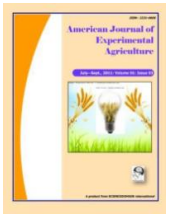

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Plant Regeneration from Shoot Tips-derived Callus of Ginger (*Zingiber officinale* Rosc.)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAI and GHD designed the study, wrote the protocol and wrote the first draft of the manuscript and reviewed the experimental design and all drafts of the manuscript. Author GHD performed the statistical analysis. Authors VMM and BMK managed the analyses of the study and identified the plants. All authors read and approved the final manuscript.

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ABSTRACT

A protocol was described to regenerate plants from callus culture of ginger (*Zingiber officinale* Rosc.). Callus cultures were induced from ginger shoot tips inverted cultured on semi-solid MS medium supplemented with 2, 4-D (0.5-1.0 mg L^{-1}) and BA (0.5- 1.0 mg L^{-1}). Maximum callus induction was obtained on MS medium supplemented with BA (0.5 mg L^{-1}) either alone or with 2,4-D (0.5 – 1.0 mg L^{-1}), while the highest callus weight was on MS medium supplemented with BA (0.5 mg L^{-1}) and 2,4-D (0.5 mg L^{-1}). Induced callus was tested for regeneration on MS medium supplemented with different concentrations of 2,4-D and BA and the best regeneration was observed on a medium containing 2.5 mg L^{-1} BA. The regenerated plants were rooted and successfully established in the field after few days of acclimatization.

Keywords: Ginger; callus induction; plant regeneration; shoot tips.

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1. INTRODUCTION

Zingiber officinale Rosc. (Ginger) of the family *Zingiberaceae* is an important tropical horticultural plant, values all over the world as an important spices for its medicinal properties. The *Zingiberaceae* is an herbaceous moderate sized family of relatively advanced monocotyledonous plant of the order *Zingiberales*. Zingiberaceous plants are rhizomatous, perennial and aromatic herbs often of large size, bearing flowers either terminally on aerial leaf shoots or from ground level [1]. Ginger's pungent aromatic rhizome is consumed all over the world as a spice, culinary herb, condiment, home remedy, and medicinal agent [2]. It is vegetatively propagated through underground rhizomes, with a very low multiplication rate [3]. The common problems in increasing ginger production are soil-borne disease infection and degeneration of rhizomes [2], and the breeding of ginger has been severely hampered by poor flowering and seed set [4], and even the rhizome cannot be stored for long time as it is susceptible to fungal diseases, which affect the quality of tubers [5]. *In vitro* culture techniques, especially meristem culture, provide an alternative way of plant propagation and maintenance of disease free germplasm banks [6]. There are many reports on ginger tissue culture. [7] Reported that maximum six shoots per bud from *in vitro* culture, with low survival under field conditions. Organ culture [3], somatic embryogenesis [4] and [8], organogenesis [9], protoplast culture [10], microrhizome production [11], shoot tips culture [12], rhizome buds cultured at different size [13] and germplasm preservation [14] have been conducted. Ginger seed production also was suggested by [15] by *in vitro* propagation methods, however these methods are not efficient, expensive and do not meet ginger germplasm conservation requirements.

The present work aimed to describe an efficient protocol for; calli production; shoot regeneration, keeping in view the potential action against various ailments and its regeneration problems in natural environment. Micropropagation of this medicinal important plant was accomplished to provide stable supply of the *in vitro* raised plantlets for the mass production for this important medicinal plant in Iraqi Kurdistan Region and to become a powerful tool for future studies.

