Journal of science faculty at Sirte University
JSFSU
2021
Peer-review Periodical Journal

جامعة سرت
Sirte University
1989

ISSN INTERNATIONAL STANDARD SERIAL NUMBER

eISSN: 2789-858X

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

SJFSSJ

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA

VOLUME 3 ISSUE 2 OCTOBER 2023

Bi-annual, Peer- Reviewed, Indexed, and Open Accessed e-Journal

Legal Deposit Number@NationaL Library (Benghazi): 990/2021

1.02/2022

jsfsu@su.edu.ly
journal.su.edu.ly/index.php/JSFSU

Scientific Journal for the Faculty of Science-Sirte University Vol. 3, No. 2 (2023) 154-161

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

In vitro Micropropagation of Ginger plant (*Zingiber officinale*)

Ahmed Shaaban, Noman Elnfishy, Zineb Aween, Elshabany Abdelah and Elmundr Abughnia

Libyan Center for Biotechnology Research, plant Tissue Culture Department.

DOI: https://doi.org/10.37375/sjfssu.v3i2.1659

ARTICLE INFO:

Received: 30 September 2023

Accepted: 14 October 2023

Published: 26 October 2023

Keywords: Micropropagation, Plant growth regulators, Tissue culture, Zingiber officinale

A B S T R A C T

The study was conducted for the purpose of micro propagation of ginger plant (Zingiber officinale) through use of plant tissue culture technique to identify the best plant micro propagation conditions. The plant samples were sterilized superficially by immersing them in the Clorox solution, and then the sterilized plants were cultured in MS media in order to obtain free contamination culture. After the sufficient number of ginger plants had obtained the plants were replanted in MS media supplemented with several concentrations of (BA and NAA) for the purpose of obtaining the best vegetative growths. The treatments were divided into four treatments as T1 (control treatment contains MS without hormones), T2 (MS +0.1mg/l NAA+1mg/l BA), T3 (MS+0.1mg/l NAA+2mg/l BA) and T4 (MS+ 1mg/l NAA). The results of this study showed that addition of 0.1mgNAA with 1 or 2mg/l BA improve the vegetative growth of ginger plant. It is also proved that the average of plant length, number of brunches and number of leaves was significantly higher in T2 and T3 compared with other two treatments. Furthermore, results proved that combination between NAA and BA gave the best vegetative growth. For the root site, the results showed that the culture media (MS) plus 1 mg / L of the growth regulator (NAA) resulted in the highest of root growth. A cording to our results addition 0.1mg NAA with 1 or 2mg/l BA would be the best choice for In vitro Micropropagation of ginger plan.

1 Introduction

Recently medicinal plants are receiving a high attention throughout the world due to that people are returned to the natural for their diseases treatments whereas, the high demand on medicinal plants nowadays led to decrease in numbers of medicinal plants on the planet. In general, medicinal plants consider as a rich resources of ingredients that can be used in drug development. Thereby obtaining high amount of medicinal plant is became as the main aim for pharmacology sector due to its important role in drugs industries. In fact, medicinal and aromatic plants mainly propagated by normal vegetative propagation methods. However, the propagation method of medicinal plant should be improved and new technologies are needed in order to obtain more quantities every year to face the high demand on these plants in the globule market. Particularly the traditional methods of propagation face some challenges, which make these methods more difficult to apply. Therefore, prevention of contamination from different source of bacteria and fungi is necessary for successful culture of medicinal plants by using in vitro propagation (Dadi *et al*., 2020) also, in vitro Micropropagation is a method useful for the multiplication of selected genotypes and chemo types of some medicinal and aromatic plant species (Rout *et al*., 2001). In vitro propagation methods are essential components of plant genetic resource management and become extremely important for the conservation of rare

