully regenerated complete plantlet compared to the other treatments. It was hypothesized that BAP + IAA treatment would regenerate more plantlets as the incubation time increased since some of the explants cultured in BAP + IAA already displayed the formation of shoots and roots. In term of explant types, cotyledon showed faster regeneration compared to the hypocotyl. The same result was reported in Grozeva et al. (2019).

For the TDZ + IAA treatment, the condition worsened after three weeks incubation. Some of the explants remained as callus or formed compact mass and did not undergo further morphological changes. Several explants also did not survive the condition. This observation was supported by a study where the continuous exposure of explants to the TDZ can result in the loss of regenerative capability (Fatima et al., 2015). In their study, the explants were able to survive and regenerate for shoot proliferation after a series of subculturing the explants on the hormone-free medium (MS0). Bairu et al. (2011) in their study stated that the physical and morphological changes in undifferentiated callus might be due to the presence of the somaclonal variation. Several authors have recorded positive response for explant regeneration supplemented with BAP + IAA. The hormonal combination displayed synergistic effect as the presence of either cytokinin or auxin alone is less likely to achieve maximum result for the bud formation (Grozeva et al., 2019). A study by Mendi et al. (2010) reported higher regeneration per explant as the culture media is added with both BAP and IAA, that represented cytokinin and auxin respectively compared to regeneration with BAP alone.

TDZ is classified as the diphenylurea-type cytokinin but its effect is quite distinctive from BAP. The result from this study showed that better height and shoot formation were observed in explants within MS media supplemented with BAP compared to TDZ (Figure 2).

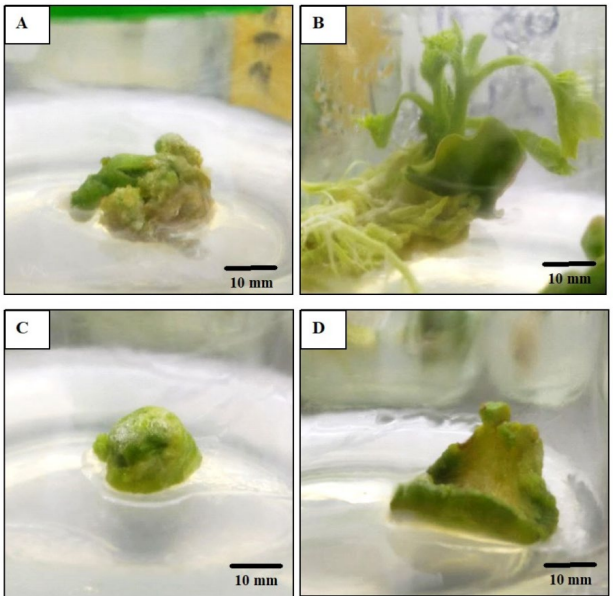

Figure 2: Explant culture after 3 weeks incubation. **A**: Hypocotyl in media containing BAP, **B**: Cotyledon in media containing BAP, **C**: Hypocotyl in media containing TDZ and **D**: Cotyledon in media containing TDZ

Table 2 shows that most of the explants treated with TDZ produced 0.00 ± 0.00 mm plant height and zero number of shoots. The data shows that most of TDZ treatments did not regenerate any plantlets. Compared to TDZ, explant treated with BAP produced notable plantlet heights and shoots. Similar result was obtained in a study by Fatima et al. (2015). In tissue culture technology, the high competency of BAP compared to the other cytokinin in shoot induction and proliferation by using various of explants was well established. Its nucleotides and riboside are natural occurring cytokinin, thus, it might serve as the contributing factor to its high superiority in explant cultures (Fatima et al., 2015).

Table 2: Mean height of cotyledon and hypocotyl (mm) and mean number of shoots formed in media containing different hormone treatment after 3 weeks.

