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EFFICIENT *IN VITRO* REGENERATION OF *ZINGIBER OFFICINALE* ROSC. VAR. WHITE THROUGH SHOOT TIPS CULTURE

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Abstract

This study was conducted in plant tissue culture Laboratory, Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Iraq, during seasons growth 2018-2019 to develop an efficient protocol for mass propagation of *Zingiber officinale* Rosc. Surface sterilized sprouting buds 1-1.5 cm from rhizomes were cultured on Murashige and Skoog MS medium supplemented with BA and NAA. Results revealed that highest multiplication occurred when the MS medium supplied with 3 mg. L^{-1} BA+ 0.5 mg. L^{-1} NAA. This treatment was highly significant in number and length of shoots and roots per explant in comparison with other treatments reached 10.00, 5.00 cm respectively. High BA concentration (7,10 mg.L^{-1}). reduced shoots number and length and caused abnormalities in shoots on *In vitro* culture. Also, this high concentration caused a good quality of callus in the base of shoots. Regenerated plantlets showed highly with a 40% survival rate.

***Keywords*:** *In vitro*, white ginger, shoot tips, plantlets.

Introduction

Ginger (*Zingiber officinale)* is a monocot plant belongs to the family Zingiberaceae. *Z. officinale* Rosc. It is an important tropical horticulture plant values all over the world as important spices for its medicinal properties (Portnoi *et al.,* 2003; Shaik and Kanth, 2018). There are two varieties of ginger: *Z. officinale* var. Rosc. (white ginger) and *Z. officinale* var. rubrum (red ginger) have been identified in Malaysia and neighboring south-east Asian countries and has spread to East Africa and the Caribbean (Weiss, 2002; Solanky *et al.,* 2013). Ginger is widely used in a variety of foods because of its nutritional composition and flavoring compounds. Ginger rhizomes are a rich source of carbohydrates, vitamins, minerals and iron (Dhanik *et al.,* 2017).

Z. officinale Rosc. normally propagates by underground rhizomes, with alow proliferation rate, easily infected by soil-borne pathogens such as bacterial or fungal and mycoplasma diseases, for example, *Pythium aphanidematum* causing soft rot, *Fusarium oxysporum* causing yellow of the leaf and *Pseudomonas solanancearum* causing bacterial wilt. These pathogenic factors cause heavy losses in yield (Guo and Zhang, 2005; Kavyashree, 2009). In addition, the Rhizome has a dormancy period and only sprouts during the monsoon that only 5 to 6 plants can be obtained from one single rhizome in a year (Solanky *et al*., 2013). Tissue culture is the only methodology that can produce a large quantity of clonal plants in a short time with high phytosanitary quality (Silva *et al*., 2014). Therefore, it's important to generate disease-free clones in large numbers in a short time (Zuraida *et al*., 2016). Multiple shoots are an efficient method of plant regeneration allowing rapid production of the large number of plants within a short period, so far *in vitro* regeneration of shoots from shoot tips has already recorded by many of researchers got positive results on other Zingiberaceae species such as (Singh *et al*., 2015; Zuraida *et al*., 2016; Chan and Thong, 2004; Yusuf *et al*., 2011). The present study aimed to investigate the effect of growth regulators (cytokinins and auxins) to formulate an efficient system for shoot multiplication, *in vitro* rooting and acclimatization of *Z. officinale*.

Materials and Method

The study was conducted in plant tissue culture laboratory at the Department of Horticulture and landscape design, College of Agriculture – Basrah in southern Iraq-University during period 2018-2019 to study the following:

Effect of different concentration of BA on shoot Multiplication:

Healthy sprouts with active buds (shoot tip) 1-1.5 cm length were collected from the rhizome of *Z. officinale* (Fig1 - A, B, C). These active buds were rinsed several times with distilled water mixed with detergent solution Tween-20 for 25-30 minute, and then rinsed several times with distilled water, then dipped in 20% Clorox (1% free chlorine) with 3-5 drop of Tween -20 for 20 minutes, and rinsed 3-5 times in distilled water under sterile laminar flow- air cabinet. These buds used as an explants for *in vitro* culture, These explants were planted in MS medium (Murashiga and Skoog,1962) obtained from American caisson Labs company about 4.33 $g.l^{-1}$ of MS salts, Then supplemented with organic compounds (Table 1) and different concentration of BA 0, 3, 5, 7, 10 mg.L^{-1} and 0.5 mg.L^{-1} NAA for a shoot–root induction in one step. The PH of MS medium was adjusted by sodium hydroxide and hydrochloric acid solution at one normality concentration, then added the agar at concentration 6 gm. l^{-1} to MS medium. The MS medium was heated on magnetic stirrer hotplate at 90 ^{o}C. 20 ml medium was spoured in to test tube culture and closed by using medical cotton and aluminum foil, then sterilized by autoclave at 121 ^{o}C and 1.05 $kg.cm^{-2}$ for 20 minutes. These cultures were grown in a growth room at 25±2 (16 hours light and 8 hours darkness). Every treatment was replicated 10 times. Data was recorded after 16 weeks of culture. The study characteristics were:

