OPEN ACCESS Science International

ISSN 2305-1884
DOI: 10.17311/sciintl.2018.1.10

Review Article
Factor Controlling Micropropagation of Fruit Trees: A Review

S.A.M. Hassan and Nagwa S. Zayed

Biotechnology Lab., Department of Pomology, National Research Centre, Postal Code 12622, Dokki, Giza, Egypt

Abstract

There were several factors controlling micropropagation of fruit trees such as explant type and size, surface sterilization , phenol exudation and its control, different culture medium, different media (strength, type and state), carbon source and additives, light and temperature, plant growth regulators, pH and agriculture media. The data indicated that smaller explants 0.5 mm such as shoot tip culture lead to establishment stage of *in vitro* culture which will be free from viruses free of the most plant species. The best surface sterilization was obtained when the explants were sterilized by using (10-30%) clorox solution with two drops of tween 20 for 15-20 min. According to explant type (shoot tip, axillary bud, one node cutting, leaf disc, flower buds and seeds). The incorporations of antioxidant agent (150 mg L^{-1} citric acid+100 mg L^{-1} ascorbic acid) for 2-24 h prior culturing in the medium is the most treatments for controlling phenol exudation. The pH of the media was adjusted to 5.7 gave better response in growth and proliferation of explant and induction of roots in many explant species. The cultured explants were incubated under 16 h of artificial light and 8 h of darkness at average temperature of 25-28°C in several explant species. Sucrose is the most common carbon source and energy because sugar is reported as a source of energy and carbon in inducing growth and development during all different stages of micropropagation. Culture at full medium strength supported better growth and proliferation in most explant species. Also, liquid medium is the best medium state during *in vitro* establishment stage when the phenolic products released from culture explants such as (Date palm, Mango, apple and some explant types) and rooting stage. The plant growth regulators such as (auxins, cytokinins and gibberellins) are required in very minute quantities. Both BAP and 2ip have been preferred for used *in vitro* proliferation media for most fruit micropropagation. In addition, NAA and IAB are the least stable *in vitro* rooting media. Also, gibberellins are used less frequently compared to auxin and cytokinins. Using combination of agriculture media consists of vermiculite: sand: peatmoss (1:1:1) is the best suitable media during *in vivo* acclimatization stage in the most plantlets.

Key words: Micropropagation, fruit tree, explant type, medium, plant growth regulator

Citation: S.A.M. Hassan and Nagwa S. Zayed, 2018. Factor controlling micropropagation of fruit trees: A review. Sci. Int., 6: 1-10.

Corresponding Author: S.A.M. Hassan, Department of Pomology, National Research Center, Dokki, Giza, Egypt Tel: 00201141242384

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Micropropagation is one from tissue culture which allows the production of large number of plants from small pieces of the mother plant in relatively short period of time and limited space. Micropropagation as recent technique is mainly used for bulk rapid propagation of several commercial plant species included date palm[1]. Micropropagation technology offers mass propagation and clean planting material. Micropropagation has the primary advantage of producing a disease which can be solved by propagation of banana[2]. Tissue culture micropropagation has been employed to aid in the clonal propagation of numerous plant species. Tissue culture technique may offer a possible method to produce a large number of genetically uniform palms identical to other plants and normal fruit after 4 years from planting and production of date palm plants free from diseased, almost 100% survival rate compared with vegetative of shoots due to the presence of a strong root system on them[1]. Surface sterilization is the most important step in preparation of explants for *in vitro* because controlling bacteria and fungi contamination of most fruit plants from field sources is very different[3]. The successful applications on micropropagation of date palm during *in vitro* acclimatized depending on the appearance of number of leaves before transplanting in the greenhouse[4]. Thus, the ultimate goal of this review was to find out the factors controlling micropropagation of fruit trees.

Mother plant (explants type, genotype and explants age): The highest shoot number and optimal shoot regeneration were obtained when cultured on MS medium supplemented with 6.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA on leaf sections of pyrus communism[5]. A protocol for in micro propagation of mature plants of citrus limon using nodal shoot segments[6]. The problem of phenolic exudation and contamination of guava c.v. "Banaras" was not observed when somatic embryo derived young and aseptic plantlets were used as explant source[7].

Using shoot tips of three fig cultivars (Aboudi, Gizy and Sultany) plantlets from the establishment stage were cultured individually on Murashige and Skoog (MS) medium supplemented with 0.5 mg L^{-1} 6-Benzylaminopurine were increasing explants development and reducing necrosis and browning[8]. In the same time, Emam[9] found that shoot tips of pear rootstock was successfully developed when cultured on MS medium as compared with one-nodal cutting. Strosse *et al.*[10] concluded that shoot tips from young suckers in banana was successfully developed when cultured on MS medium during the establishment stage. Also, Sutherland *et al.*[11] found that shoot tips explants in banana were superior increasing proliferation of homogenous and diseases free plantlets. Whereas, Baiea[12] found that leaf discs explants of two peach rootstocks were successfully developed when cultured on MS medium (Fig. 1).