Corresponding author: **E-mail:** ahmeduv@yahoo.com

pants (Sidhu, 2011). Generally In vitro propagation of important plants can offer considerable benefits, including rapid cultivation of species that have limited reproductive capacity and exist in threatened habitats (Ziba *et al*., 2016).Micropropagation is the process of vegetative growth and multiplication from viable and regenerative cells in various plant tissue culture techniques (Zhou and Wu, 2006). Moreover, in vitro propagation large numbers of identical plants can be produced within a limited space and time, which can be used as planting materials this technique is being used in the propagation of different medicinal plants (Dadi *et al*., 2020). Storage facilities maybe established at any geographical location and cultures are not subject to environmental disturbance such as temperature fluctuation, cyclones, insect, pests, and pathogen (Sharma and Malik, 2014). Application of the tissue culture protocols will facilitate research in to the enhanced production of antitumor compounds through different biotechnological strategies, such as plant cell, tissue and organ cultures, and large – scale cultivation in bioreactors. In vitro plant regeneration depends on the type of growth regulators used and explants source while, in vitro cultured explants respond differently according to the growth regulators added to the cultured medium, thus contributing to the germplasm conservation of this endangered and valuable medicinal species in the wild (Ziba *et al*., 2016). Recently large numbers of medicinal plants are being harvested from their wild habitat and due to this over harvesting several species have become extinct plants (Kumari and Priya, 2020). Ginger (Zingiber officinale Roscoe) is belongs to the family of Zingiberaceae. This plant is an important tropical horticultural plant, valued throughout the countries as an important spice for its nutritional and medicinal properties (Devina *et al*., 2016). This plant is an important tropical horticultural plant, value throughout the countries as an important spice for its nutritional and medicinal properties. Ginger is vegetative propagated through use of rhizome sand mainly cultivated in many countries including India, China, Japan, Indonesia, Australia, Nigeria and West Indies (Ravindran and Nirmal, 2005). The plant has been used as medicinal plant to treat cancer diseases (Zang *et al*., 2021). Ginger is a perennial herb and commercially grown in many tropical regions and is a native to tropical south east of Asia (Serasan and Sileshi, 2011). This plant is an unfertile species that failed to set seed. Ginger is a herbaceous perennial grown as an annual crop. These are plants of tropical and subtropical regions distributed mainly in East Asia. Several authors have quoted different figures for the total number of genera and species but it is probably appropriate to quote the world record at least51 genera and 1500 species (Newman, 2001). Ginger has many fibrous roots, pseudo stem with leaves, and an underground stem rhizome. It is one of the top 20selling herbal supplements in the United States (Gang and Ma, 2008). The rhizome is the tissue used for a lot of purposes, such as vegetative propagation and the storage of food materials (Ravindran and Nirmal Babu, 2005). Ginger is vegetatively propagated through underground rhizomes and mainly cultivated in many countries including India, China, Japan, Indonesia, Australia, Nigeria and West Indies (Ravindran and Nirmal, 2005). Propagation of zingier using traditional methods are facing some problems such as appearance of plant disease, luck of rhizomes, limited to a specific season of the year and conservation of zingier available all the year. However, collection may occur at any time independent of flowering period for each species (this assumes that seed material is not required) (Nada opal *et al*., 2011) while, there is the potential of virus elimination from contaminated tissue through meristem culture (Behera *et al*., 2010). Thereby In vitro propagation has long been recognized as an efficient means for rapid clonal multiplication and conservation. In fact in vitro culture became as the best method as a continuous source of supply of disease free planting material for commercial utilization. (Kambaska and Santilata, 2009). Du to its anti-inflammatory properties, ginger is used as a medicinal plant to treat a wide area of illnesses and disorders, such as arthritis, in flammatory bowel disease, cancer, Alzheimer's disease and the common cold. Moreover, this plant success fully used as anti-allergic, antiemetic, anti-hepatotoxic, anti-flammatory, anti-nauseate, antiseptic, antitussive, cardio vascular, digestive, and hypoglycemic activities (Duke et al., 2003). The plant cannot be sexually propagated duo to that the rhizomes are used for its vegetative propagation (Nair, 2019). Moreover, the rhizome of this plant considers as an economical part and using large quantities of rhizomes as starting material for propagation negatively affects its supply in the globule market (Nisaretal, 2021). Ginger is reproductive and is only propagated vegetative (Ravindran and Nirmal Babu, 2005). On the other hand, ginger plant affected by several diseases which may act the propagation of this plant and then decrease the number of plant every year, rhizome rot caused by Pythium spp. And Ralstonia solanacearu mare major diseases affecting ginger.