Hormones	Explant	Mean of the plantlet height (mm ± SE)	Mean of the number of shoots formed
0.5 mg/L BAP	Cotyledon	10.00 ± 6.12	3.75 ± 2.18
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00
1.0 mg/L BAP	Cotyledon	11.25 ± 6.58	2.25 ± 1.44
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00
1.5 mg/L BAP	Cotyledon	6.25 ± 6.25	1.75 ± 1.75
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00
0.5 mg/L TDZ	Cotyledon	0.00 ± 0.00	0.00 ± 0.00
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00
1.0 mg/L TDZ	Cotyledon	2.50 ± 2.50	0.50 ± 0.50
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00
1.5 mg/L TDZ	Cotyledon	0.00 ± 0.00	0.00 ± 0.00
	Hypocotyl	0.00 ± 0.00	0.00 ± 0.00

eISSN Number: 2462-2389

This study tried to understand the activity of both BAP and TDZ as this variety of *C. melo* L. (reticulatus var.) always displayed differences in their organogenesis (Ficcadenti & Rotino., 1995). This study suggested that the BAP was far superior than the TDZ in term of organogenesis. The TDZ might be applicable, but repeated sub-culturing in MS0 should be done to aid the regeneration (Fatima *et al.,* 2015). The concentration of TDZ used in the experiment might be quite high and not suitable for the *C. melo* regeneration. Kehie and Kumaria (2012) in their study claimed that high concentration of TDZ can be toxic towards the explants cultured.

Further experiment was conducted with other explants using BAP (0.5 mg/L, 1.0 mg/L and 1.5 mg/L). The cotyledons, hypocotyls, nodal explants, petioles and young shoots were excised from the seedlings of seeds cultured in MS media supplemented with BAP at different concentrations. All explants were cultured in MS0 media for two weeks before subculturing in new media containing plant growth regulators or hormones. The explants were sub cultured in new media containing BAP with constant addition of 0.5 mg/L IAA, where the BAP concentrations used were similar to the original media of the explants during the seed culture. After four weeks incubation, the morphological changes of the explant cultured can be clearly observed. The cotyledons in all treatment (A1, A2 and A3) developed plantlets with significant heights and number of shoots. Similar observation was observed in nodal explants. The plantlets from cotyledons and nodal explants were regenerated *via* direct regeneration without any formation of callus. The direct shoot regeneration was the preferred method for mass production *via* tissue culture because the chances of somaclonal variation is low (Debnath, 2018). Contrarily, the hypocotyls, petioles and shoot tips mostly developed callus (Figure 3).

In term of the explant type, it was observed from the cultures that the cotyledons and nodal explants showed the best plantlets regeneration after four weeks incubation. One-way ANOVA ($p<0.05$) revealed that there were statistically significant differences in the height of regenerated plantlets in all media (A1, A2 and A3) between at least two (explants) groups respectively ((F (4, 15) = [24.991], (p=1.75E-06), (F (4, 15) = [9.173], (p=0.00059) and (F (4, 15) = [11.016], (p=0.000226)). This study rejected the null hypothesis stating that all group means were equal for all explants (within A1, A2 and A3).

The Post Hoc DMRT was conducted to determine where the significant differences between the explants occurred. In A1 media (Table 3), cotyledon (20.00 ± 3.24 mm^{a}) showed the most significant height of regenerated plantlets followed by nodal explant (14.25 ± 1.49 mm^{b}) as significant differences in the mean values with petiole, hypocotyl and shoot was observed. No significant mean difference was detected within those explants (hypocotyl, petiole and shoot). For A2 media (Table 4), both cotyledon (29.00 ± 3.81 mm^{a}) and nodal explant (21.25 ± 5.31 mm^{ab}) showed significant height of regenerated plantlets, while the A3 media's cotyledon (28.50 ± 3.62 mm^{a}) showed the most significant height of regenerated plantlets followed by nodal explant (12.50 ± 1.04 mm^{b}) (Table 5). Most petioles, hypocotyls and shoot tips in this experiment did not demonstrate excellent plantlets' heights compared to the cotyledons and nodal explants (Figure 4).

