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# Callus induction, direct and indirect organogenesis of ginger (*Zingiber officinale* Rosc)

**Ammar Mohammed Ahmed Ali[1]*, Mawahib ElAmin Mohamed El-Nour[2] and Sakina Mohamed Yagi[3]**

[1]Department of Biology, Faculty of Education, Hajjah University, Yemen.
[2]Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, Sudan.
[3]Botany Department, Faculty of Science, University of Khartoum, Sudan.

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**The present study aimed to induce callus, direct and indirect organogenesis of ginger (*Zingiber officinale* Rosc) by using Murashige and Skoog (MS) medium fortified with different concentrations and combinations of growth regulators. Shoot tip, *in vitro* leaf and root segments were used as explants to induce callus by MS medium containing (0.00 as control, 0.5, 1.00, 2.00 and 3.00 mg/L) of 2,4-dichloro-phenoxyacetic acid (2,4-D). Callus induced was subcultured on MS+2,4-D at different concentrations (0.5, 1.00, 2.00 and 3.00 mg/L) and one concentration 0.5 mg/L of 6-benzyl amino purine (BAP) was used. The sprouting buds (about 1 to 1.5 cm) were used as explants for direct shoots and roots induction by MS medium + 2.00, 3.00 and 4.5 mg/L of BAP. Callus induced by 1.00 mg/L 2,4-D was regenerated on MS + 0.5 mg/L 2,4-D to obtain a green callus, this callus was transferred to MS medium with combinations of 0.5 mg/L 1-naphthaleneacetic acid (NAA) with different concentrations of BAP (1.00, 2.00,3.00 and 4.00 mg/L) for indirect organogenesis. The results reveals that, for callus induction, callus was only induced from shoot tip explant in all concentrations of 2,4-D. The highest callus fresh weight was obtained by 1.00 mg/L of 2,4-D (1.302 ± 0.09) g than that induced by other treatment ($p < 0.05$). In the case of callus induced by subculture, the highest callus fresh weight initiated was 1.509 ± 0.00 g by 0.5 mg/L 2,4-D. For direct organogenesis, 4.5 mg/L BAP showed the highest number of *in vitro* shoots and roots, 4 ± 0.35 shoots and 15 ± 0.46 roots per explants. For indirect organogenesis, the best shoots and roots initiated were 2 ± 0.21 shoots and 22 ± 0.33 roots by combination of 1.00 mg/L BAP+0.5 mg/L NAA.**

**Key words:** Callus induction, growth regulators, *Zingiber officinale* Rosc, organogenesis.

## INTRODUCTION

Plant tissue culture is the *in vitro* aseptic culture of any part of plant or whole plant under controlled nutritional and environmental conditions. This technique depends mainly on the concept of totipotentiality of plant cells,

*Corresponding author. E-mail: ammarsood21@gmail.com.

**Table 1.** MS medium supplemented with different concentrations and combinations of plant growth regulators.

| Type of culture | Treatment | Phytohormone | Concentration mg/L |
|---|---|---|---|
| Callus induction | 1 | 2,4-D | 0, 0.5, 1, 2, 3 |
| Callus subculture | 2 | 2,4-D | 0, 0.5, 1, 2, 3 |
| | 3 | BAP | 0, 0.5, 0.5, 0.5, 0.5 |
| | | 2,4-D | 0, 0.5, 1, 2, 3 |
| Direct organogenesis | 4 | BAP | 0, 2, 3, 4.5 |
| Indirect organogenesis | 5 | NAA | 0, 0.5, 0.5, 0.5 |
| | | BAP | 0, 2, 3, 4 |

which refers to the ability of a single cell to express the full genome by cell division (Hussain et al., 2012). *In vitro* culture techniques provide an alternative means of plant propagation and a tool for crop improvement (Guo and Zhang, 2005). In many tissue culture, experiments prior to successful agri-biotechnological research on crops, reliable callus induction and efficient *in vitro* regeneration system is urgently required (Thingbaijam and Huidrom, 2014). Through tissue culture, diseases-free clones could be obtained with a rapid multiplication rate (Guo and Zhang, 2005).