The utility, the various method of propagation includes efficient cost, effective method of in vitro multiplication is essential for improvement of ginger. However, plant tissue culture technology exists as one of the most important methods being used to obtain moor plants and avoid the problems which may face with vegetative propagation. Although several studies have proved the successfully use of tissue culture techniques propagation method of ginger (Serasan and Sileshi, 2011), it is very important to develop an effective protocol because the efficiency of tissue culture is dependent on several factors, such as plant growth regulators, physiological state of the explants, etc. The plant cannot be sexually propagated duo to that its rhizomes are used for its vegetative propagation (Nair, 2019). Moreover, the rhizome of this plant considers as an economical part and using large quantities of rhizomes as starting material for propagation negatively affects its supply in the globule market (Nisar *et al*., 2021). Ginger is reproductive and is only propagated vegetative (Ravindran and Nirmal Babu, 2005). On the other hand, ginger plant affected by several diseases which may act the propagation of this plant and then decrease the number of planted plants every year, rhizome rot caused by Pythium spp. And Ralstonia solanace arum are major diseases affecting ginger. The utility, the various method of propagation includes efficient cost, effective method of in vitro multiplication is essential for improvement of ginger. However, plant tissue culture technology exists as one of the most important methods being used to obtain moor plants and avoid the problems which may face with vegetative propagation. Although several studies have proved the successfully use of tissue culture techniques propagation method of ginger (Serasan and Sileshi, 2011), it is very important to develop an effective protocol because the efficiency of tissue culture is dependent on several factors, such as plant growth regulators, physiological state of the explants, etc.

The present study was carried out to develop an effective protocol for in vitro propagation and plant regeneration of ginger. Thereby plant tissue culture technology came as alternative method for propagation of some medicinal plants.

2 Materials and Methods

This study was conducted in the laboratory of plant tissue culture entered the Libyan Center for biotechnology research located in Tripoli Libya.

Explants preparation

Fresh ginger rhizomes were brought from the local market. Directly ginger rhizomes were placed in a dark Place away from any light source in order to induce the plant to produce buds. After 30 to 40 days, the grown buds were taken for next stage.

Media preparation

The culture media is mainly used to provide the necessary plant needs of the macro and micronutrients beside source of carbon, vitamins and growth regulators needed for the study, whereas MS media has been selected and used to obtain an efficiency protocol forging Micropropagation.

Establishment of contamination-free tissue culture

This stage conducted for the purpose of obtaining clean MS media free of contaminations for the development of single nodes of ginger plant, whereas this stage started with preparation of Half basal of Murashige and Skoog 1962 (MS) salts nutrient medium with vitamins and supplemented with30g L-1 sucrose was used for in vitro seedlings germination. All cultures pH was adjusted to 5.8 with 1N KOH or 1N HCl, then with 7g L-1 agar prior to autoclaving at 121ºC and 1.2kg cm-2 for 15 minutes. Directly jars contained culture media transferred and put in an autoclave at a temperature of 121°C and an atmospheric pressure of 1.02 bar for 15 minutes for the sterilization stage.

Table (1) MS media preparation

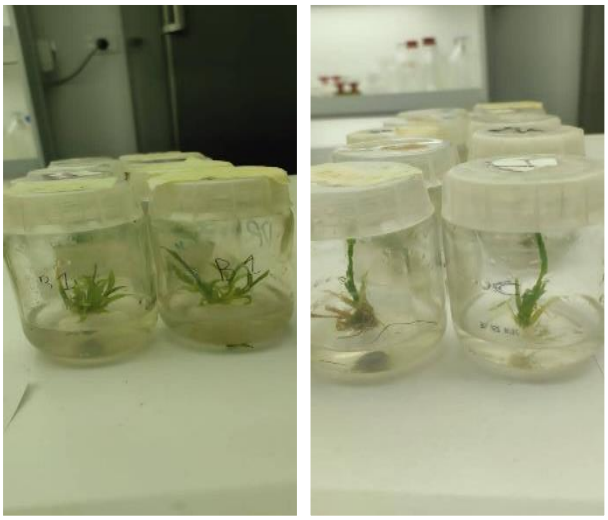
Macro elements		**Micro elements**	
Salts	mg/l	Salts	mg/l
$NH_4. NO_3$	1650	H_3BO_3	6.2
KNO_3	1900	$MnSO_4. H_2O$	16.9
$CaCl_2.2H_2O$	440	$Zn\ SO_4.7H_2O$	8.6
$Mg\ SO_4.7H_2O$	370	KI	0.83
$KH_2\ PO_4$	170	$NaMOO_4.2H_2O$	0.25
Vitamins		$CUSO_4\ .5H_2O$	0.025
Myo-inositol	100	$CO\ CL_2.6H_2O$	0.025
Nicotinic acid	0.5	NaEDTA. $2H_2O$	37.3
Thiamine. HCl	0.1	$FeSO_4. 7H_2O$	27.8
Pyridoxine. HCL	0.5		
Agar	7000		
Sucrose	30000		