The best number of shoots (5.35 shoots/responding explant) of *Jatropha curcas* explants occurred from axillary buds[13]. Similarity shoot tip, stem and axillary bud explants were successfully used for micropropagation of *Jatropha Curcas*[14]. In the same, Sumalatha[15] Found that shoot tip as the explant was successfully used for micropropagation of banana plants. The best shoot proliferation of Jojoba distinguished clone from shoot tip and nodal segments[16].

The nodal segments as the explants were successfully used for callus induction as compared to leaf and root segments of citrus plants[17]. In the same time, somatic embryo derived young and aseptic plantlets were used as explants source were successfully used for micropropagation of guava c.v."Banarasi"[7].

The same time, the longest roots were obtained when the c.v. Golden delicious control and by genotype 4566 were cultured on WPM medium as compared with the genotypes 4608, Gi47 7/4 and C/907[18].

Surface sterilization of explants: Banana plants were surface disinfected by 0.1% $HgCl_2$ solution containing tween 20 for 5 min and washed thoroughly with sterile, deionized water[15].

Meanwhile, sterilize shoot tip and nodal bud using sodium hypchlorite Clorox (NaOCl) for 20 min which gave good explants survival in pomegranate[19]. Using a combination of NaOCl and Na methiolate for 20 min are the two most successfully sterilize axially bud and segments in pomegranate which gave good explant survival (65%)[20]. In the same time that using NaOCl (0.75%) gave the maximum survival percentage (46.66%) followed by NaOCl (1.0%) with 26.66% survival percentage as compared with NaOCl (1.25%) with the minimum survival (6.6%) of Jack fruit[21] .

In another study during trials on *in vitro* propagation of mangoes, using combination of 10% sodium hypochlorite "Clorox" (sodium hypochlorite 5.25%) NaOCl and 0.05% mercuric chloride ($HgCl_2$) for dipping durations 7 and 10 min give the highest survival percentages and lower contamination percentage shown in Fig. 2. Also, using 10% sodium hypochlorite was successful in surface sterilization and gave good explant in very low visual contamination of rootstocks Mariana (*Prunus mariana*)[22].

In addition, surface sterilization gave good explanation for grape tissue (*Vitis vinifera*) successfully by using combination of 10% sodium hypochlorite and surfactant drops for 10 min[23]. Three doses, 0.05, 0.1 and 0.2% of aqueous sodium hypochlorite or mercuric chloride solution separately for

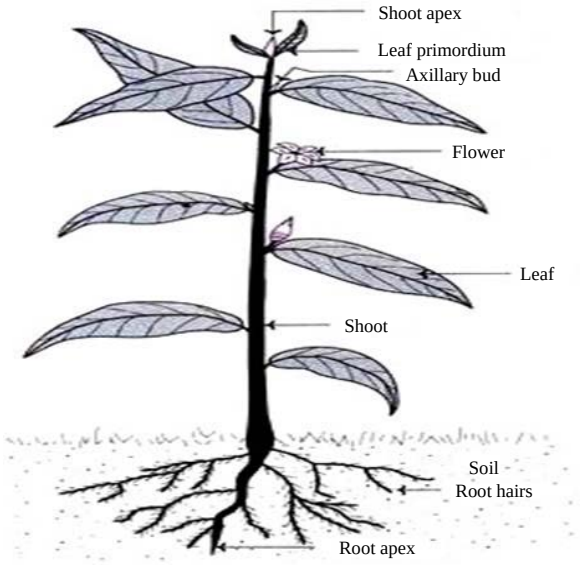

Fig. 1: Different types of explant

Fig. 2: Different surface sterilization of Jojoba

10 min were used as surface sterilize. It was found that using 0.1-0.2% mercuric chloride increased aseptic culture establishment but inhibited by break due to toxicity to explant of *Bambusa tulda*[24]. In the same time dipping explants in 15% of sodium hypochlorite for 15 min was effective in sterilizing grape root stocks[25].

Phenol exudation and its control: It was mentioned that in Fig. 3a, b, using activated charcoal in the cultural medium induced initiation proliferation of woody plant but the negative effect of activated charcoal by adsorbing growth regulators and lowering pH of the medium[26]. In the same time dark treatment for few days of initiated shoots reduces the release of phenolic compounds from the shoots of apple

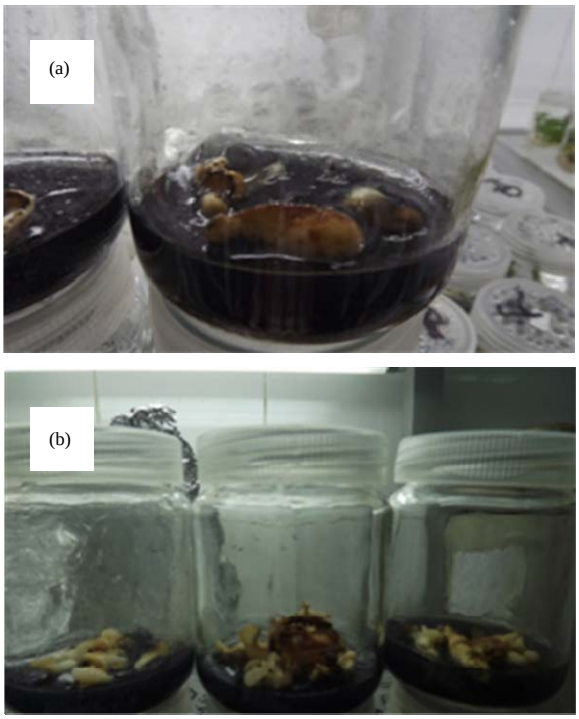

Fig. 3(a-b): Effect of anti oxidant on phenol oxidation of date palm, (a) Control and (b) Antioxidant solution 150 mg L^{-1} Citric + 100 g L^{-1} ascorbic