2. MATERIALS AND METHODS

2.1 Callus Induction

This investigation was conducted at Plant Tissue Culture Lab. at the Scientific Research Center, Faculty of Science at the University of Duhok. Shoot tips of *in vitro* cultured ginger on MS medium [16] supplemented with 1.0 mg/l BA were cut and placed in inverted form on the culture medium. Various concentrations of 2,4-dichloro-phenoxyacetic acid (2,4-D) and benzylaminopurine (BA) (0.0, 0.5, 1.0 mg/l) in MS medium containing 3% sucrose, 100 mg/l inositol were evaluated for inducing callus. The pH of the medium was adjusted to 5.7±0.1 with 1N NaOH or HCl, prior to the addition of agar (7. 0) g/l. The medium was brought up to the final volume, subsequently, 25 ml of medium was dispensed into 250 ml Mason jars and capped with colorless PVP covers and fitted with rubber bunds. The medium was sterilized by autoclaving for 15 min at 121°C temperature under 1.04 kg/cm² and allowed to solidify under room temperature. Three explants were cultured in each vessel with five replications. The cultures were incubated in the culture room under 25± 1°C temperature and 16 hours daily exposure to 1000 Lux cool white light, followed by 8 hours of darkness. The results were recorded after 8 weeks of culture.

2.2 Plant Regeneration

Two months old callus cultures were transferred to MS medium supplemented with BA (0.0, 2.5, and 5 mg/l) and 2, 4-D (0.0, 0.2 and 0.4 mg/l) either alone or in combination. After eight weeks the shoots length, number of shoots and leaves/ explant were recorded. The plantlets were suppurated from the callus and cultured on MS medium was supplemented with 2.5 mg/L kinetin for both multiplication and rooting stages [17].

Eventually, for hardening stage, a number of well and successfully rooted plantlets were removed from culture vessels and their roots were washed with distilled water and immersed in Benlate fungicide (0.1% for 10 minutes). Finally, they were planted in pots containing steam sterilized soil mixture of peat moss: loam: stryrofoam (1:1:0.5) under tightly controlled atmosphere of the greenhouse.

2.3 Data Analysis

All the comparison between means was carried out according to Duncan's multiple range test ($P < 0.05$) using a computerized program of SAS [18].

3. RESULTS AND DISCUSSION

After 10 days of culture, there was a notable enlargement of inverted shoot tips with a whitish to brown colour and fragile texture (Fig. 1A). The callus growth was slow and highly sensitive to mechanical force or fragile, the surface was covered with small hairy structure.

The caulogenic response of the shoot tips to various concentrations and combinations of cytokinins and auxins is shown in (Fig. 2) For callus induction the highest and best callus induction (100%) was noticed on MS medium containing BA either alone or in combination with 2,4-D (Fig. 1, B), while those devoid of BA showed lowest response. Similar results were obtained by [19] and [9] where they reported that 2, 4-D is the most effective auxin for callus induction in ginger and turmeric [20].

The highest callus weight (1.43 g) showed in (Fig. 3) observed on MS medium supplemented with 0.5 mg/l from BA and 2,4-D (Fig. 1, C) followed by MS medium containing only BA at concentration 0.5 mg/l (1.34 g) , no significance different between the treatments. While the least weight (0.17 g) was observed on MS medium devoid of growth regulators.

The well developed, white and fragile callus was transferred to regeneration medium (Fig. 4). The highest mean number of regenerated shoots (6.0 shoots/explants) was observed on MS media containing BA 2.5 mg/l (Fig. 1, D) followed by MS medium supplemented with BA 5.0 mg/l which was 5.0 shoots/explant, but did not different significantly. Similar results were recorded by [20] they observed that 5.0 mg/l BA produced the highest number of shoots form callus in *Curcuma attenuate*, [21] recorded that BA combination with NAA or TDZ was effective for shoot regeneration from anther-derived -callus of *Curcuma attenuate*. The MS medium fortified with BA and 2, 4-D showed the lowest mean numbers of shoots. Similar results were recorded by [19], where 1 to 3 mg/l of BA was the best concentrations for shoot regeneration from callus tissues. [9] State that 2, 4-D at lower concentration would enhances organogenesis. Since there is no significant difference between 2.5 and 5.0 mg/l of BA, it is advisable to use the lowest concentration to reduce the mass production cost.