Surface Sterilization of Explants

After growing buds reaching length of 1-2 cm the grown buds were collected from giber rhizomes and prepared for surface sterilization stage. The collected buds were cut into parts of suitable lengths then placed under running water for 30minutes to remove surface contaminants from soil and insect residues ,afterwards samples were transferred to the laminar airflow cabinet for sterilization by using ethanol with 70% concentration for two minutes then ginger buds sterilized by sodium hypochlorite (Clorox) with concentrations 2.5% and for 20 minutes with keeping stirring of the samples time to time for sterilization from bacteria and fungus. finally, samples were washed by using sterilized distill water for three times with five minutes each time to remove the toxic effect of sodium hypochlorite solution (Aazami et al.,2010).

Culture stage

This stage started by planted the selected and sterilized buds in containers supplemented with MS media, whereas 2-3 buds placed in each container for the purpose of obtaining tissue cultures free of contamination, afterwards samples were transferred to growth room and incubated at 16 hour light /day and 8 hour dark/day the light intensity was (1-s1Mm 30 µ) by using fluorescent lamps , temperature 25±2C◦ and 40% humidity, while all the conditions were under control and the samples incubated for four to six weeks to be ready for use in next stage of the study



Figure(1): regenerated shoot lets of ginger plant after 45 days of culture.

Subculture Stage

After the whole plants being obtained through tissue culture technique the subculture stage started by transfer the obtained plants to cabinet room for subculture operations for the purpose of evaluate the effect of plant growth regulators on ginger propagated plants which already obtained from the previous stage figure (2). The plants were re-planted in MS media supplemented with different concentrations of plant regulators as follows:
First treatment (T1) control treatment MS media without added hormones
Second treatment (T2) MS media + 1mg/l BA + 0.1mg/l NAA
Third treatment (T3) MS media + 2mg/l BA + 0.1mg/l NAA
Fourth treatment (T4) MS media + 1mg/l NAA

While two three plants were planted in each jar then all plant samples incubated in the growth room under the seam conditions which written above then after 45 days the results were taken and recorded.

Figure (2): plants incubated in growth room.

3 Results

This study conducted to determine the best protocol which can be successfully used for In vitro propagation of ginger plant. Thereby carefully, assessments must be done to obtain the best method. However, several studies were conducted in order to obtain satiable method for Micropropagation of ginger plant and medicinal plants in general. In fact, the efficiency of used method in Micropropagation depend on the ability of used culture media which usually contain a combination between oxen and cytokines to improve and enhance the plant growth as hole through parameters such as plant height, number of leaves and roots which

has been evaluated in this study. However, the result of this study were as follow.

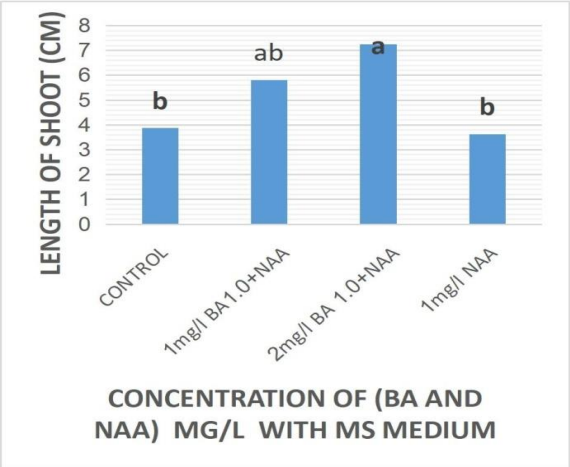


Figure (3): effect of plant growth regulators on plant length of ginger.

Plant Length Parameter

The results of this study figure (2) showed that the treatments T2 and T3 which contain a combination between NAA and BA , whereas (T2) contains MS media + 1mg/l BA + 0.1mg/l NAA and (T3) contains MS media + 2mg/l BA + 0.1mg/l NAA gave the best results for plant length this obtained results proved that use of MS media with a combination between NAA and BA led to obtain plants with satiable growth but the concentration of added NAA and BA must be selected carefully and tested, whereas in this study 0.1 NAA, 1 and 2 mg/l were added this results proved the positive role of NAA and BA growth regulators on plant growth improvement. Furthermore, plant length obtained from treatment of T2 and T3 was significantly higher than the other treatments which are.

