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# SHOOT RECLAMATION OF CHAYOTE FROM NODES THROUGH MICROPROPAGATION

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## ABSTRACT

*Sechium edule* commonly called Chayote, is known for its fruits young leaves, shoots, stems and tuberous roots around the world. Chayote has great economic significance. In order to avoid the heterogeneity due to genetic diversity (as the seeds are recalcitrant and thus cannot be stored), micropropagation becomes the basic tool to produce innumerable identical clones, thus retrieving the homogeneity. Hence, chayote has been chosen to provide high yield of micropropagation productivity. In this study, nodes and seeds were taken. The rate of mortality and the rate of shoot generation were monitored. The observations of shoot regeneration by nodal explant and shoot tip explant were recorded. Several combinations with the auxins and cytokines were trialed. The best bud break was obtained at 74% sodium hypochlorite with less rate of contamination when compared to other trials. On the other hand, the shoot length and number of shoots were observed to be the highest using IAA and BAP combination which recorded a length of 1.67 cm and number of shoots were around 5 compared to the other combinations. This study on success rate of shoot regeneration through micropropagation using nodal explants helps in the establishment of direct organogenesis and to eliminate the somaclonal variation and to overcome the heterogeneity in the fruits.

**KEYWORDS:** *Sechium edule*, micropropagation, auxins, cytokines, nodal explants, shoot regeneration.

## 1. INTRODUCTION

Chayote or *Sechium edule*, which belongs to the *cucurbitacea* family is an herbaceous perennial, monoecious climber. It is an important part of traditional diets across Meso America. Its young leaves, shoots, stems and tuberous roots are edible and its fruits are consumed in many countries.[1] Its name varies according to regions like chow-chow, isqush, piskut, sikut.[2]

Micropropagation is an essential biotechnological tool which is highly preferred in recent years. It has become a commercially viable method mainly used for *invitro* clonal propagation. It is widely used for herbaceous and woody plants. As *Sechium edule* belongs to *cucurbitacea*, it also develops tendrils for support.[3] Chayote fruit bears single large seed and mature in 28-35 days.

Micropropagation helps us in conserving the plants and also to produce numerous clones. This technique requires shorter period of time as compared with normal condition. Moreover it is very effective for recalcitrant seeds. There are reports on micropropagation done with the nodal plants, shoot tip explants and embryo.[4] Tissue culture is maintained in sterile condition. Thus, it helps preventing contamination, which is one of its major advantages. Higher yields, mass multiplication, innovation of new varieties, elimination of diseases are the other advantages of micropropagation. Therefore, due to these reasons, the micro propagation of chayote is developed in "*invitro*" plant tissue culture technology. Chayote usually grows well in warm climate and long summer days. Soil with ample moisture and rich in organic matter is more preferred for growing these plants. The pH range of soil can be of 6.0 to 6.8.[5]

Chayote is rich in fiber, potassium, calcium, iron and vitamin C. Its fruits and seeds, especially are highly rich in vitamins and amino acids. It also contains the main hydro soluble antioxidant i.e., ascorbic acid or vitamin C. Therefore, the reactive species in plasma and tissue can be detoxified.[6] Its fruits and vegetables are fresh and therefore it has high moisture content (89-95%) and especially its young stem and tuberous root has high calorific content.[7] Meso America exist greatest genetic diversity and so its nutritional values are well known among the local customers. Due to these reasons, it is a very important crop.[8] This is one of the vegetables people prefer as it is most affordable especially for low income families.[9] It is found that the chayote plant can be stored for a very long periods, when it is buried in a dry area. It is also found that seeds do not be dried.[10]

The chayote has many medicinal uses and thus several studies were conducted on its biological activities. It is proved that *Sechium edule* in the aqueous extract of its fruit exhibits hypotensive effect and anti- ulcer property from its extract. Another study reported that it was tested against strains of multi resistant staphylococci and enterococci. It showed antimicrobial efficacy with alcoholic extract and tincture.[11]

*Sechium edule* has been reported to have antibacterial, antioxidants, antihypertensive and antiepileptic activities.[1] It is used in Mexico as a renal disease therapeutic and to control high blood pressure.[2]

**Figure 1: Chayote plant and chayote fruit and seed.**

The nodes and the seeds of the chayote are collected and rinsed several times in sterile water. The explant taken from the chayote plant is subjected to surface sterilization. Surface sterilization is done because of common contamination is caused by dust particles, bacteria, fungi and insects.