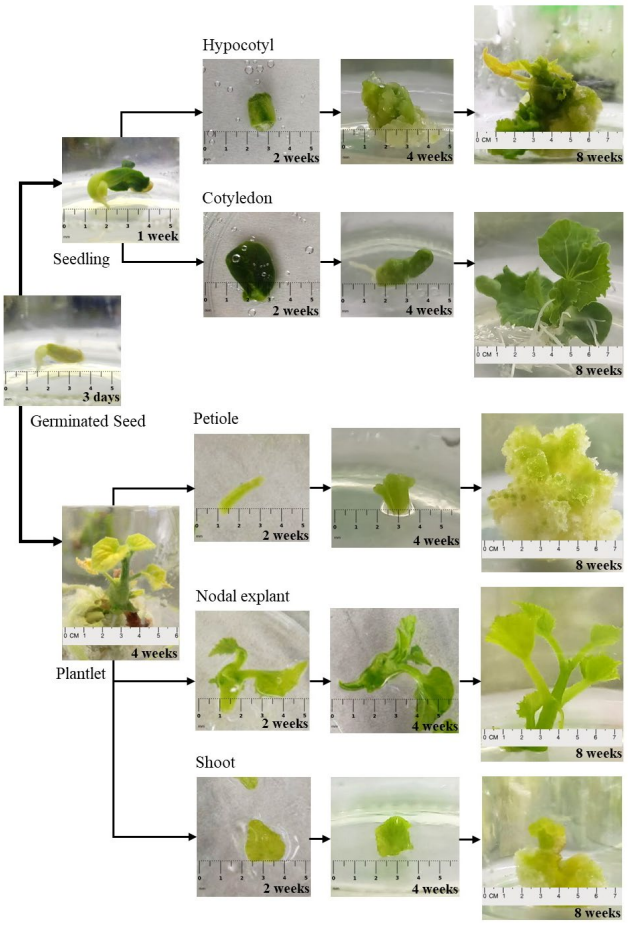

Figure 3: Various explants cultured in media containing BAP at several growth stages

Table 3: Mean height of plantlets from cotyledon, hypocotyl, nodal explant, petiole and shoot (mm) and mean number of shoots formed after 6 weeks in media A1

Explants	Media	Hormones	Mean height of plantlets (mm)	Mean number of shoots formed
Hypocotyl	A1	0.5 mg/L BAP + 0.5 mg/L IAA	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Cotyledon			20.00 ± 3.24^{a}	7.25 ± 1.65^{b}
Nodal explant			14.25 ± 1.49^{b}	11.50 ± 1.71^{a}
Petiole			3.25 ± 1.97^{c}	0.75 ± 0.48^{c}
Shoot tips			0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

eISSN Number: 2462-2389

Table 4: Mean height of plantlets from cotyledon, hypocotyl, nodal explant, petiole and shoot (mm) and mean number of shoots formed after 6 weeks in media A2

Explants	Media	Hormones	Mean height of plantlets (mm)	Mean number of shoots formed
Hypocotyl	A2	1.0 mg/L BAP + 0.5 mg/L IAA	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
Cotyledon			29.00 ± 3.81^{a}	6.25 ± 0.75^{ab}
Nodal explant			21.25 ± 5.31^{ab}	8.50 ± 2.53^{a}
Petiole			7.00 ± 4.53^{bc}	5.75 ± 2.58^{ab}
Shoot tips			9.00 ± 3.32^{cd}	4.25 ± 2.66^{ab}

Table 5: Mean height of plantlets from cotyledon, hypocotyl, nodal explant, petiole and shoot (mm) and mean number of shoots formed after 6 weeks in media A3