Ginger (*Zingiber officinale* Rosc) is a slender, perennial rhizomatous herb (Kathi, 1999). The rhizome of *Z. officinale* Rosc has long served culinary and medicinal uses (Afzal et al., 2001). *Z. officinale* Rosc normally propagates by underground rhizomes, with a low proliferation rate, easily infected by soil-borne pathogens such as bacterial wilt (*Pseudomonas solanacearum*), soft rot (*Pythium aphanidermatum*) and nematodes (*Meloidogyne* spp.); these pathogenic factors are readily transmitted through traditional cultivation practices, which cause heavy losses in yield (Guo and Zhang, 2005). Therefore, it was deemed important to overcome these problems through cultivated this crop under favourable conditions. Plant tissue culture technique can be used as a solution for these problems and a proper alternative to produce disease-free clones of ginger plant by direct and indirect organogenesis.

The present study aimed to investigate effect of growth regulators on callus induction, direct and indirect organogenesis of *Z. officinale* Rosc by different explants.

## MATERIALS AND METHODS

This investigation was conducted in Plant Tissue Culture Laboratory at Department of Biology and Biotechnology, Faculty of Science and Technology, AL-Neelain University, Khartoum, Sudan.

### Plant

The healthy fresh rhizome of *Z. officinale* Rosc were acquired from the local market of Khartoum city.

### Preparation of explants

Healthy and clean *Z. officinale* rhizomes were incubated in the dark at 25±2°C for three weeks for sprouting buds, buds about 1 to 1.5 cm were excised and used as explants for direct shoots and roots induction, while shoot tip about 0.5 cm was used as explant for callus induction. All explants were washed with soap and water several times, then dipped in 70% alcohol for 60 second. This was followed by soaking in 20% clorox (0.5% free chlorine) with two to three drops of Tween-20 for 15 min, and rinsed three to five times in sterile distilled water. Finally, the explants were treated with 0.1% ($HgCl_2$ W/V) for 10 min and washed thoroughly 5 to 6 times with sterile distilled water.

### Culture medium

The sterilized explants were planted in (MS) medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of plant growth regulators as follows in Table 1. All cultures were incubated at 25±2°C, photoperiod of 16/8 h light/dark for two months for callus induction and three months for direct and indirect shoots and roots formation. All cultures were subcultured at four weeks intervals.

### Data analysis

The data were expressed as mean ± Standard deviation of three replicates. Statistical analysis was performed with SPSS software (Griffth, 2007). Means with significant differences were performed using Duncan least significant difference (LSD) at $p<0.05$ probability level of significance.

## RESULTS AND DISCUSSION

### Callus induction

The shoot tip, *in vitro* leaf and root segments were implanted on MS medium with 0.00 as control, 0.5, 1.00, 2.00 and 3.00 mg/L of auxin 2,4-D for callus induction. Callus was initiated from shoot tip explants, this callus was initiated by meristematic dome after the fourth week

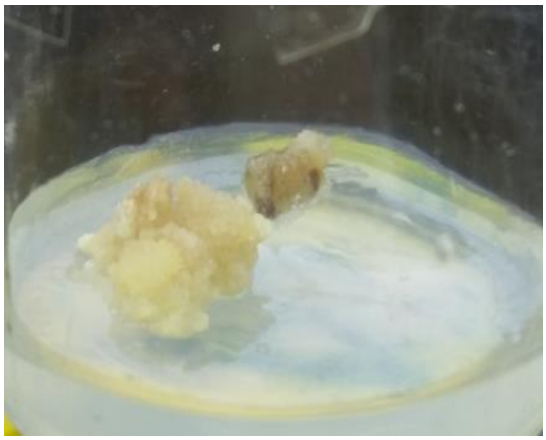

**Figure 1.** Callus induced by shoot tip explant after eight weeks.

**Table 2.** Effect plant growth regulators on callus induced from shoot tip explants of *Z. officinale* Rosc after 8 weeks using MS medium.

| Type of culture | PGR. in basal MS medium mg/L | | Parameter | | |
|---|---|---|---|---|---|
| | 2,4-D | BAP | Fresh weight of callus (g) | Color of callus | Texture of callus |
| Callus induction | 0.00 | 0.00 | - | - | - |
| | 0.5 | 0.00 | 0.601±0.04 | Creamy | Compact |
| | 1.0 | 0.00 | 1.302±0.09 | Creamy | Compact |
| | 2.0 | 0.00 | 0.370±0.05 | Brown | Friable |
| | 3.0 | 0.00 | 0.319±0.01 | Brown | Friable |
| Callus subculture | 0.00 | 0.00 | - | - | - |
| | 0.5 | 0.00 | 1.509±0.00 | Creamy | Friable |
| | 1.0 | 0.00 | 1.05±0.07 | Creamy | Friable |
| | 2.0 | 0.00 | 0.70±0.28 | Creamy | Friable |
| | 3.0 | 0.00 | 0.59±0.09 | Creamy | Friable |
| | 0.00 | 0.00 | - | - | - |
| | 0.5 | 0.5 | 0.594±0.60 | Creamy | Friable |
| | 1.0 | 0.5 | 0.5±0.65 | Creamy | Friable |
| | 2.0 | 0.5 | 0.47±0.65 | Creamy | Friable |
| | 3.0 | 0.5 | 0.48±0.67 | Creamy | Friable |