root stocks[27]. Similarly attempted *in vitro* culture of pomegranate c.v. Mridula, nodal segments with sterile wax decreased the phenol exudation and lead to good explant percentage of cultural establishment[28]. Using ascorbic acid was the most successfully method to reduce oxidation during the establishment stage of apple[18].

Different methods such as keeping explants in dark after culture, adding antioxidants such as citric acid (100 mg L^{-1}) and ascorbic acid (150 mg L^{-1}) for 30 min to the medium as well as adding activated charcoal (3 g L^{-1}) to the medium. These treatments reduce phenolic compound production and browning of olive explants[29].

Medium (type, strength and state): It was reported that full strength of Murashige and Skoog medium is more suitable for establishment of coffee plantlets[30]. Number of leaves and shoot length of fig plants was induced on double strength Murashige and Skoog medium showing in Fig. 4(a-d). Meanwhile, the highest value of shoot number was obtained on half MS strength medium[31]. Also, Mukherjee *et al.*[32] mentioned that shoot proliferation from nodal explants of grape root stock was induced on half strength Murashige and Skoog medium.

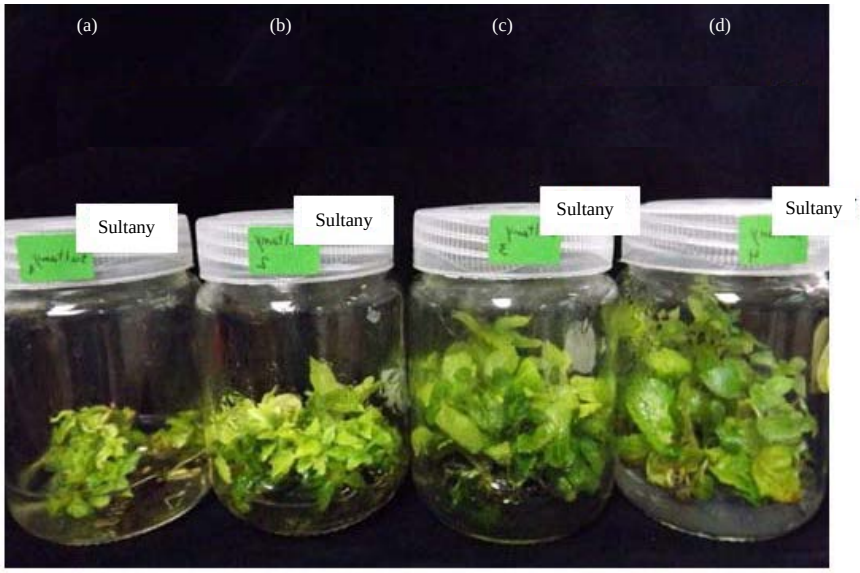

Fig. 4: Effect of medium strength of sultany fig cultivar, (a) Full strength, (b) Double strength, (C) One-half and (d) One-quarter

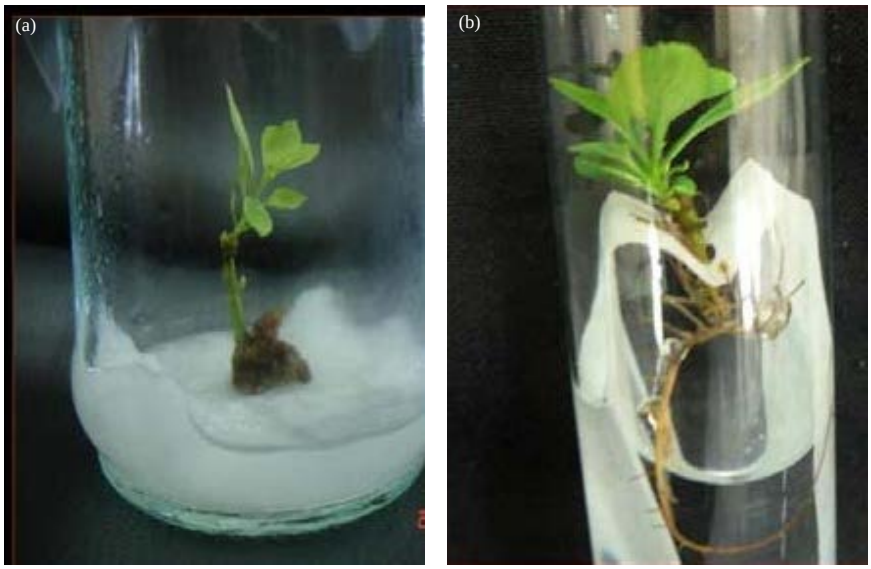

Fig. 5: Effect of liquid media on apple rootstock

On the highest proliferation rates and shoot growth of apple as shown in Fig. 5 were also obtained with MS medium replacing Fe-EDTA by Fe-EDDHA as iron source. In the same case, woody plant medium (WPM) is the most important to avoid contamination by fungi and bacteria as well as phenolic oxidation during the establishment stage[18].