T4 and control treatment, whereas the plants were grown in T3 reached a length of 7.2cm and plants grown in T2 reached plant length of 5.9 cm but no significant differences were found among those treatments.

This obtained results proved the positive effect of NAA and BA when added with satiable concentrations also addition of NAA alone to MS media was not able to improve plant growth and the same was found in control treatment. Our results were in the same line with Kheiry et al. (2018) who found that addition of BA and NAA improves ginger growth under use of plant tissue culture technology.

Number of Leaves

For number of leaves parameter figure (4) almost the same results as in plant length have been found. The results of this study showed that the treatments of T2 and T3 gave the highest number of leaves, which indicate the positive effect of NAA and BA hormones. This results proved that addition of NAA and BA to MS media increases the number of leaves formed by the cultured plant this also explains

that BA growth regulator induce the plant to produce more leaves which led to improvement in plant growth in general. The results of this study showed also that number of leaves produced in T2 and T3 was significantly than number of leaves produced in T4 and T1 (control treatment). While T2 and T3 reached an average of 28 and 31 leaves respectively, whereas no significantly differences were found between T2 and T3.

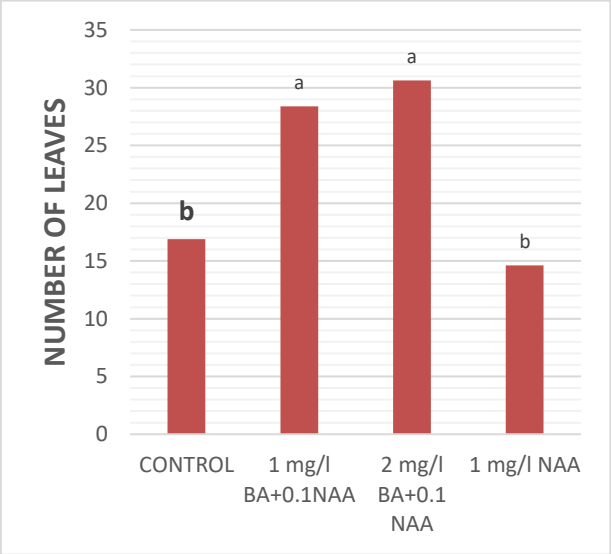


Figure (4): effect of plant growth regulators on number of leaves

The results showed also that T1 and T4 were not efficient compared with T2 and T3 which explain the positive role of plant growth regulators BA. Furthermore, according to our obtained results we found that the addition of 2mg/l BA increased the number of leaves and plant length which proves that this concentration is extremely satiable for ginger Micropropagation this has been proved by several studies. On other hand NAA when added to MS media without addition of BA as in T4 was not efficient and the number of leaves was low.

Number of Brunch's Parameter

Plant tissue culture technology has a major impact on both agriculture and industry sector by providing a large number of plants in short period of time to demand also it has contributed greatly to the advancement of agriculture science in recent times and it is an indispensable tool in modern agriculture nowadays (Garica *et al*., 2010). However, number of brunches increase the number of obtained plant through plant tissue culture and several hormones are used to increase number of brunches production. Our results figure (5) proved that addition of BA increases the number of brunches. The results showed that treatment of T2 (MS+0.5 mg/l NAA and1mg/l BA) and T3 (MS+ 0.5mg/l NAA and1mg/2 BA) gave the highest number of brunches. The results showed that the treatment 0.5 NAA+ 2mg BA were significantly higher than the rest of the treatments, especially the control treatment (MS), which demonstrated the positive effect of growth regulators on the growth of the ginger plant, and demonstrates the extent of the success of ginger plant propagation using cultivation technology.

Plant tissue and obtaining appropriate lengths for the cultivated plants at a specific time in preparation for transferring them to the replanting stage and the acclimatization stage which, succeeded in growing a plant that grows using the technique of vegetative cultivation (Villanor, c. c, 2010). The average of number of brunches in those treatments was significantly higher than other two treatments, which areT1and T4. While T2 and T3 produced, number of brunches reached to7and 6.2 brunches respectively and no significantly, differences were found betweenT2 and T3while, the lowest number of brunches were obtained inT1and T4. These results proved that T1and T4 were not satiable for ginger Micropropagation. However, no significant differences were found among T1and T4. The present results were in complete agreement with those indicated in studies of Khiry *et al*., (2020) that use of 2mg/l BA in combination with NAA improved the vegetative growth of ginger plant. The researcher reported also that present a satiable response of zingier plant to Micropropagation by tissue culture technology in all the treatments even control treatment moreover the results showed that the treatment of 2mg/l BA gave the highest average of obtained number of brunches and root system growth. necessitates This results proved that application of tissue culture techniques as a solution to these problems (Nayak and Naik, 2006).