## 2. MATERIALS AND METHODOLOGY

The chayote vegetables were collected from the market in Bengaluru, India. These were allowed to mature for a few days. They were sprouted within a week, then the sprouted chayote vegetables were planted (15 – 20 days) at Genewin Biotech, Hosur, Tamil Nadu, India.

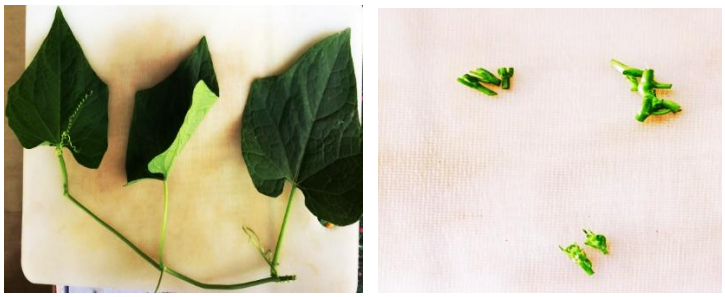

**Figure 2: Chayote leaves and chayote nodes.**

The plants grew well. The shoots were having 5-6 nodes (Figure 2). They were taken from the matured sprouted vegetables. The seeds were also collected from the plant.

The collected seeds and nodes were disinfected by rinsing them in 70% solution of ethyl alcohol for 30 seconds and with 50% of sodium hypochlorite solution for 12 minutes. Then finally, they are rinsed in sterile water thrice. This was maintained at a temperature of -27 ±2̊ C for 16 hours in the growth chamber.

The explants were then treated with the antifungal agent and streptomycin. They are also treated with Tween 20 reagent. Then surface sterilization was done with 70% of ethanol for 1 minute and 2% of sodium hypochlorite for 20 minutes and rinsed with sterilized triple distilled water for five times and is followed with 0.1% of mercuric chloride for five minutes and again rinsed in sterile distilled water.

The explants were then treated with the 0.1% mercuric chloride at time interval of 5, 10, 15, 20 and 25 min. Then, the treated explants were again sterilized with 4% Sodium Hypochlorite at a time interval of 5, 10, 15 and 20 min.

By comparing success rate with 0.1% mercuric chloride and 4% sodium hypochlorite with the same time interval, effectiveness of the explant of the chayote plant has been tabulated. After surface sterilization, the initiation process is carried out.

### 2.1. Initiation

The induced sign of nodes in explants was taken and it was chopped from the shoot segment. The Murashige and Skoog media is the commonly preferred media for the plant tissue culture which comprise of macronutrients, micronutrients, and also some organic elements.

The explants were inoculated in the MS (Murashige and Skoog, 1962) medium. The medium was supplemented with the 3% sucrose, BAP (concentration range – 0.5 1, 2, µm) IAA and with the combination of Indole-3-Acetic Acid and 6-Benzyl aminopurine (IAA+BAP). The pH of the MS medium was adjusted to 5.8 followed by autoclaving. Shoot regeneration was observed regularly and the values were recorded.

## 3. RESULTS

### 3.1. Effect of mercuric chloride

By using 0.1% of mercuric chloride as the surface sterilant, it was observed that the lower rate of shoot regeneration was 73% mercuric chloride at 15 min. it was tabulated in Table 1.

### 3.2. Effect of sodium hypochlorite

By using sodium hypochlorite as the surface sterilant, it was observed the lower shoot regeneration and bud break is obtained by using 74% using sodium hypochlorite for 20 min. It was tabulated in Table 2.