Similarly the highest shoot proliferation on *Citrus Limon* was obtained with MS medium with lower concentrations of ammonium and potassium nitrates[6].

On the other hand, used 3/4 medium strength was the most suitable medium for development of Jojoba clones[16]. acclimatization of plantlets derived from tissue culture confirmed the efficiency and importance of this method. The subculture of rooted shoots into MS salts solution and increasing light intensity enhanced the plantlet photosynthesis and then changing it from heterotrophic to autotrophic status of date palm[33].

The highest survival percentage (70-86%) of date palm was obtained on half medium strength after 1 month pre

acclimatization stage as compared with the direct transfer to the greenhouse ranging from 12-28%, Mazri[34]. Also, one-half strength MS medium was the most suitable medium for the maximum number of roots and root length of almond plantlets, c.v. Nonpareil[35].

The highest rooting with higher number of roots (10.33 roots/shoot) per micro shoot found on half strength MS basal medium in pomegranate[19]. was obtained[36] found that the best significant improvement of greening and explants development per micro flower bud on full and one-half medium strength compared with one-quarter medium strength of pear "Le Conte" flower bud.

In respect that several factors affecting on *in vitro* root formation of date palm c.v. Boufeggous plantlets such as with solid or liquid medium just before acclimatization[37]. While, the used of liquid medium is more suitable for medium state (solid or semisolid media) before the transfer of plantlets to the greenhouse of Barhee date palm[38]. Also, use of a liquid medium before the transfer of plantlets to the greenhouse of date palm c.v. Najda was very effective in improve shoot quality[34]. The highest survival percentage of *in vitro* acclimatized date palm c.v. Barhee was recorded with plantlets growing on liquid Ms medium compared with the other medium status[39].

The highest rooting percentages on apple was observed when the plantlets were cultured in liquid organized medium supplemented with IBA at 25 µM on all genotypes including M. domestic c.v Golden delicious[18].

Supporting media: The best number of shoots and shoot length in pineapple plantlets were induced to form shoots on MS medium containing 2 g L^{-1} gelrite. Meanwhile, an agar at the rate of 7 g L^{-1} was better than gelrite during multiplication stage[40]. Similar , increased response with the decrease in agar from 0.8-0.4% in the medium improve the *in vitro* root and shoot parameters as compared with the other supporting structures banana (musa spp) c.v. Grand naine plantlets[41]. Also, added to the media with gelling specialist agar at 5-8 g L^{-1} in the medium were successfully used for microprogation of banana plants[15].

Carbon source and additive: It was revealed that medium supplementation with 30 g L^{-1} sucrose gave the best result of shoot tip explants of papaya[42]. Similarly, sucrose at the rate of 30 g L^{-1} concentration as carbon source was proved to be best resolute on *in vitro* rooting of banana (Musa spp) c.v Grand naine plantlets[41].

In the same time using of MS medium supplemented with 20 g L^{-1} fructose induced shoot proliferation of the two fig cultivars (Sultany and Aboudi) as compared with the explants an media without sucrose shown in Fig. 6(a-d)[31].

In the respect, addition of both fructose and glucose to Murashige and Skoog medium resulted in improving shoot length and leaf number of jojoba plants. While, sucrose proved to be the best carbon source for shoot number[16]. On the same time, supplemented the culture medium with 30-60 g L^{-1} sucrose encouraged maximum explant development and shoot growth of date palm while fructose produced the highest values of dry weights as compared with other carbon sources treatments such as glucose, sucrose and maltose[43].

The same addition also, one-half medium strength treatment enhanced the proliferation percentage and shoot number compared with the other treatments[36].

Whereas, addition of either adenine sulphate (80 mg L^{-1}) or yeast extract (300 mg L^{-1}) was effective in highest callus production and reduced necrosis and browning of both Nemagaurd and Okinawa root stock[12]. The same, olive medium (OM) supplementation with 50 ml L^{-1} coconut water gave the best result of the highest proliferation rates with an average of 3.4 new explants on each 30 days in olive[44]. While used sucrose concentrations at the rates of (30-40 g L^{-1}) as a carbon sources gave the best results for microprogagation of banana pants[15].

Light, temperature conditions and pH requirements: *In vitro* plantlets of date palm c.v. Barhi are generally grown under 2000 lux, through using different numbers of white cool fluorescent lamps for 16 h of light and 8 h of dark, improved most parameters under study such as root length, root number, shoot length and greening as compared with the other light intensities. Meanwhile, increasing shoot thickness was recorded when the cultures were subjected to light intensity of 3000 lux[4]. *In vitro* plantlets of date palm are generally grown under high light intensity enhanced the plantlet photosynthesis[33].