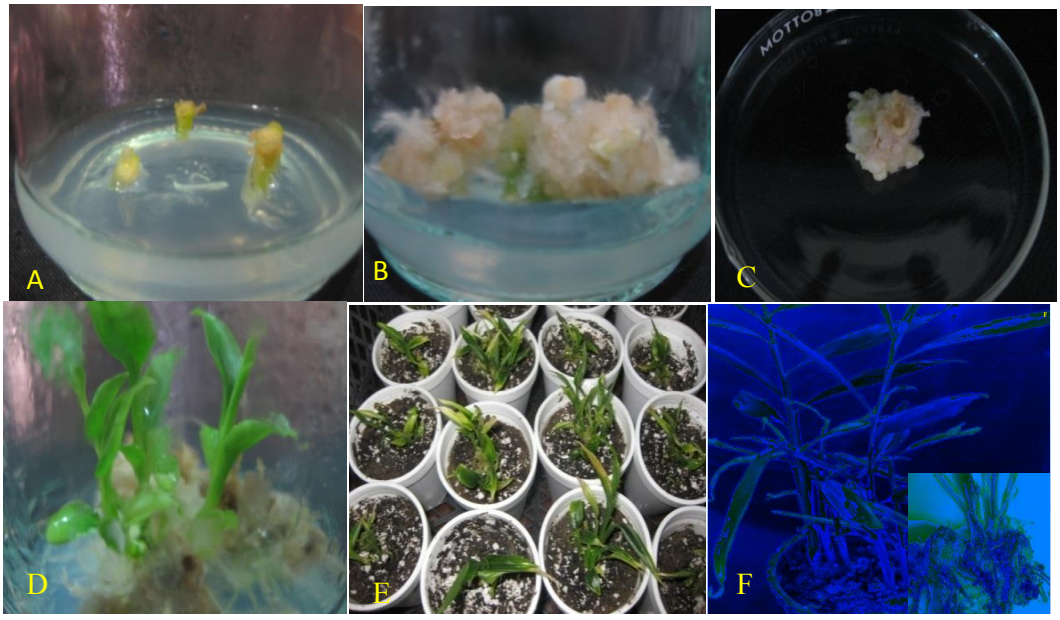

Fig. 1A. Shoot tip culture in inverted in MS medium, B. Callus induction in MS medium supplemented with BA and 2, 4-D after 8 weeks, C. High callus weight in MS medium supplemented with 0.5 mg/l BA and 2, 4-D, D. plantlets regenerated from the callus, E Acclimatization of plantlets in the soil, F. Hardened plant ready for transfer to field with formed rhizome

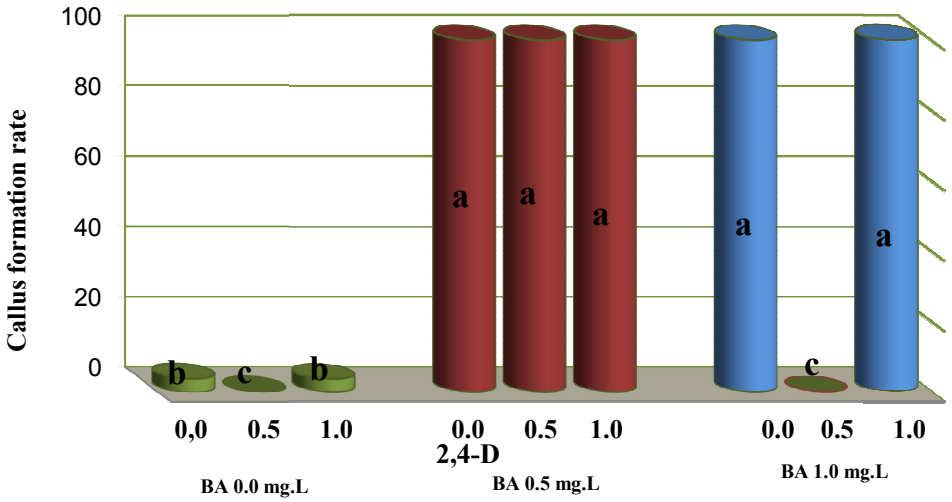

Fig. 2. Effects of different concentrations of BA and 2, 4-D on callus formation from shoot tips of *Zingiber officinale*