### 3.3. Comparing the effect of mercuric chloride and sodium hypochlorite

By comparing Table 1 and Table 2, the best bud break obtained was using sodium hypochlorite. Considering the lower value of shoot regeneration, sodium hypochlorite (74%) gives the higher value than the mercuric chloride (73%).

Therefore as a result of surface sterilization of the aimed node in the MS media prepared, the shoot regeneration and bud break is obtained using Sodium Hypochlorite.

### 3.4. Effects of growth hormones [Combination of auxins and cytokines]

It was observed that the effects of combination of auxins (IAA) and cytokines (BAP) in MS media on the shoot proliferation were evaluated. The yield of both combination of IAA and BAP concentration gives the maximum length and number of shoots or nodes to be 1.67±0.28 and 4.89±0.73. Thus, as a result, the best regenerated explant of *Sechium edule* is obtained by the combination of both auxins and cytokines shown (Figure 3).

**Figure 3: Regenerated explants using auxins and cytokines combination.**

### 3.5. Surface Sterilization

#### 3.5.1. Using Mercuric chloride

**Table 1: Effect of mercuric chloride.**

| Mercuric chloride treatment (minute) | Total number of explants taken | Rate of mortality (%) | Rate of shoot regeneration (%) | mean (%) |
|---|---|---|---|---|
| 5 | 25 | 89 | 80-85 | 82 |
| 10 | | 87 | 89-95 | 92 |
| 15 | | 82 | 70-75 | 73 |
| 20 | | 87 | 75-78 | 77 |
| 25 | | 88 | 80-82 | 82 |

#### 3.5.2. Using Sodium hypochlorite

**Table 2: Effect of sodium hypochlorite.**

| Sodium hypochlorite treatment (minute) | Total number of explants taken | Rate of mortality (%) | Rate of shoot regeneration (%) | Mean (%) |
|---|---|---|---|---|
| 5 | 25 | 96.5 | 90-92 | 86.5 |
| 10 | | 90 | 90-94 | 88 |
| 15 | | 80.8 | 85-90 | 86 |
| **20** | | **68.4** | **70-75** | **74** |

**% Mortality = Explants contamination / Total number of Explants** x **100**

**Figure 4:a. Mortality explants with seed.**

**Figure 4.b. Mortality explants with nodes.**

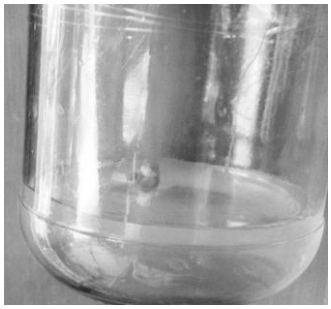

**Figure 5: Best bud break – sodium hypochlorite at 20 min.**

### 3.5.3. Initiation Outcome

**Table 3: Comparing the effect of mercuric chloride and sodium hypochlorite.**

| IAA (µm) | BAP (µm) | IAA+BAP (µm) | Length of shoots/nodes (cm) (mean ±shoot explant) | Number of shoots/nodes (mean ±shoot explant) |
|---|---|---|---|---|
| 0.5 | 0 | 0 | 1.03±0.22 [a] | 3.83±0.78 [c] |
| 1.0 | 0 | 0 | 0.67±0.12 [b a] | 1.29±0.90 [a] |
| 2.0 | 0 | 0 | 1.38±0.14 [a c] | 4.35±0.78 [d] |
| 0 | 0.5 | 0 | 0.61±0.09 [a] | 1.66 ± 0.37 [a] |
| 0 | 1.0 | 0 | 0.62 ± 0.09 [a] | 2.33 ± 0.41 [a] |
| 0 | 2.0 | 0 | 0.63 ± 0.09 [a] | 2.36 ± 0.40 [a] |
| 0 | 0 | 0.1+2.0 | 0.39 ± 0.14 [c b] | 1.28 ±0.37 [b] |
| 0 | 0 | 0.2+2.0 | **1.67±0.28 [a]** | **4.89±0.73 [b]** |
| 0 | 0 | 0.3+2.0 | 1.21 ± 0.27 [a] | 2.11 ± 0.11 [a c] |

## 4. DISCUSSION

The present study was carried out to obtain shoot reclamation from nodes through micropropagation in *Sechium edule*. Results of the best bud break could be obtained by using surface sterilants like sodium hypochlorite. The rate of shoot regeneration obtained using sodium hypochlorite (74%) which is greater than the rate obtained by mercuric chloride (73%). Thus, by comparing both sterilants, sodium hypochlorite is found to be more effective.