Similarly, tasted *in vitro* plantlets under high light intensity (4000-12000 lux) and temperature (26-36°C) might cause charring of leaves and wilting of plantlets[45]. Also, *in vitro* root formation of date palm c.v. Barhi, the best results for shoot thickness was recorded when the cultures were subjected to a light intensity of 3000 lux . However, number of root, root length, number of leaves and greening were recorded when the cultures were subjected to light intensity of 2000 lux[39].

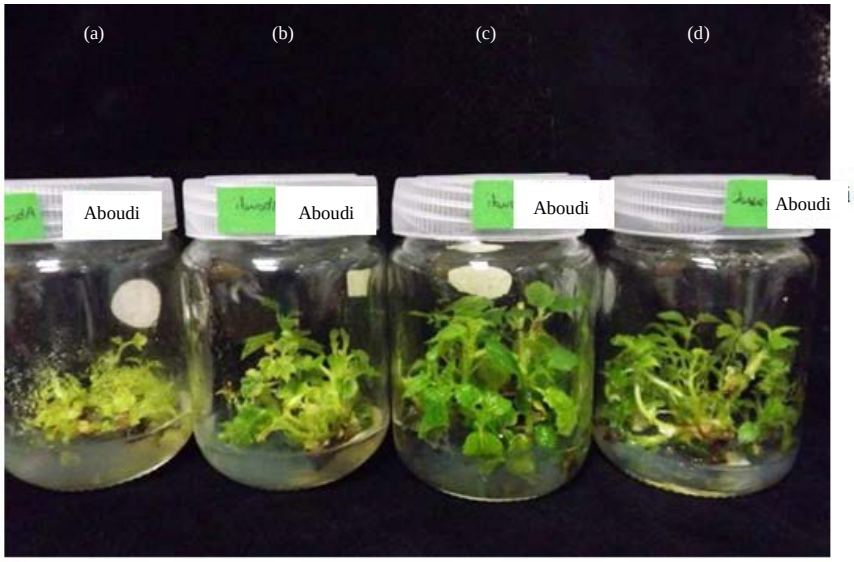

Fig. 6: Effect of carbon source of Aboudy Fig cultivar, (a) Glucose, (b) Fructose, (c) Sorbitol and (d) Sucrose

The same result found that several factors affecting the *in vitro* root formation of most fruit trees, the best results for both root number and root length were recorded when the culture were subjected to light intensity of (4.000-12000 lux) and temperature (26-36°C) might cause charring of leaves and increasing of plantlets[45]. While, during acclimatization plantlets of date palm, increasing light intensity increased the plantlets photosynthesis[33].

On the other hand *in vitro* plantlets of banana were generally grown under temperature 28°C and presented of light to 12-16 h[15]. Also, cold pretreatment of explant for 3 days at 5°C encouraged the best responses of peach root stocks[12].

The best root and minimum time for root initiation with longest length at PH 5.5 during the preparation of the medium on *in vitro* rooting of Banana plantlets c.v. Grand naine were obtained by Ahmed *et al.*[41].

Plant growth regulator: The supplementation of the culture medium with BA (0.5 mg L^{-1}) and IBA (0.25 mg L^{-1}) induced the highest number of shoots after 30 days of culture on *Jatropha Curcas*[14]. The supplementation of the subculture medium with 0.5 mg L^{-1} BA and 0.5 mg L^{-1} IBA were necessary for differentiation of the shoot bud as shown in Fig. 7a-c. While only 1.0 mg L^{-1} IBA induced the shoot elongation of *Jatropha Curcas*[46]. similarly, the supplementation of the culture medium with 6.00 mg L^{-1} IBA induced the best multiplication of coffee plantlets, in the some cases, number of roots and root length/plantlets were greatest with 3.0 g L^{-1} IBA[30].

In another study the supplementation of the culture medium with 3 mg L^{-1} BA and 0.5 mg L^{-1} NAA induced the regeneration response (71.89%) from model segment in citrus plants. In some cases, maximum rooting percentage (71%) was obtained with modal segment derived callus cultured on Ms medium supplemented with 0.5 mg L^{-1} NAA and 3 mg L^{-1} BA[17]. While the supplementation of the culture medium with 2.0 mg L^{-1} BA and 0.0, 0.5 or 1.0 IBA mg L^{-1} induced the best number of regenerated plantlets of either Nemagaurd or Okinawa rootstock[12].

The same supplementation of the culture Ms medium with 1.0 mg L^{-1} BA induced the best multiplication of almond plantlets c.v. Nonpareil. In the same cases, number of roots and root length/plantlets was greatest with 8.0 mg L^{-1} indole acetic acid[35].

Similarly, the supplementation of the culture medium with 1.0 mg L^{-1} BAP combined with 1.0 mg IBA was the best for *in vitro* culture initiation of apple plantlets[27]. Also, the supplementation of the culture medium with 1.0 and 1.5 mg L^{-1} BAP combined with 1.0 mg L^{-1} IBA induced the survival percentage (96.7%) of MM 106 and (93.3%) Anna apple plantlets[47].