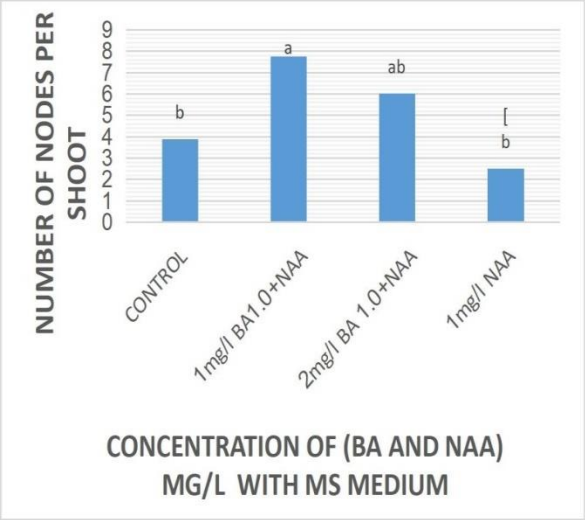


Figure (5) effect of plant growth regulators on number of brunches.

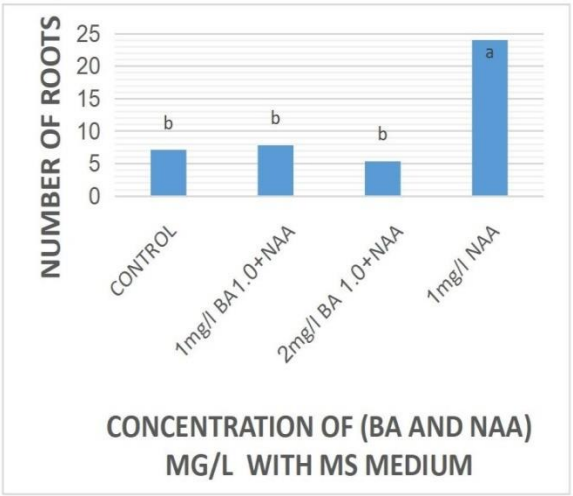


Figure (6) Effect of plant growth regulators on number of roots

4 Discussion

Number of Roots Parameter

It is well known that NAA induce the plants to produce roots and several studies proved that use of NAA improves plant root formation. Particularly NAA largely used for roots formation.

The results of our study figure (6) showed that T4 treatment which contains 1mg/l NAA without addition of BA gave the highest number of roots. Furthermore, the

number of roots produced through T4 was significantly higher than the other three treatments which are T1, T2, T3. Whereas the average of number of roots in this treatment arrived to 23 roots. Although T4 treatment produced the highest number of roots but plant growth in this treatment was lower than T2 and T3. However, the number of brunches and leaves was lower than T2and T3 because of the absence of BA. This obtained results proved that use of NAA + BA gave plant growth batter than NAA alone. Addition of NAA increases the number of roots but did not improve the plant growth in general. Our results showed also that treatments T1 ,T2and T4 gave the lowest number of roots and no significant differences were found among those treatments.

Length of Roots

For roots length parameter the results of our study figure (7) showed that all the used treatments were in the same level and no significant differences were among all treatments. These results explained that all the treatments were in the same performance in case of roots length.

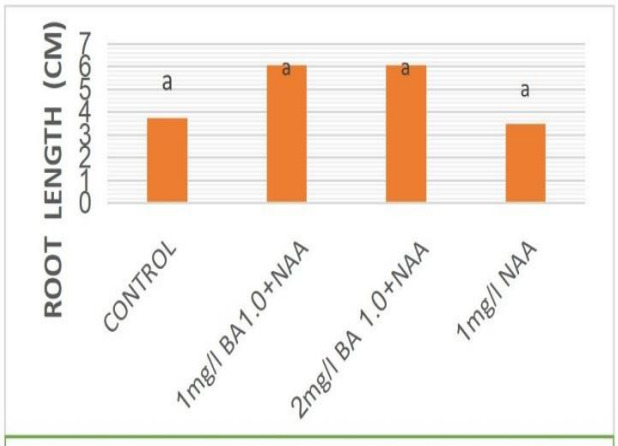


Figure (7) Effect of plant growth regulators on roots leng

5 Conclusion

Ginger plant showed a medical importance. The plant used in human disease recovery. Thereby more quantities are needed. Propagation of ginger plant faces problems especially with plan diseases that is why in vitro Micropropagation extremely needed. Our study proved that use of plant tissue culture technology is clearly successful. The results of this study showed that addition of BA growth regulator improves the plant growth in general and the treatments supplemented with BA gave the best results.