1. Number of Shoot per explant
2. Length of Shoot per cm
3. Number of root per shoot

Table 1 : The chemical composition additives to MS medium

Seq	Chemical material	Quantity mg.L^{-1}
1-	Sucrose	30000
2-	Adenine sulfate	40
3-	Activated charcoal	500
4-	Thiamine	1
5-	Citric acid	150
6-	Ascorbic acid	100
7-	Orthophosphate Sodium	170

Acclimatization

Shoot-root micropropagates were removed from the medium (Fig 1-K), then washed under running tap water to remove solid MS medium, and transported them to jars containing sterilized water (Fig 1- L) for 10-15 days water was changed every two days and jars were covered with glasses. These shoots–root micropropagated were planted in plastic pots 5cm in diameter containing autoclave soil mix (peat moss and ground sand 1:2), and covered with glasses for 15-20 days (Fig 1-M-N), the glasses cover was removed gradually to harden and kept them growth room under condition (Temperature 25±2 ºC) 24 h photoperiod with 1500 Lux light intensity. The precentag of survival was recorded after 12 weeks by using the flowing equation :

$$\text{Percentage of frequency} = \frac{\text{Number of plants showing response}}{\text{Total number of plants}} \times 100$$

Statistical design and analysis:

A completely randomized design was used. The data was subjected to the analysis of variance and mean values were compared using LSD at 1% (SPSS, 2016).

Results and Discussion

The sterile sprouting buds (shoot tip) about 1-1.5 cm of *Z. officinale* were cultured vertically on MS medium supplemented with different concentrations of Cytokinin BA 0, 3, 5, 7, 10 mg. L^{-1} in combination with 0.5 mg.L^{-1} NAA on shoots Multiplication (Fig 1-D). *In vitro*, roots were initiated after 20-25 days from culture time earlier than shoots which were initiated after 40-50 days because *Z. officinale* plants have a dormancy period (Solanky *et al*., 2013) (Fig 1-E). Microshoots and roots were successfully induced in all treatments of BA in one step compared to MS control medium which have no BA in side it, these results proved that a combination of BA + NAA supports root formation or will shorten the time for plant regeneration, a similar finding was reported by Nkere and Mbanaso (2010). When they propagated ginger by indirect organogenesis method, also similar results were reported by Singh *et al*. (2015), When cultured shoot tip of *Curcuma caesia* on MS medium supplemented with a combination BAP + NAA produced both shoots and roots (Fig 1- F, G). Shoots, roots number per explant and shoots, roots length were measured for 16 weeks *in vitro* plants.

BA at concentration 3.0 mg. L^{-1} recorded the highest number and length of shoots (10.00, 5.16) cm and number of roots 5.00 per explant respectively (Table 2). It was significantly with other treatments in multiple shoots induction. It was observed that Cytokinine was required in optimal quantity for shoot proliferation.

In the same table, Results also showed a lowest number and length of *in vitro* shoots (1.17, 0.75) cm and roots 0.00 per explant respectively is shown in medium supplemented with 0.00 mg.L^{-1} BA + 0.5 mg .L^{-1} NAA. This results were due to the lower concentration or lack of BA in the medium supported poor regeneration response and produced a single shoot (Short, 1986). However, the height concentration of BA (7, 10 mg.L^{-1}) was suggested to reduce length and number of shoots and to cause abnormalities in shoots on *in vitro* culture (Fig. 1- H) (Jafari *et al*., 2011; Sathyagowri and Seran, 2011). Results also indicated that callus formation was found on the apical and the end of multiple shoots have grown in medium supplemented with a high concentration of BA 7, 10 mg. L^{-1} (Fig1- G, I, J), The reason may be to increase the concentration of BA in MS medium due to be supra-optimal for cell division stimulation and callus formation (Al-Taha, 2008).