Explants	Media	Hormones	Mean height of plantlets (mm)	Mean number of shoots formed
Hypocotyl	A3	1.5 mg/L BAP + 0.5 mg/L IAA	7.00 ± 7.00^{bc}	2.75 ± 2.75^{b}
Cotyledon			28.50 ± 3.62^{a}	12.25 ± 1.25^{a}
Nodal explant			12.50 ± 1.04^{b}	9.25 ± 1.32^{a}
Petiole			0.00 ± 0.00^{bc}	0.25 ± 0.25^{b}
Shoot tips			0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

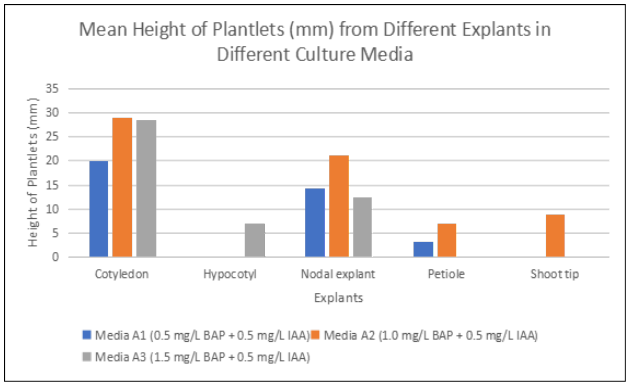

Figure 4: Mean height of plantlets (mm) from different explants in different culture media

In term of shoot formation, the one-way ANOVA ($p<0.05$) conducted to analyse the effect of different types of explants used on the number of shoots formed revealed that there were statistically significant differences in the number of shoots formed within explants in media A1 and A3 between at least two (explants) groups respectively ((F (4, 15) = [23.261], (p=2.76E-06) and (F (4, 15) = [14.107], (p=5.72E-05)). This study rejected the null hypothesis stating that all group means were equal for explants in A1 and A3 media.

The Post Hoc DMRT was conducted to determine where the significant differences between the explants occurred. It was found in A1 media (Table 3) that nodal explant (11.50 ± 1.71^{a}) showed the most significant shoot formation followed by cotyledon (7.25 ± 1.65^{b}), petioles (0.75 ± 0.48^{c}), hypocotyl (0.00 ± 0.00^{c}) and shoot tip (0.00 ± 0.00^{c}). Similar to the observation for plantlets' height, no significant mean different was detected within those three explants (hypocotyl, petiole and shoot). For the A2 media (F (4, 15) = [2.442] (p=0.092)), it did not show significant differences in the number of shoots formed within the explant in A2 media, thus, this study accepted the null hypothesis stating that all group means were equal for A2 media. DMRT was conducted to show the insignificant mean different between the explants (Table 4). Within A3 media (Table 5), both cotyledon (12.25 ± 1.25^{a}) and nodal explant (9.25 ± 1.32^{a}) showed the most significant shoot formation followed hypocotyl (2.75 ± 2.75^{b}), petiole (0.25 ± 0.25^{b}) and shoot tip (0.00 ± 0.00^{c}). Most petioles, hypocotyls and shoot tips in this experiment demonstrated lower shoot formation compared to the cotyledons and nodal explants (Figure 5).

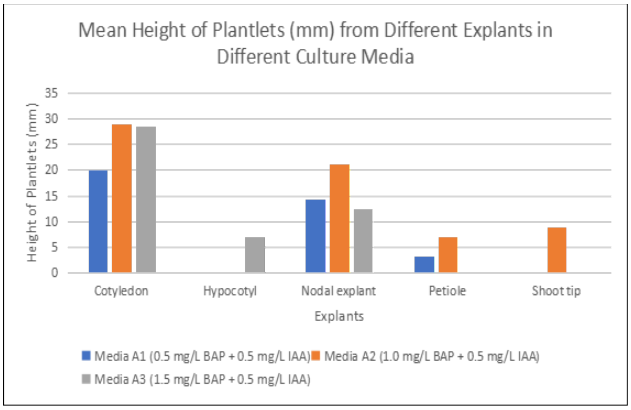

Figure 5: Mean height of plantlets (mm) from different explants in different culture media