Conflict of Interest: The authors declare that there are no conflicts of interest.

References

Behera K. K., Pani and S. Sahoo, "Effect of plant growth regulator on in vitro multiplication of turmeric (Curcuma longa L cv. Ranga)," International Journal of Biological Technology, Vol. 1, No. 1, 2010, pp. 16-23.

Dadi T L, Dejene T B and Hatabtamu G M. (2020). Micro propagation of medicinal plants. international journal of plant breeding and crop science. vol 7(2) pp. 796-802. Issn :2167.

Devina D, Teoh Y J and Jualang A G. (2016). I n vitro propagation of Zingiber officinal. Transactions science and technology. (1 – 2) ,162-167.

Garcia-Gonzales R, Quiroz K, Carrasco B, Caligari P (2010) Plant tissue culture: Current status, opportunities and challenges. Cien. Inv. Agr. 37(3): 5-30.

Kambaska, K. B. and Santilata, S. (2009). Effect of Plant Growth Regulator on Micropropagation of Ginger (Zingiber officinale Rosc.). Journal of Agricultural Technology 5(2):271-280.

Kheiry K, Ahmed S, Elmundr A, Mhammed A, Salem H and Areje S. (2020). Micropropagation of Zingiber officinal Roscoe. journal of Misurata university for agriculture science 1:1 38-50. ISSN 2708: 8588.

Kumar S. and Singh, N. (2009). Micropropagation of Prosopis cineraria. Druce – amulti purposed esert tree. Researcher,1(3):9-13.

Kumari R. and Priya (2020). Optimization of Concentrations of Plant Growth Regulators for In Vitro Multiple Shoot Formation and Efficient Root Induction in PhylanodifloraL. (Lippia nodiflora L.) An Important Medicinal Plant. Indian J. Sci. Res.10(2):39-44. medicinal plants. Biotechnology Adv 18:91-

Murashige T, Skoog F (1962) A revised medium for the rapid growth and Murashinge T and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures plant1:473 –497.

Nayak, S. and Naik, P.K. (2006). Factors Effecting In Vitro Micro Rhizome Formation and Growth In Curcuma longaL. and Improved Field Performance of Micro Propagated Plants, ScienceAsia.32:31-37.

Newman, M. (2001). Nomenclatural notes on Zingiberaceae. Rapid clonal propagation of ginger (Zingiber officinales Rosc.). Japan J.Breed.38: 437-442.

Ravindran, P. N. & Nirmal Babu, K. (2005). Ginger the Genus Zingiber. United States of America: CRC Press. Tissue culture and biotechnology of Ginger. Photochemistry 25:1333

Rout, G.R., S.K. Palai, S. Samantaray and P. Das. (2001). Effect of growth regulator and culture conditions on shoot multiplication and rhizome formation in ginger

(Zingiber officinale Rosc.). In vitro Cell. Dev. Biol.-Plant, 37: 814-819

Sarasan V., Kite, G. C., Sileshi, Gudeta. W. S. Philip C.S. (2011). Applications of photochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticide plants and generating income for the rural poor. Plant Cell Rep 30, 1163–1172.https://doi.org/10.1007/s00299-011-1047-5.

Sharma K and Malik B. (2014). In vitro conservation of Zingier officinal through rhizome. journal of pharmacy and biological science. ISSN:2319-7676.Vol 9: pp39-40.

Sidhu, Y. (2011) 'In vitro Micropropagation of medicinal plants by tissue culture', The Plymouth Student Scientist, 4(1), p. 432-449.

Villamor, C.C., Influence of media strength and sources of nitrogen on Micropropagation of ginger, *Zingiber officinale* Rosc. E-Int. Sci. Res. J., 2: 150-155(2010).

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

Scientific Journal Impact Factor

TOGETHER WE REACH THE GOAL

jsfsu@su.edu.ly

journal.su.edu.ly/index.php/JSFSU