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In vitro Shoot Micro Propagation of Medicinal Applications and Ornamental Value of *Cestrum nocturnum L.*

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Abstract

Background/Objectives: *Cestrum nocturnum L.* a night Blooming jasmine belongs to Solanaceae family widely circulated all over tropical as well as subtropical areas of the World. It is mainly popular for ornamental fragrant flowering and hedge plant but also sometimes for traditional medicinal purpose. Due to strongest smelling characteristics of the plants, it is used in many industries for making Perfumes, essential Oils, Soaps, Candles, Body Oils, etc. The existence of natural plants of economic importance are threatened due to rapid urban development, including industrialization, residential development, educational, commercial etc., reduce the land for cultivation. Hence plant tissue culture protocol may be adapted for production and utilization of economically popular plants, including *C. nocturnum* involving limited space and short period of time. **Methods:** Shoot tip explants of naturally grown *C. nocturnum* were excised sterilized and endued on 'Murashige and Skoog' (MS) medium enriched changed concentration of BA, NAA, as well as GA3 singly or in combination. Excised micro shoots were examined for root development on 0.5 MS using IBA, NAA as well as IAA separately. **Findings:** The highest amount of multiple bud were observed in low concentration of BA (01.50 milligram × l^{-1}), resulted no. of shoot 4.40 as well as 4.20/explant, no. of leaves 15.40 as well as 4.20/explant as well as size of different shoot 5.360 as well as 4.860 cm. The concentration of IBA and IAA were found to be best for root formation in micro shoots (13.20, 6.80 roots/micro shoots) as well as root size (8.39, 5.73 cm) individually. **Application:** There are many opportunity of plant tissue culture which offer marvelous chances in plant propagation, plant development as well as creation of plants with necessary agronomical features. Finally often hardening plantlets were gradually adjusted to natural condition and acclimatized with 90% success. This established protocol could help plant cell biotechnology, horticulture, medical and industrial sector of the country.

Keywords: *Cestrum nocturnum*, Micro Propagation, MS Salts Medium, Night Jasmine, Tissue Culture, Transplantable Plantlets

1. Introduction

There are more than 300 species contain genus Cestrum mostly in Asia, Europe, Africa as well as most of them are grown in warm 'subtropical' as well as 'tropical regions'. *Cestrum nocturnum* is a member of the family Solanaceae. It is a strongly scented flower that blooms at night thus alternatively known as 'lady of the night', 'Queen of the night' and 'night blooming Jasmine'[1]. Jasmines generally grow in all types of soils. However, they are better adapted to rich loamy or dry sandy and irrigated soil. In soil with more clay, the vegetative growth is vigorous but flower production is lest in amount, while in soil with gravel, the plants exhibit stunted growth. Water logging

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or excessive watering is detrimental to the plants. Now it is probable to improve techniques for virus and micro-organisms free plant regeneration, salinity forbearance, herbicide resistance, infection confrontation, frost resistance and combination of large amount protein content as well as genetically engineer plants for necessary characters. Hybridisation, clonal selection, mutation and ploidy breeding have been attempted as plant improvement methods. Plant extracts have shown larvicidal activity[2] counter to the mosquito Aedesa egypti while displaying no poisonousness to fish[3]. Plant extracts causes hematological changes in the freshwater fish when exposed to sub lethal concentration of this plant[4]. Pharmaceutical studies showed that the leaves of the plant have important pain killing as well as bactericidal activity[5,6]. It is also documented that plants have local anesthetic effect, cardiac arrhythmic effect and inhibitory effect on central nerve system[7]. Mature foliage contains a 'calcinogenic glycoside' that guides to vitamin D poisonousness as well as is responsible for elevated serum calcium level[8]. Several of glycosides for example '(25R)-spirost-5-ene-2R, 3 ,-diol pentaglycosides (nocturnoside A), (25R)-spirost-5-en-3-,-ol tetraglycoside (nocturnoside B)' as well as phenolic glucosides (cesternosides A as well as B)[9], two new glycosides of flavonol group as well as seven saponins of steroid groups together among them four are new along with eight new glycosides of steroid group have been separated from the leaves of *C. nocturnum*. The volatile oil extracted from the plant is recognized as 'mosquito-repellent' therefore *C. nocturnum* is utilized to inhibit 'malaria' in some African region[10]. Some phytochemical analysis showed the existence of significant 'bioactive compounds' in dissimilar portions of the plant: Such as 'alkaloids, flavonol glycosides, steroidal saponins, fatty acids, essential oils, phenols' as well as few others[11]. Practitioners utilize the plant externally for skin disorders, but some technical information demonstrates that it gives a wide range of 'pharmacological activity' when administered systemically or in separated organ preparations. For example, it is in the habit of treat arterial hypotension and as an analgesic, abortive, diuretic, antispasmodic, dyspeptic, antiviral, as well as smooth muscle relaxant; it also gives negative inotropic as well aschronotropicactivities[12–15]. Maximum of the species of Cestrum have set up some uses in traditional medicine. Cestrum parqui is utilized in Chilean traditional medicine as antifebrile as well as for the treatment of disease as well as inflammation[16]. Therefore, we have taken on to examine the antifungal action of the necessary oil as well as organic components extracted from the flowers of *C. nocturnum* grown in all over Bangladesh as well as the end results are reported in this regard. Though leaves of *C. nocturnum* have important pharmacological effect in burns as well as swellings, they have been utilized as traditional medicine. It is also utilized for treatment of epilepsy in addition as stupefying charm medicine in. Tissue culture has a lot of use in amount of zones of herbal science, together with simple physiology, creation of ordinary conservation breeding, recovery of transgenic plants and also in propagation[17].