Nevertheless, the cotyledons from A2 media successfully developed plantlets with both roots and shoots, thus, the plantlets were prepared for acclimatization. The other plantlets regenerated without roots were further cultured in rooting media containing 1.0 mg/L IBA (Devendra *et al.,* 2009), to induce the root formation before being acclimatized (Kehie & Kumaria, 2012). IBA was used replacing the IAA because it promoted thick and long root compared to IAA as proved by many studies on Cucurbitaceae species (Devendra *et al.,* 2009). Similar to Devendra et al. (2009), the roots emerged within approximately two weeks and several plantlets achieved good rooting system after four weeks of culture. The plantlets with developed shoots and roots were prepared for acclimatization. The plantlets were

eISSN Number: 2462-2389

adapted in non-tissue culture environment whether in wet tissue or sterilized cocopeat (Patra & Beura, 2016) after they were removed from culture media and treated with fungicide. The plantlets were kept in adaptation environment for one week in tissue culture temperature and gradually at increased temperature from time to time (two weeks). The plantlet was placed in closed jar and the lid was removed for 1-2 hour every day before being acclimatized at the actual planting area (Figure 6).

Figure 6: Acclimatized plantlets after a series of adaptation

Based on the study conducted, hypocotyls, shoots and petioles mostly undergo indirect regeneration *via* callus while very small number regenerated plantlets *via* direct regeneration in the presence of BAP + IAA treatments. Regeneration of the plantlets *via* callus was reliable for genetically stable plant material (Nunez-Palenius *et al.,* 2008), however, it made it more susceptible to tissue culture-induced variation (TCIV) such as somaclonal variation (Debnath, 2018).

4. CONCLUSION

This study concluded that the clones of *C. melo* L. Glamour cv. were successfully regenerated from the explants culture of this plant. This study successfully determined the most suitable cytokinin for optimal growth of the *C. melo* in various stages. In this study, the cytokinin BAP had shown the best overall effects towards the regeneration of the *C. melo* L. plant in the culture medium compared to TDZ. The positive effect of TDZ on the explants can only be observed during seed culture as it provided significant morphological responses during seedling growth. Further exposure of explants to TDZ in culture media especially after the explants were excised and sub-cultured had resulted in the loss of regenerative capability of the explants. This study also found that the cotyledon and nodal explants were among the explants that showed the best plantlet regeneration. This study observed that the *C. melo* explants displayed distinct regeneration rate in term of plantlet height and number of shoots formed depending on the concentration of cytokinin (BAP) added to the media.

REFERENCES

Anderson W. C. (1980). Mass propagation by tissue culture. *In: Principles and techniques. Agricultural research results, United States Department of Agriculture*, pp 1–9.

Bairu, M. W., Aremu, A. O., & van Staden, J. (2011). Somaclonal variation in plants: Causes and detection methods. *Plant Growth Regulation*, *63*(2), 147–173.

Bhatia, S. (2015). Chapter 1- History and Scope of Plant Biotechnology. *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences,* 1–30. Academic Press.

Bridgen, M. P., Van Houtven, W., & Eeckhaut, T. (2018). Plant Tissue Culture Techniques for Breeding. *Springer International Publishing AG, part of Springer Nature 2018*, 127–144.

Chandra, S., Bandopadhyay, R., Kumar, V., & Chandra, R. (2010). Acclimatization of tissue cultured plantlets: From laboratory to land. *Biotechnology Letters*, *32*(9), 1199–1205.

Debnath, S. C., & Ghosh, A. (2022). Phenotypic variation and epigenetic insight into tissue culture berry crops. *Frontiers in Plant Science*, *13*(December), 1–14.

Debnath S. C. (2018). Thidiazuron in micropropagation of small fruits. Thidiazuron: from urea derivative to plant growth regulator. *Springer Nature Singapore Pte Ltd, Singapore*, pp 139–158.