In addition, supplementation of Ms medium with 0.5 mg L^{-1} BAP induced the shoot proliferation (5.37 shoots/explant) of a dwarfing cherry root stock[48]. Similar results recorded that the supplementation of the Olive medium with 2.22 mg L^{-1} BAP induced the best proliferation rates with average of 3.4 new explants after 30 days. In the same cases, rooting percentage 85% was obtained by OM with 3 g L^{-1} IBA,[44]. In the same time, the highly cytokinin

Fig. 7: Effect of growth regulators, (a) BAP, (b) IBA and (c) Agriculture media

Fig. 8: Effect of agricultural media Pomegranate

induced the best shoot multiplication of jojoba. Also, callus induction was greatest with Auxins alone or combined with cytokinin[16].

Cytokinin 6 benzyl aminopyrine (BAP) has been found essential for obtaining good shoot proliferation in pomegranate. In the same cases, Murashige and Skoog (MS) medium containing 2.0 mg L^{-1} IBA has been found essential for obtaining good rooting during *in vitro* rooting[19]. Highest frequency of shoot proliferation was obtained in culture medium Quorin and lepoivre medium supplemented with BA at the rate of 0.4 mg L^{-1} and IBA 0.05 mg L^{-1} [20].

Also, the highest rooting percentages (70.37) with dual auxin i.e. IBA and NAA supplemented to the rooting medium[28] found that the highest rooting percentages (93.33%) and longer roots (3.29 cm) were obtained in culture medium added 0.5 mg L^{-1} IBA. In the same cases, high frequency shoot elongation was also obtained with MS medium supplemented with higher concentration of cherry laurel prunus laurocerasus L[49].

Moreover, high frequency shoot proliferation was obtained on the basal medium supplemented with lower concentrations of BAP of sweet cherry cultivar "Lapins". On the other hand, good shoot elongation was also obtained with a higher concentration of BAP[50]. Increased shoot proliferation of olive was also obtained with increasing BAP concentration up to 2.1 mg L^{-1} in the media. The effect of high concentrations of BAP may be prevented by supplemented GA3 to the culture media during proliferation stage[29]. The highest shoots and number of shoots (4.66) per proliferated explant were obtained on MS culture medium supplemented with BAP at the rate of 1.5 mg L^{-1}) of Jack fruit[21].

Agricultural media: Data in Fig. 8, the highest survival percentages of plantlets, plant height (cm), plant thickness (cm) and number of leaves/plant occurred when agricultural media during acclimatization consists of a mixture of sand and peat moss (1: 1) was used of coffee plantlets[30]. Also, date palm c.v. Barhee plantlets were successfully acclimatized when transferred to pots containing a mixture of (25%

vermiculite+50% and + 25% peat moss)[39]. While, rooted shoots (plantlets) were transplanted in small pots containing a mixture of peat moss and perlite (2:1) and placed in plastic tunnels or in a greenhouse. The survival percentage was 85% after 3 months when the plants were transferred to bigger pots of Maktom c.v. Date palm[33].

In addition, the highest survival percentage (95%) of olive plantlets were obtained during acclimatized when transferred to pots containing a mixture of vermiculite: Perlite 3: 1 (V/V) substrate[44]. In the same cases, the highest survival percentage (85%) after 3 months of date palm c.v. Maktom plantlets during acclimatization stage when transplanted in a small pots containing a mixture of peat moss and perlite (2: 1) and placed in plastic tunnels in a greenhouse[33].

CONCLUSION

This study indicated that smaller explants 0.5 mm such as shoot tip culture will be free from viruses free of the most plant species. The incorporations of antioxidant agent prior culturing in the medium is the most treatments for controlling phenol exudation. The pH of the media was adjusted to 5.7 gave better response in growth and proliferation of explant and induction of roots in many explant species. Sugar is reported as a source of energy and carbon in inducing growth and development during of micropropagation. Culture at full medium strength supported better growth and proliferation in most explant species. The plant growth regulators such as (auxins, cytokinins and gibberellins) are required in very minute quantities.

SIGNIFICANCE STATEMENTS

This study discovers the factor that controls the micropropagation that can be beneficial for *in vitro* propagation of fruit tree. This study will help the researcher to uncover the critical areas of success of culturing explant in vitro culture and low cost process of propagation that many researchers were not able to explore. Thus a new theory on how to overcome many factors that are effective on explant success may be arrived at.