*PGR= Plant growth regulator, mean ± standard deviation.

of culture; no callus was observed when the leaf and root segments were used as explants (Figure 1). These results agree with El-Nabarawy et al. (2015), they used different type of explants (shoot tip, leaves and rhizome segments) to induce callus from *Z. officinale* Rosc. They found that callus was only observed from shoot tip explants after 30 day of culture. Seyyedyousefi et al. (2013) found that, callus induction is hard and time consuming in many monocotyledons like *Alstroemeria* sp.

The results in Table 2 show the mean of fresh weight of callus induced by different concentrations of 2,4-D. The highest mean of fresh weight of callus was induced by 1.00 mg/L 2,4-D (1.302 ± 0.09 g) (Figure 2C) followed by 0.5 mg/L (0.601 ± 0.04 g) (Figure 2B) and 2.00 mg/L (0.370 ± 0.05 g) (Figure 2D). 3.00 mg/L of 2,4-D induced lowest amount of fresh weight of callus (0.319 ± 0.01 g) (Figure 2E). Significant differences ($p<0.05$) were detected among the effect of different concentrations of 2,4-D on callus induction. Resmi and Shylaja (2010) showed that 1.00 mg/L 2,4-D recorded the highest callusing (56.37%) and callus growth by *Z. officinale* Rosc shoot tip explants, also Ma and Gang (2006) reported the presence of 2,4-D at 0.5 to 1.5 mg/L in the culture medium resulted in callus growth for *Z. officinale*

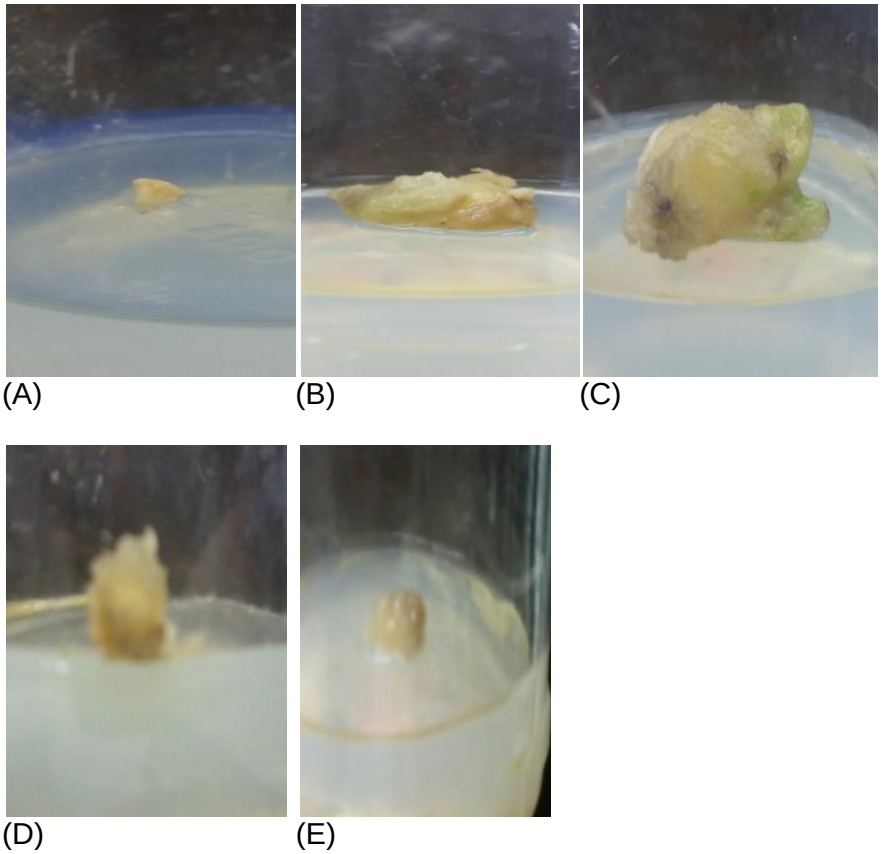


**Figure 2.** Callus induced from shoot tip explants by MS medium supplemented with 2,4-D hormone, (A) 0.00 mg/L as control, (B) 0.5 mg/L, (C) 1.00 mg/L, (D) 2,00 mg/L and (E) 3.00 mg/L.