Devendra, N. K., Subhash, B., & Seetharam, Y. N. (2009). Callus growth and plant regeneration in *Momordica dioica* (Roxb.) Willd. Cucurbitaceae. *Journal of Sustainable Agriculture*, *3*(4), 743–748.

Fatima, N., Ahmad, N., Ahmad, I., & Anis, M. (2015). Interactive Effects of Growth Regulators, Carbon Sources, pH on Plant Regeneration and Assessment of Genetic Fidelity Using Single Primer Amplification Reaction (SPARS) Techniques in *Withania somnifera* L. *Applied Biochemistry and Biotechnology*, *177*(1), 118–136.

Ficcadenti, N., Veronese, P., Sestili, S., Crino, P., Lucretti, S., Schiavi, M., & Saccardo, F. (1995). Influence of genotype on the induction of haploidy in *Cucumis melo* L. by using irradiated pollen. *J Genet Breed*, *49*, 359–364.

Fitri, M., Nazar, K., Meidyawati, R., & Azmi, R. (2020). Antibacterial effect of xanthorrhizol (*Curcuma xanthorrhiza* roxb.) against the biofilm of fusobacterium nucleatum. *International Journal of Applied Pharmaceutics*, *12*(Special Issue 2), 57–61.

Grozeva, S. Y., Velkov, N. V., & Ivanova, Z. V. (2019). *In vitro* Plant Regeneration of Two *Cucumis melo* L. Genotypes Using Different Explant Types and Culture Medium. *Ecologia Balkanica*, *11*(2), 193–202.

Kehie, M., & Kumaria, S. (2012). *In vitro* plantlet regeneration from nodal segments and shoot tips of Capsicum chinense Jacq. cv. Naga King Chili. 31–35.

Košmrlj, K., Kladnik, A., & Bohanec, B. (2015). Adventitious regeneration in styrian oil pumpkin in relation to the endoreduplication pattern and induced tetraploidy on fusaric acid-supplemented media. *Plant Growth Regulation*, *75*(3), 587–594.

Mendi, Y. Y., Eldoğan, S., Gutakev, R., Ipek, M., Çürük, P., & Çetiner, S. (2010). Regeneration and histological analysis of snake melon (*Cucumis melo* var. flexuosus L. Naudin) by direct organogenesis. *Turkish Journal of Agriculture and Forestry*, *34*(4), 309–317.

Miguel, J. F. (2023). Influence of sodium salicylate on adventitious organogenesis of a commercial cucumber cultivar. *Multidisciplinary Science Journal*, *5*(1).

Murashige T (1974). Plant propagation through tissue cultures. *Annu Rev Plant Physiol* 25:135–166

Naitchede, L. H., Runo, S., & Nyende, A. B. (2022). *In vitro* Rapid Regeneration of Bitter Melon (*Momordica charantia* L.) Using Direct Organogenesis in Kenya. *Universal Journal of Agricultural Research*, *10*(5), 514–525.

Nguyen, M. L., Ta, T. H. T., Huyen, T. N. B. T., & Voronina, A. V. (2019). Anther-derived callus formation in bitter melon (*Momordica charantia* L.) as influenced by microspore development stage and medium composition. *Sel'skokhozyaistvennaya Biologiya*, *54*(1), 140–148.

eISSN Number: 2462-2389

Nuñez-Palenius, H. G., Gomez-Lim, M., Ochoa-Alejo, N., Grumet, R., Lester, G., & Cantliffe, D. J. (2008). Melon fruits: Genetic diversity, physiology, and biotechnology features. *Critical Reviews in Biotechnology*, *28*(1), 13–55.

Patra, S. K., & Beura, S. (2016). Media standardization for pre-hardening and hardening of in vitro regenerated plantlets of gerbera cultivars. *Journal of Applied Horticulture*, *18*(1), 76–79.

Skoog, F., & Miller C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured. *Vitro Symp Soc Exp Biol* 11:118–131

eISSN Number: 2462-2389