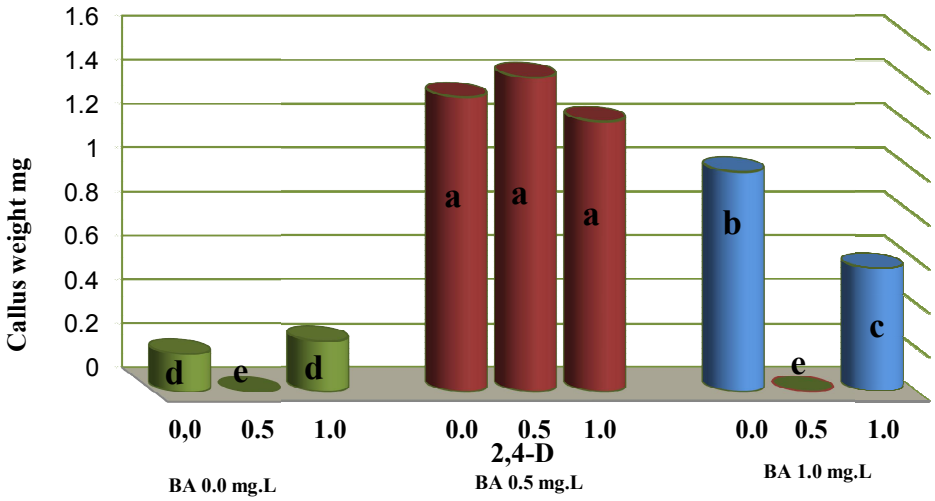

Fig. 3. Effects of different concentrations of BA and 2, 4-D on callus weight from shoot tips of *Zingiber officinale*

These results emphasize the importance of cytokinins in shoot regeneration in *in vitro* plant propagation. Cytokinins activate RNA synthesis; stimulate protein synthesis and the activities of some enzymes. Generally, herbaceous plants are highly responsive to BA treatments and most herbaceous species produce well formed shoots suitable for further shoot proliferation [22].

On the other hand, the highest mean of shoot length (6.00 cm) (Fig. 5) and highest mean of leaves number (5.0 leaves/explant) (Fig. 6) was found in a medium containing 0.2 mg/l 2, 4-D only without adding any cytokinins and this was highly significant than all other means.

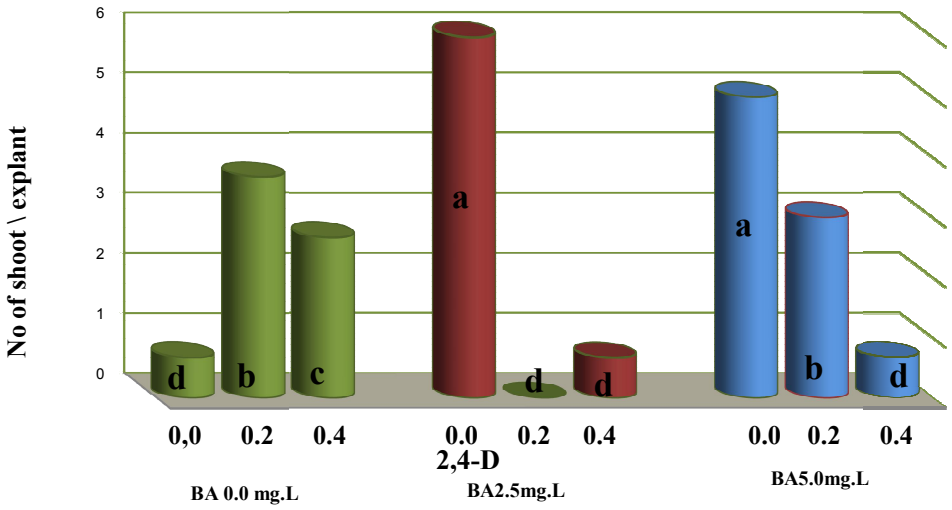

Fig. 4. Effects of different concentrations of BA and 2, 4-D in No. of shoot\explant from callus culture of *Zingiber officinale*