Rosc explants, moreover Malamug et al. (1991) and Babu et al. (1992) reported that 2,4-D is the most effective auxin for callus induction in ginger and turmeric.

Callus of *Z. officinale* was compact and showed creamy colour by 0.5 and 1.00 mg/L of 2,4-D, while the callus induced by concentration 2.0 and 3.00 mg/L 2,4-D was friable and showed brown colour. These results are similar to that obtained by Anasori and Asghari (2008); they found some callus of *Z. officinale* turned brown in colour. Cream coloured callus was subcultured after four weeks in MS medium with four concentrations of 2,4-D (0.5 , 1.00, 2.00 and 3.00 mg/L) and one concentration of BAP (0.5 mg/L) was used in combination with 2,4-D concentrations. The highest mean of fresh weight initiated by subculture among different treatments was 1.509 ± 0.00 g by MS + 0.5 mg/L 2,4-D (Figures 3 and 5). Combinations of 0.5 mg/L BAP with different concentrations of 2,4-D induced lower fresh weight of callus compared to callus initiated by subculture on medium supplemented with 2,4-D only. The best fresh weight of callus initiated by combinations of 0.5 mg/L BAP with different concentrations of 2,4-D was 0.594 ± 0.06 g by MS medium containing 0.5 mg/L BAP + 0.5 mg/L 2,4-D (Figure 4).

**Direct organogenesis**

The sterile sprouting buds (about 1 to 1.5 cm) were used as explants to induce shoots and roots on MS medium with 2.00, 3.00 and 4.5 mg/L of BAP. *In vitro* shoots and roots were successfully induced in all treatments (Figure 6). *In vitro* roots were initiated after 15 to 20 days from culture time earlier than shoots which were initiated after 30 to 40 days. The shoots and roots number/explant and shoots, roots length were measured for three months *in vitro* plants (Figures 8, 9 and 10). BAP at concentration 4.5 mg/L showed the highest number of *In vitro* shoots (4 ± 0.35) and roots (15 ± 0.46) per explant (Figures 6B and 7). These results agree with Abbas et al. (2011), they found that augmentation of MS medium+4.5 mg/L BAP recorded the highest shootlets multiplication percentage from *in vitro* propagation of *Z. officinale* using sprouting buds. Also, Nkere and Mbanaso (2010) investigated the optimizing concentrations of growth regulators for *in vitro* ginger propagation, they found combination of 0.05 mg/L NAA and 4.0 mg/L BAP gave the highest shoot regeneration rate.

Two shoots and 10 ± 0.8 roots per explants were initiated by 3.00 mg/L BAP (Figure 6C). MS medium +