2. Materials and Methods

The procedure for media preparation consists of preparation of standard solutions of highly purified chemicals and demineralized water. Many type of culture systems were supreme broadly utilized MS medium formulated by Murashige and Skoog (MS). The standard solutions are put in storage in any type of glass or plastic sampling bottles as well as put into the refrigerator till the next time requirement. Currently a day, dry powders are available in the market mainly commercial prepared for plant tissue culture medium. The culture of media is commonly purified in an autoclave at 1212C as well as 17 psi for 25 minutes. Hormones as well as additional temperature sensitive organic compounds are filter purified as well as added to the autoclaved medium.

Several experiments including the test of response of the different explants were done to propagation and studying the effect of some growth regulators at three stages of growth (initiation, multiplication and rooting), as well to determine the optimum conditions and ways of acclimatization of plant produce by tissue culture and transplant them to the soil.

2.1 Initiation Stage

Approximately 12–18 cm lengthy actively growing shoots were taken from about 1.5 years old *Cestrum nocturnum* adult plant by cutting it from the botanical garden. Shoots or Buds were defoliated as well as cleaned by washing it with water for 70 minutes in view to take out soil as well as extra superficial contamination, followed by normal pure water as well as liquid or power detergent for 25 minutes; then rinsed for four–eight minutes in distilled sterilized water. After this, these types of shorter sections were pieced into one and half centimeter long, together

with the bud of terminal (apical) end as well as particular nodes with an axillary bud. With the help of absorption in the 0.05% Mercuric Chloride ($HgCl_2$) solutions (0.05% w/v) for 8 minutes; Bud tips and nodes with auxiliary shoots were detached and sterilized. Those disinfected tissues [explants] then were soaked 4–5 times with decontaminated distilled water, as well as the finishes of explanted tissues uncovered to sterilant were cut off. The try out were carried out with ten replicates as well as the explanted tissues were placed aseptically in a 25 × 150 millimeter conical flacks or in test-tubes each contains 15 milliliter of 'Murashige and Skoog' medium enriched with of controllers for growth but not all in the same concentrations. 'Benzyladenine' with 0.0, 1.50 as well as 3.0 as well as 4.50 mg × l^{-1} was incorporated to the culture medium as well as the responses of cultured explanted tissues at the initiation stage were observed.

Ten explanted tissues were cultured in ten test-tubes (for every concentration there were an explanted tissue in every test tube). At 25.0 ± 2° C and below light conditions they were incubated of 15 light hours as well as 8.0 darkness hours. The fallouts were noted down after one month to one and half month after planting. There effects were observed on culture initiation at various concentrations of Benzyladenine and 1-Naphthaleneacetic Acid (NAA) when jointly composed. BA was utilized at 0, 1.50, 3.0 as well as 4.50 mg × l^{-1} as well as NAA at 0, 0.20, 0.40 as well as 0.60 mg × l^{-1}. For each treatment ten test tubes were utilized. Based on stage I results, the produced micro shoots on or after the interpretation were progressed to 'Murashige and Skoog' medium (proliferation period was found medium) on or after the Grade Anaction. After 6 weeks after planting; amount as well as measurement of buds was documented.

2.2 Multiplication Stage

At multiplication period; investigates incorporated the result of BA was experienced at 0, 1.50, 3.0 as well as 4.50 mg × l^{-1} to find out its effects on amount as well as measurement of new buds. Beside this, the outcome of the relations distinguishes BA as well as NAA on multiplication period was also observed. To all actions, together with the regulator; BA was added at 0, 1.50, 3.0 as well as 4.50 milligram × l^{-1}, along with NAA at 0, 0.10, 0.20 as well as 0.30 milligram × l^{-1}. GA3 was incorporated to 'Murashige and Skoog' medium at 3.0 milligram × l^{-1}. A number of separate experiments were carried out after IBA, NAA as well as IAA (0.0, 0.50, 1.0 as well as 2.0 milligram × l^{-1}) were being added and the outcomes of IBA, NAA as well as IAA added to the culture Broth on micro bud rooting were studied. Everything on these treatments was observed in 0.5 (half) strong point of MS Broth. Characteristics for example amount of micro buds, amount of roots/buds as well as root length (cm) were noted down though the rooting stage was our concern. On a monthly basis for 1.0–1.50 repeated months these evaluations were performed. At the end of 1.50 months, the outcomes were collected, be around as well as recorded as a ratio or amount for every action.