REFERENCES

1. Abduallatif, A.A., 2008. The problems facing the use of tissue culture technique in date palm (*Phoenix dactyl lifera* L.). Sci. J. King Faisal Univ., 9: 85-92.
2. Ngomuo, M., E. Mneney and P.A. Ndakidemi, 2014. The *in vitro* propagation techniques for producing banana using shoot tip cultures. Am. J. Plant Sci., 5: 1614-1622.
3. Mihaljevic, I., K. Dugalic, V. Tomas, M. Viljevac and A. Pranjic *et al*., 2013. *In vitro* sterilization procedures for micropropagation of oblacinska sour cherry. J. Agric. Sci., 58: 117-126.
4. Hagagy, N.A.A., N.S. Zayed, Y.Y. Abdel Atty and D.A.B. Youssef, 2015. Trials on acclimatization of tissue culture derived date palm plants (*Phoenix dactylifera* L.). Ann. Agric. Sci. Moshtohor, 53: 243-248.
5. Tang, H., Y. Luo and C. Liu, 2008. Plant regeneration from *in vitro* leaves of four commercial Pyrus species. Plant Soil Environ., 54: 140-148.
6. Rathore, J.S., M.S. Rathore, M. Singh, R.P. Singh and N.S. Shekhawat, 2007. Micropropagation of mature tree of Citrus limon. Indian J. Biotechnol., 6: 239-244.
7. Rai, M.K., V.S. Jaiswal and U. Jaiswal, 2009. Shoot multiplication and plant regeneration of guava (*Psidium guajava* L.) from nodal explants of *in vitro* raised plantlets. J. Fruit Ornamental Plant Res., 17: 29-38.
8. Mustafa, N.S., S.A.M. Hassan and R.A. Taha, 2016. *In vitro* studies on growth and rooting of some fig cultivars. Res. J. Pharm. Biol. Chem. Sci., 7: 124-130.
9. Emam, H.E., 2006. Physiological studies on *in vitro* propagation of pyrus communis root stock. Ph.D. Thesis, Cairo University, Egypt.
10. Strosse, H., H. Schoofs, B. Panis, E. Andre, K. Reyniers and R. Swennen, 2006. Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). Plant Sci., 170: 104-112.
11. Sutherland, R., J.V. Escalant, K. Kunert and A. Viljoen, 2005. Applications of cell and tissue culture in banana (*Musa* spp.). Proceedings of the 5th African Mycological Association and Inaugural Symposium of the African Society for Medical Mycology, January 23-26, 2005 Hartenbos, South Africa.
12. Baiea, M.H., 2009. Physiological and cytological studies on tolerance of some stone fruits root stocks to salinity and Drought by using micro-propagation. Ph.D. Thesis, Zagazig University, Egypt.
13. Sujatha, M., H.P.S. Makkar and K. Becker, 2005. Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regul., 47: 83-90.
14. Kaewpoo, M. and S. Te-chato, 2009. Influence of explant types and plant growth regulators on multiple shoot formation from *Jatropha curcas*. Sci. Asia, 35: 353-357.
15. Sumalatha, A., 2016. Plant tissue culture of Banana in laboratory. J. Bot. Sci., 3: 54-62.
16. Fayek, M.A., E.A. Shaaban, A.A. El-Obeidy and R.A. Taha, 2007. *In vitro* propagation of three femal jojoba clones (*Simmondsis chinesis* (Link) *Schneider*) using *in vitro* culture. World J. Agric. Sci., 6: 446-450.
17. Savita, V., G. Virk and A. Nagpal, 2010. Effect of explant type and different plant growth regulators on callus induction and plantlet regeneration in *Citrus jambhiri* Lush. Environ. We Int. J. Sci. Technol., 5: 97-106.