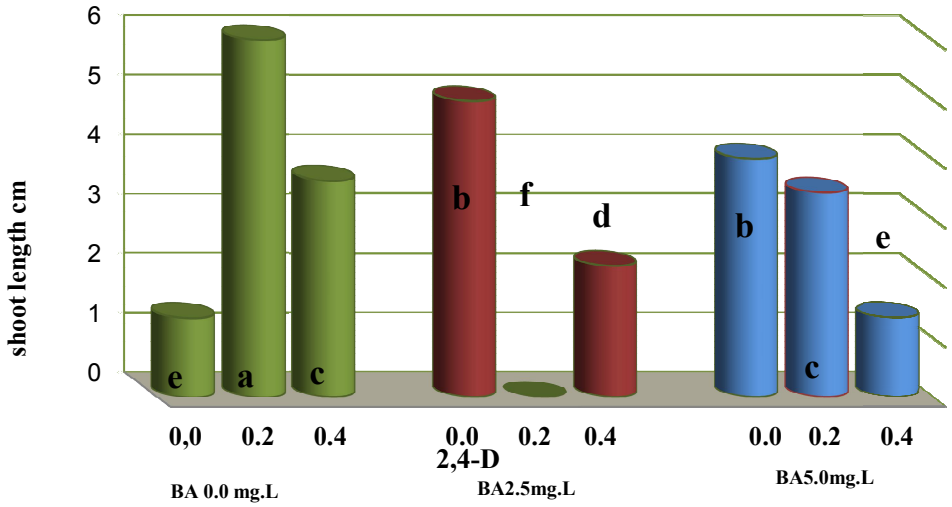

Fig. 5. Effects of different concentrations of BA and 2, 4-D in shoot length (cm) from callus culture of *Zingiber officinale*

The shoots regenerated from calli were transfer and successfully multiplied in 2.5 mg/l kinetin. Roots were also formed in the same medium or in phytohormone-free medium. These records are in agreement with those found by [17] and [23] which indicates that the endogenous auxin level in the plant itself is so sufficient that can form roots without applying any exogenous auxins. Plantlets were successfully transplanted into pots after sufficient development of roots to establish the field condition and rhizome formation in the soil (Fig. 1, E, F).

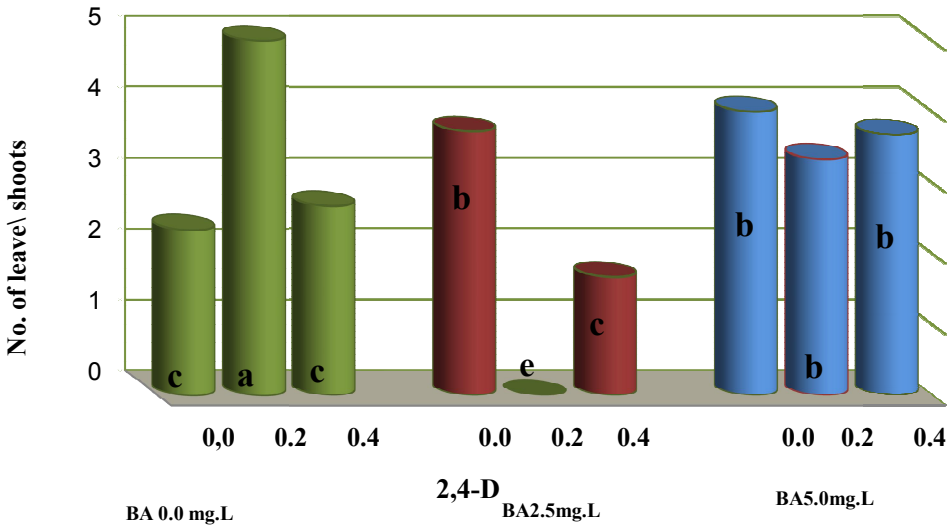

Fig. 6. Effects of different concentrations of BA and 2, 4-D in No. of leaves\ shoots from callus culture of *Zingiber officinale*

4. CONCLUSION

It was concluded that it is possible to regenerate plants from callus culture of ginger (*Zingiber officinale* Rosc.), which is induced from ginger shoot tips explants and are suitable for propagation. Plant regeneration was successfully developed for shoot tips-derived callus of ginger and these explants may be used according to their higher rate of shoot multiplication. The plant regeneration is varies dependent on phytohormone concentrations. For ginger shoot tip explants, the developed protocol is suitable for micpropagation for future work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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