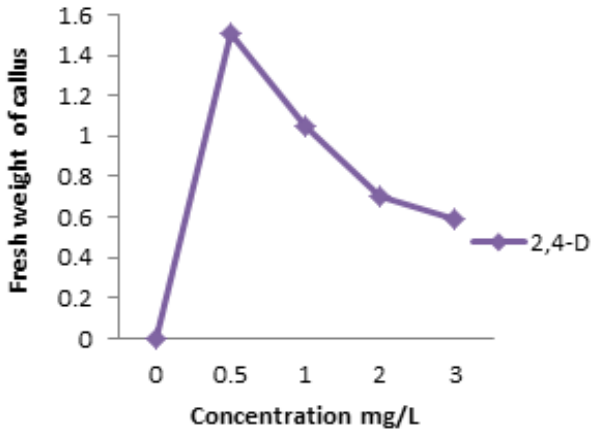


**Figure 3.** Effect 2,4-D on fresh weight of callus by subculture.

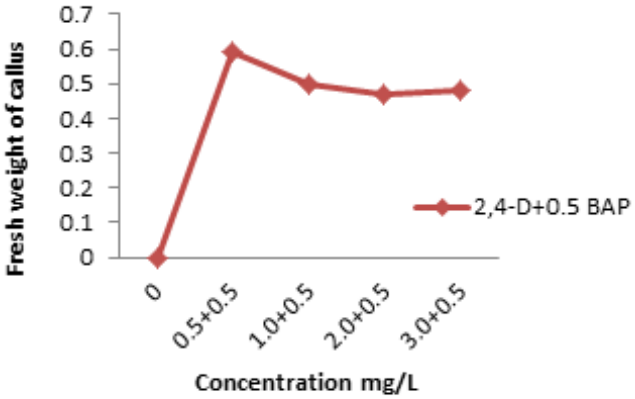


**Figure 4.** Effect combination of 0.5mg BAP+2,4-D concentrations on fresh weight of callus by subculture.

**Figure 5.** Callus subcultured on MS medium + 0.5 mg/L of 2,4-D.

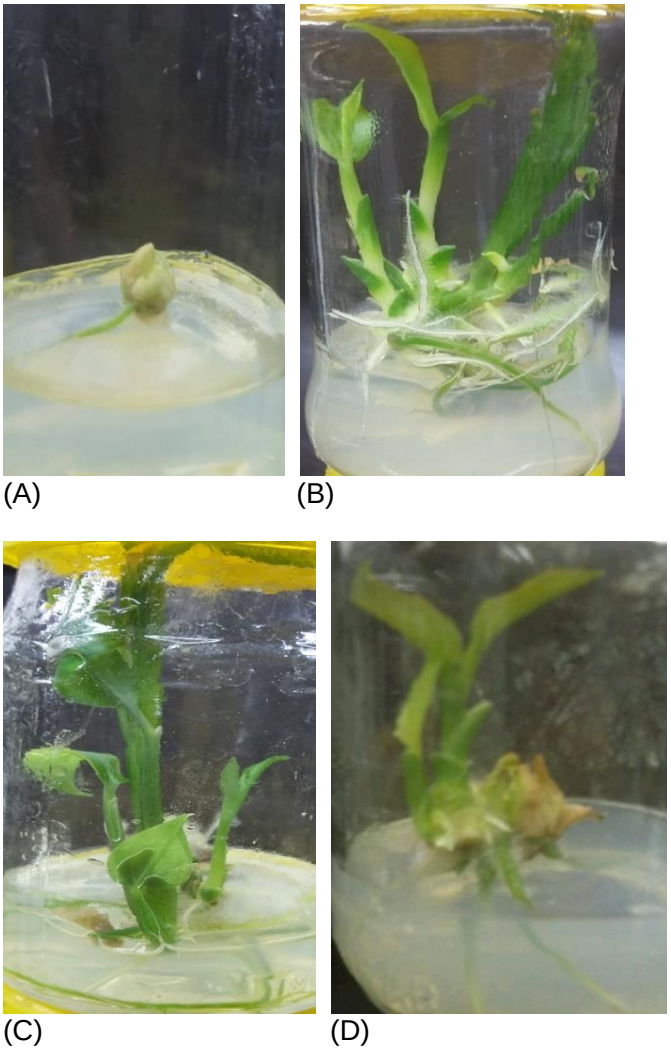


**Figure 6.** Direct organogenesis( *In vitro* shoots and roots initiation) by MS medium supplemented with, A (0.00) as control, B (4.5 mg/L BAP), C (3.00 mg/L BAP) and D (2.00 mg/L BAP).

2.00 mg/L BAP induced lowest number of *in vitro* shoots (2 ± 0.67) and roots (8 ± 0.35) per explant (Figure 6D). Shoots length is shown in Table 3 and Figure 9 and roots length is shown in Figure 10.

## Indirect organogenesis

Callus induced by 1 mg/L 2,4-D after six weeks was subcultured on MS fortified with 0.5 mg/L 2,4-D for six weeks to obtain a green coloured callus, then this callus was transferred to MS with 1.00 mg/L BAP+0.5 mg/L NAA, 2.00 mg/L BAP+0.5 mg/L NAA and 4.00 mg/L BAP+0.5 mg/L NAA to induce shoots and roots from callus (indirect organogenesis). Combination of 1.00 mg/L BAP+0.5 mg/L NAA induced the best shoots and roots initiated from callus, 2 ± 0.21 shoots and 22 ± 0.33