The result of the present study has an agreement with the other studies (Panda, 2007) related to the culturing of *in vitro Curcuma longa* L. shoot tip on MS medium supplemented with 2 or 3 mg.L^{-1} BAP and 1 mg.L^{-1} NAA produced a highest average number of the shoot (2.4 and 2.6 shoots) respectively and optimal number of root (3 and 2.6); respectively. Jala (2012). Zuraida *et al*. (2016) got a similar result when transferred *in vitro* microshoots of *Z. officinale* var. Rubrum from a medium supplement with 5 mg.L^{-1} BA to MS medium supplemented with 1.0, 3.0, 5.0 BA and 3.0, 0.5 mg.L^{-1} NAA which produced the highest number of shoots and roots , but average number of shoots (19.5 ± 2.3) per explant and mean number of roots of shoots (7.3± 2.1) produced in MS medium supplemented with 3.0 mg.L^{-1} BAP + 0.5 mg.L^{-1} NAA. Also, Sigh *et al.* (2015) got a similar result when culturing shoot tips of *Curcuma caesia* in MS medium containing 5.0 mg.L^{-1} BA + 2.0 mg.L^{-1} NAA and also 3.0 mg.L^{-1} BAP + 0.5 mg.L^{-1} NAA which produced the highest number of shoots 5.65 and 5.70 shoots per explant respectively. Panda *et al*. (2007) developed *in vitro* propagation protocol for *Curcuma longa* and gave 7.6 ± 0.3 shoots by using a medium with 3.0 mg.L^{-1} BAP.

Table 2: Average number of new shoots, roots and plant height of *Zingiber officinale* Rosc. var. White on MS medium supplemented with a combination of BA and NAA after culture for 15 weeks.

Treatment		Number of shoots Per explant	Shoot length in cm	Number of roots Per explant
BA (mg.L^{-1})	NAA (mg.L^{-1})			
0	0	1.17	0.75	0.00
3	0.5	10.00	5.16	5.00
5	0.5	5.50	3.16	1.83
7	0.5	5.33	1.66	1.33
10	0.5	3.17	0.75	0.67
LSD $_{0.01}$		1.30	1.14	0.85

Acclimatization

Finally, the healthy regenerated plantlets of *Z. officinale* which were selected from different treatments were acclimatized. The process of acclimatization continued for 5-6 months (Fig1- L, M, N) and the rate of survival was 40 %, because the most of the healthy plant have a low proliferation rate of roots, in addition to afine roots were formed in this method of propagation (shoot, root induction) which was easily infected by soil-borne pathogens such as bacterial and root-knot nematode (*Meloidogyne incognita*), These

pathogenic factors cause heavy losses in yield (Guo and Zhang, 2005; Kavyashree, 2009). Similar results were reported by Al-Taha *et al*. (2018) in their study on *Schlumbergera russelliana* which may result in the death of plants when they acclimated it.

Conclusions

In the present study, sprouting rhizome bud (shoot tips) of *Z. officinale* Rosc. var. White. showed that the highest significant values *in vitro* shoots, roots number and length was induced from shoot tip about 1-1.5 cm long were used as explant by 3.0 mg.l^{-1} BA + 0.5 mg.l^{-1} NAA. Also when the concentration of BA was increased (5, 7, 10 mg.l^{-1}) in MS medium gave the best quality of callus. In this investigation, the mortality rate of the plantlets with low developed roots that were acclimatized and hardened was low.

Fig. 1 : *In vitro* micropropagation of *Zingiber officinale* Rosc. var. White (A) Budding rhizomes. (B, C) Shoot tips explants. (D) Shoot tips about 1–2 cm length and 1–1.5 cm width cultured vertically on MS medium (E) Callus and root formation from shoot tip cultured *in vitro* (F) Primary initiated microshoots after 12 weeks. (G) Shoot regeneration cultured on MS medium supplemented with different concentration of BA (0.0, 3.0, 5.0, 7.0, 10.0 mg.L^{-1}) +0.5 mg .L^{-1} NAA after 16 weeks. (I) Callus initiation from shoots tips after 16 weeks. (J) Microscope picture for primary callus. (H) Abnormalities branches in concentration 7,10 BA mg. L^{-1} + 0.5 NAA mg .L^{-1}. (K) Maturation of plantlets. (L) Tissue culture-derived complete planted with well-developed roots ready for hardening. (M) Primary hardening in pots. (N) Acclimatized plants after 5-6 months.

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