Further studies were carried out by subjecting the *Sechium edule* shoots and nodes regenerated by explants using growth hormones. Results indicated that neither IAA nor BAP alone proved beneficial for regenerated explants of shoot and nodes. Maximum length and number of nodes or shoots (1.67±0.28) and (4.89 ± 0.73) were obtained by a combination of both IAA and BAP. The cytokines BAP were found to be superior to IAA. BAP has the property of slow degradation and its activity will not change when autoclaved.[12,13]

## 5. CONCLUSION

From the above results on shoot reclamation of *Sechium edule* from the nodes through micropropagation, this study suggest the result on maximum shoot regeneration bud break using sodium hypochlorite and growth hormones with the combination of IAA and BAP. Therefore, nodal explant help in the establishment of direct organogenesis to estimate the somoclonal variation and to overcome heterogeneity in the fruits.

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## 7. REFERENCES

1. Kaushik K. Antibacterial activity of *Sechium edule* (Jacq.) Swartz against gram negative food borne bacteria, Adv. Appl. Sci. Res., 2013; 4(2): 259-261.
2. Mishra LK, Das P. Nutritional Evaluation of Squash (*Sechium Edule*) Germplasms Collected from Garo Hills of Meghalaya, Int. J. Agric. Env. Biotech., 2015; 8(4): 971-975.
3. Arévalo-galarza JCL, Avendaño-arrazate CH, Soto-hernández M, et al. Production, Genetics, Postharvest Management and Pharmacological Characteristics of Sechium edule (Jacq.) Sw. Global Science Book, 2007; 1(1): 41-53.
4. Thilagam D, Kumudini BS, Manohar SH. *invitro* nodal explants and assessment of clonal fidelity, Int. J. Agric. Sci. Res., 2016; 6(5): 285-292.
5. Kaushik K, Mishra LK, Das P, et al. *Sechium edule*. South African J Bot., 2017; 6(5): 971-975.
6. Graciela de J. Albarracin, Romina V. Lucero Lupez, Mirta Lucas de Arellano, Eduardo Marchesvky, Nora L. Escudero, NPAIJ, 2010; 6(2): 94-101.
7. Vela-g G. Chayote (*Sechium edule*) Phytochemical and pharmacological approaches, 2017; (October) 979-991.
8. Sw J, Rodríguez-larramendi LA, Guevara-hernández F, et al. Traditional knowledge on integrated pest and weed management in chayote (*Sechium edule* (Jacq.) Sw.) crops from localities of chiapas, Mexico, Acta Agronomica, 2017; 66(4): 466-472.
9. Acevedo-hernandez G. Genetic fidelity assessment in plants of *Sechium edule* regenerated via organogenesis. South African J Bot., 2017; 112(September): 118-122.
10. Ana Abdelnour-Esquivel, Florent Engelmann. Cryopreservation of chayote (*sechium edule* jacq. sw.) zygotic embryos and shoot-tips from *invitro* plantlets., CryoLetters, 2002; 23: 281-282.
11. Ragasa CY, Biona K, Shen C. Chemical constituents of *Sechium edule* (Jacq.) Swartz., Der Pharma Chemica, 2014; 6(5): 251-255.

12. Meena MC, Meena R, Culture T. *invitro* multiplication of *helicteres isora* through nodal stem segment explant from mature plant. *Wjpps*, 2018; 7(8): 707-715.
13. Daga M. studies on tissue and anther culture of tree species. Ph.D. Thesis, J.N.V. University, Jodhpur, 1994.