**Figure 7.** *in vitro* shoots and roots initiated by MS medium supplemented with 4.5 mg/l BAP.

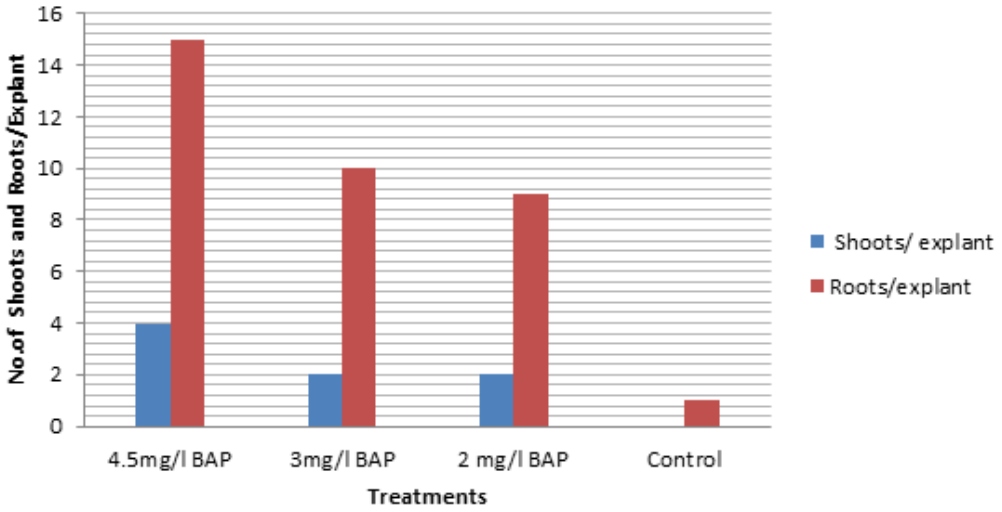


**Figure 8.** Effect of growth regulator (BAP) added to MS medium on shoots and roots induced/explant of *Z. officinale* for three months of culture.