18. Ciccotti, A.M., C. Bisognin, I. Battocletti, A. Salvadori, M. Herdemertens and W. Jarausch, 2008. Micropropagation of apple proliferation-resistant apomictic *Malus sieboldii* genotypes. Agron. Res., 6: 445-458.
19. Singh, S.K., A. Singh, N.V. Singh and D. Ramajayam, 2010. Pomegranate tissue culture and biotechnology. Fruit Vegetable Cereal Sci. Biotechnol., 4: 35-44.
20. Damiano, C., M.D.A. Padro and A. Frattarelli, 2008. Propagation and establishment *in vitro* of myrtle (*Myrtus communis* L.), pomegranate (*Punica granatum* L.) and mulberry (*Morus alba* L.). Propagation Ornamental Plants, 8: 3-8.
21. Khan, F.R., H. Ur-Rahman, N.A. Abbasi, M. Ibrahim and G. Abbas, 2010. *In vitro* shoot and root proliferation of jack fruit as affected by different concentrations of growth regulators. Sarhad J. Agric., 26: 533-538.
22. Amiri, S., S. Ashtari, H.B. Abdullah, S.A. Nazari, E. Khodadadi, E. Khodadadi and M. Sabzi, 2013. Control contamination during micropropagation process of rootstock Mariana (*Prunus mariana*). Ann. Biol. Res., 4: 149-151.
23. Sim, S.T., 2006. Virus elimination from grape selections using tissue culture. FPS Grape Program Newsletter, November 2006, pp: 30-31.
24. Mishra, Y., P.K. Patel S. Yadav, F. Shirin and S.A. Ansari, 2008. A micropropagation system for cloning of *Bambusa tulda* Roxb. Scient. Hortic., 115: 315-318.
25. Elbotaty, E.M.A., 2012. Production of developed grape rootstocks using *in vitro* mutations. Ph.D. Thesis, Cairo University, Egypt.
26. Feyissa, T., M. Welander and L. Negash, 2005. Micropropagation of Hagenia abyssinica: A multipurpose tree. Plant Cell Tissue Organ Culture, 80: 119-127.
27. Dalal, M.A., B. Das, A.K. Sharma, M.A. Mir and A.S. Sounduri, 2006. *In vitro* cloning of apple (*Malus domestica Borkh*) employing forced shoot tip cultures of M9 rootstock. Indian J. Biotechnol., 5: 543-550.
28. Singh, N.V., S.K. Singh and V.B. Patel, 2007. *In-vitro* axillary shoot proliferation and clonal propagation of 'G 137' pomegranate (*Punica granatum*). Indian J. Agric. Sci., 77: 505-508.
29. Ahmed, A.R. and R.S. Ali, 2012. *In vitro* microporpagation of olive (*Aeaeuropaea* L.) Mission by nodal segments. J. Bol. Environ., 6: 155-159.
30. Abd El Gawad, N.M., H.A. Mahdy and E.S. Boshra, 2012. *In vitro* micropropageation protocol and acclimatization of coffee trees (*Coffea arabical*). J. Plant Prod. Mansoura Univ., 3: 109-116.
31. Mustafa, N.S., R.A. Taha, S.A.M. Hassan and N.S.M. Zaid, 2013. Effect of medium strength and carbon source on *in vitro* shoot multiplication of two *Ficus carica* cultivars. J. Applied Sci. Res., 9: 3068-3074.
32. Mukherjee, P., N. Husain, S.C. Misra and V.S. Rao, 2010. *In vitro* propagation of a grape rootstock, deGrasset (*Vitis champinii Planch.*): Effects of medium compositions and plant growth regulators. Scient. Hortic., 126: 13-19.
33. Khierallah, H.S.M. and S.M. Bader, 2007. Micropropagation of date palm (*Phoenix dactylifera* L.) var. Maktoom through direct organogenesis. Acta Hortic., 736: 213-224.
34. Mazri, M.A., 2012. Effect of liquid media and *in vitro* pre-acclimatization stage on shoot elongation and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Najda. J. Ornament Hortic. Plants, 2: 225-231.
35. Isikalan, C., F.A. Akbas, S. Namli, E. Tilkat and D. Basaran, 2008. *In vitro* micropropagation of almond (*Amygdalus communis* L. cv. Nonpareil). Afr. J. Biotechnol., 7: 1875-1880.
36. Yehia, T.A., A.A. Hegazi, M.A. Saleh, D.M. Ahmed, N.S. Zaied and S.A. Hassan, 2012. Physiological studies on development flower bud *in vitro* of Le Conte pear trees. Middle-East J. Sci. Res., 12: 864-869.
37. Othmani, A., C. Bayoudh, N. Drira, M. Marrakchi and M. Trifi, 2009. Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant Cell Tissue Organ Culture, 97: 71-79.
38. Fki, L., N. Bouaziz, W. Kriaa, R. Benjemaa-Masmoudi, R. Gargouri-Bouzid, A. Rival and N. Drira, 2011. Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. J. Plant Physiol., 168: 1694-1700.
39. Youssef, D.A.B., 2015. Physiological studies on acclimatization and growth behavior of date palm plants resultant from tissue culture. M.Sc. Thesis, Zagazig University, Egypt.
40. Abd El Gawad, N.M.A., N.S. Zaied and M.A. Saleh, 2010. A comparative study on different carbon source concentrations and gelling a gent on *in vitro* proliferation of pineapple (*Ananas colossus*). Nat. Sci., 8: 127-130.
41. Ahmed, S., A. Sharma, B. Bhushan, A.K. Singh and V.K. Wali, 2014. Effect of carbohydrate source, pH and supporting media on *in vitro* rooting of banana (*Musa* spp.) cv. Grand naine plantlets. Afr. J. Agric. Res., 9: 1135-1140.
42. Kabir, A.H., M.A. Bari, A.K.M.N. Huda, M.A. Rezvy and I. Mahfuz, 2007. Effect of growth regulators and carbon sources on axillary shoot proliferation from shoot-tip explant and successful transplantation of papaya (*Carica papaya* L.). Biotechnology, 6: 268-272.
43. Al-Khateeb, A.A., 2008. Regulation of *in vitro* bud formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi by different carbon sources. Bioresour. Technol., 99: 6550-6555.
44. Peixe, A., A. Raposo, R. Lourenco, H. Cardoso and E. Macedo, 2007. Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. Scient. Hortic., 113: 1-7.

45. Chandra, S.V.B., O.V. Kumar and R. Chandra, 2010. Acclimation of tissue cultured plants from laboratory to land. Biotec Letts., 32: 1199-1205.
46. Jha, T.B., P. Mukherjee and M.M. Datta, 2007. Somatic embryogenesis in *Jatropha curcas* Linn., an important biofuel plant. Plant Biotechnol. Rep., 1: 135-140.
47. Guadi, D., 2011. Micropropagation of two apple (*Malus domestica* Borkh) varieties from shoot tip explants. M.Sc. Thesis, Addis Ababa University, Ethiopia.
48. Mahdavian, M., N. Bouzari and H. Abdollahi, 2011. Effects of media and plant growth regulators on micropropagation of a dwarfing cherry rootstock (PHL-A). Biharean Biol., 5: 86-90.
49. Sulusoglu, M. and A. Cavusoglu, 2013. Micropropagation of cherry laurel *Prunus laurocerasus* L. J. Food Agric. Environ., 11: 576-579.
50. Ruzic, D.V. and T.I. Vujovic, 2008. The effects of cytokinin types and their concentration on *in vitro* multiplication of sweet cherry cv. Lapins (*Prunus avium* L.). Hortscience, 35: 12-21.