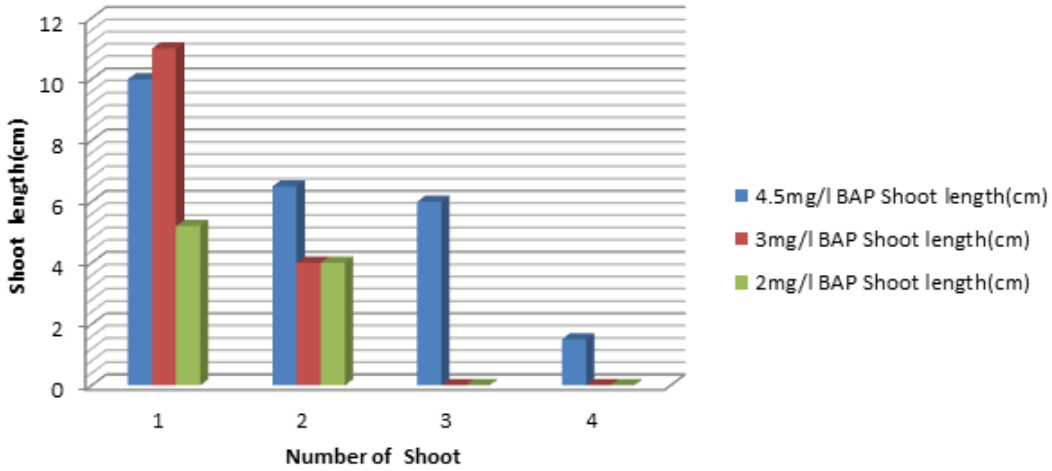


**Figure 9.** Effect of BAP on shoots length (cm) of *Z. officinale* for three months of culture.

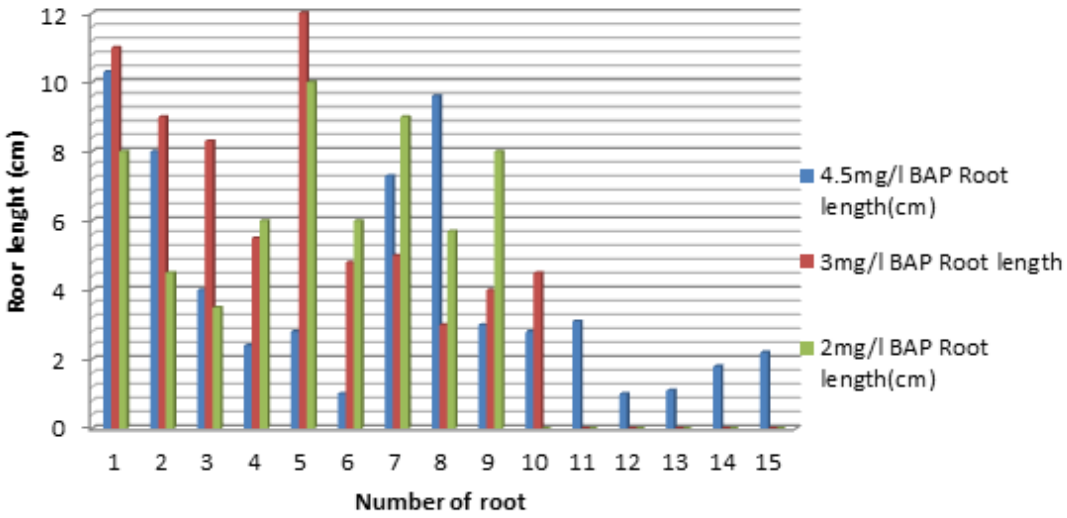


**Figure 10.** Effect BAP on roots length (cm) of *Z. officinale* for three months of culture.

**Table 3.** Effect BAP on shoot length (cm) of *Z. officinale* for three months of culture.

| PGR | No. shoot | Shoot length (cm) |
|---|---|---|
| 2 mg/l BAP | 1 | 5.2 |
| | 2 | 4 |
| 3 mg/l BAP | 1 | 11 |
| | 2 | 4 |
| 4.5 mg/l BAP | 1 | 10 |
| | 2 | 6.5 |
| | 3 | 6 |
| | 4 | 1.5 |

roots (Figure 11A and 12), compared to other treatments, which were one shoot and 7 ± 0.00 roots by combination of 2.00 mg/L BAP+0.5 mg/L NAA (Figure 11B), one shoot and 20 + 0.15 roots were initiated by 3.00 mg/L BAP + 0.5 NAA (Figure 11C), while the combination of 4.00 mg/L BAP + 0.5 mg/L NAA induced 21 roots and no shooting was observed during three months of culture (Figure 11D). Our findings are in harmony with Solanky et al. (2013) who investigated *in vitro* regeneration of ginger through callus culture, they found that maximum shoot regeneration per gram of callus was achieved from the MS media containing 1 mg/L BAP with 1 mg/L Kin 82.24% shooting with 8.62 number of shoots under 2000 lux light intensity. Taha et al. (2013) reported that the highest number of regenerated shootlets of callus derived from ginger leaves were (1.67) by MS+1 mg/L BAP, and the lowest number of regenerated shootlets (0.4) was recorded with 4 mg/L BAP.

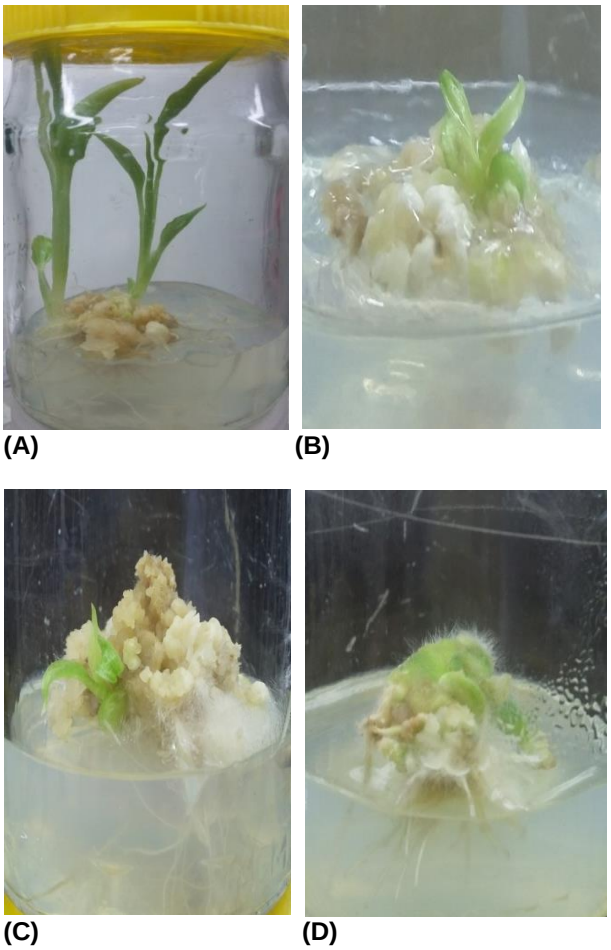


**Figure 11.** Indirect organogenesis (*In vitro* shoots and roots initiation) on MS medium supplemented with combinations of: (A)1.00 mg/L BAP+0.5 mg/L NAA, (B)2.00 mg/L BAP+ 0.5 mg/L NAA, (C) 3.00 BAP+0.5 mg/L NAA and(D) 4.00 BAP+0.5 mg/L NAA.

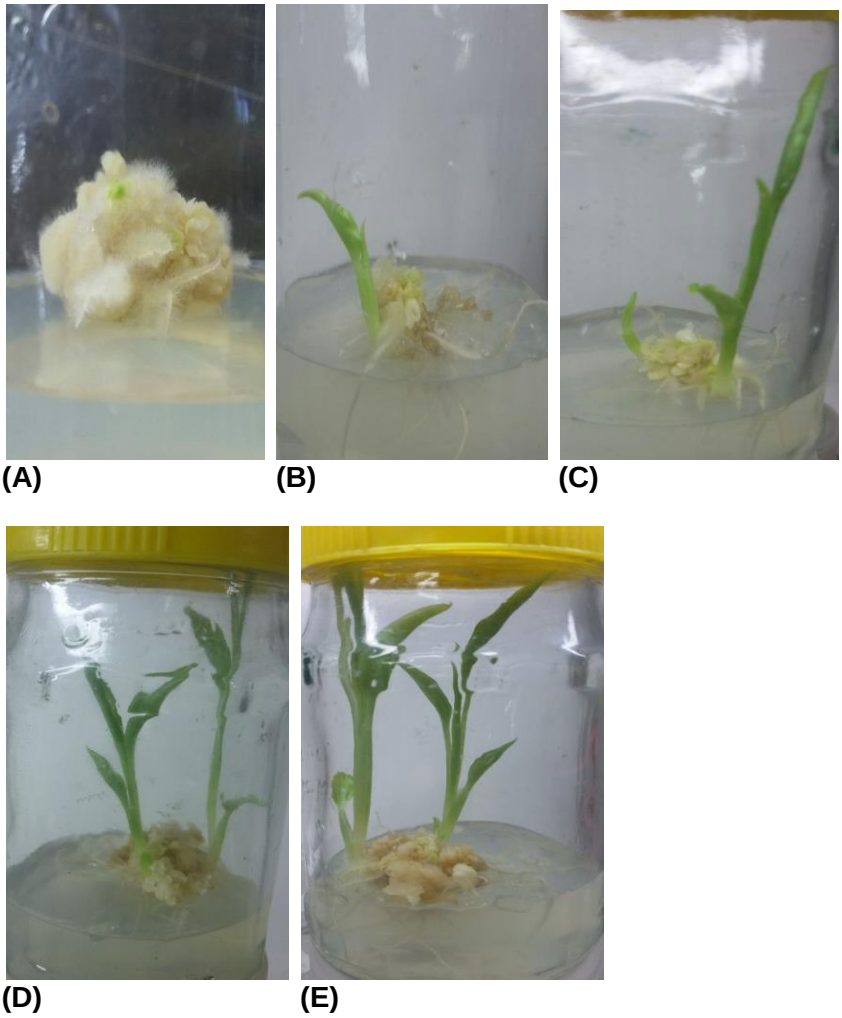


**Figure 12.** Development the best indirect organogenesis (*In vitro* shoots and roots initiation) on MS combined with 1.00 mg/L BAP+0.5 mg/L NAA during three month of culture. (A) Callus subcultured on MS + 0.5 mg/L 2,4-D for six weeks (B, C, D and E), *in vitro* shoots and roots initiation during three months of culture.

The medium containing BAP+NAA enhanced roots formation. Similar finding was reported by Nkere and Mbanaso (2010), they investigated optimizing concentrations of growth regulators for *in vitro* ginger propagation; they revealed that combination of BAP+NAA supports root formation.

## Conclusion

From the study, we can conclude that callus was only induced from shoot tip explant. The highest significant value of callus fresh weight was induced from shoot tip explants by 1.00 mg/L of 2,4-D. A suitable concentration to regenerate callus by subculture was 0.5 mg/L 2,4-D. Direct organogenesis showed highest number of *in vitro* shoots and roots by 4.5 mg/L BAP, and the best shoots and roots from indirect organogenesis were induced by combination of 1.00 mg/L BAP+0.5 mg/L NAA.

## Conflict of Interests

The authors have not declared any conflict of interests.

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