2.3 Rooting Stage and Acclimatization

More than a few micro plants were carefully chosen on or after those that displayed respectable vegetative progress later 1.50–2.0 months on or after *Cestrum nocturnum* shoot rooting depicted in Figure 1. Later then selected micro plants were cleaned by washing it in normal pure water to wash out the agar gelon or after the roots, which might cause infection later. After that they were soaked in 0.10% solution of Benlate fungicide and later planted in artificial pots contained a purified mixture of peat moss as well as canal soil (1:1). In view to maintain high humidity in the culture situation a light elastic protection which permits light penetration as well as contains a lot of air passing option were used to cover the pots.

Micro plants were properly watered as well as a MS salts containing solution were given with 0.250 of original strength. After two weeks from planting the elastic cover was detached on or after time to time. The micro plants were then moved for transplantation and later 0.10% Benlate fungicide was sprayed as necessary after elapsing four weeks.

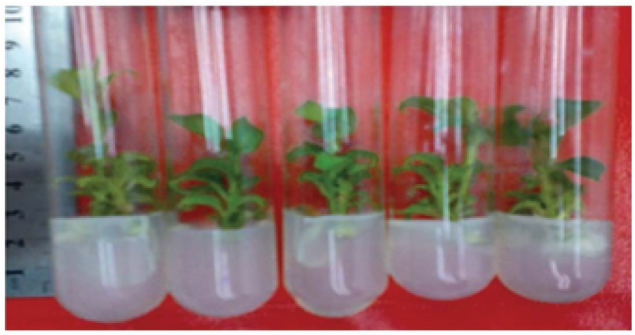

Figure 1. Initiation stage of multiplication of Shoot of *Cestrum nocturnum* (after4–6 weeks growth) on MS Broth enriched with BA+NAA at various concentrations.

3. Observation and Results

Stage 1: Initiation

3.1 Interpretations of BA as well as NAA Concentrations during Explants Development

Outcome of various concentrations of BA, NAA as well as their relative effects on the ratio of reaction of lateral as well as station shoots cut out on or after easy trimming of Cestrum grown on MS Broth. BA (1.50 milligram × l^{-1}) this concentration was meaningfully finished for lateral buds, the further concentrations for both lateral as well as terminal shoots as well as provided the maximum feedback percentage (100%) was noticed. Treatment of lateral buds of 1.50 mg × l^{-1} BA with best of NAA concentrations provides the maximum response (100%). Beside this, for the terminal shoots, upper percentages of effects resulted on or after treating 1.50 milligram l^{-1} BA with 0.0, 0.20 milligram × l^{-1} NAA concentration. Figure 2 depicts the outcomes of BA, NAA concentration variation as well as their effects and also types of shoots on the mean number of buds, leaves as well as the size of new buds at initiation period. It is proved that side shoot or stem (lateral shoots) formed further new buds and a higher amount of leaves as well assize of new buds in comparison with those on or after terminal shoots. Deficiency of cytokinin in the lateral buds may be cause[18]. BA conc. at 01.50 milligram × l^{-1} helps in obtaining the greatest extent of buds and leaves and also the greatest extent of bud size in lateral as well as terminal shoots (5.20, 4.0 shoots/explant, 18, 13.20 leaves/explant as well as 6.00, 5.50 centimeter; on the given sequence). This confirms the requirement existence of cytokinin (BA) in initiation Broth. It is already been mentioned in various issued articles written on tissue culture techniques of various fruit plants like pear[19], plum[20] and walnut. Taking the effect of NAA in consideration, it may be stated clearly that the highest values of number of new buds, amount of leaves as well assize of new buds were produced at 0.20 mg × l^{-1} NAA for both lateral as well as terminal shoots. Observing the relations between BA, NAA as well as categories of shoots, it is clear that the highest number of amount of buds, amount of leaves as well assize of new buds were gained on or after the relations with the low concentrations of individual regulators of growth for individual lateral as well as terminal shoots.

Figure 2. First stage of root formation of *Cestrum nocturnumon* MS Broth (4–6 weeks of growth) nourished with different concentrations IBA, NAA as well as IAA.

The action produced an important rise in the mean number of fresh buds, mean amount of leaves as well as average size of new buds on lateral shoots in comparison with those on or after terminal shoots. But with the help of treatment that is free of regulators of plant progress (02.60 cm, 02.20 cm (shoots/explants), 07.20 cm, 06.60 cm (leaves/explants), as well as 4.88 cm, 3.44 cm) the least amount of newly grown buds, average amount of newly grown leaves as well as average size of newly grown buds formed. These results agrees with finding of other researcher[21], as they commented that utilization of cytokinins as well as auxins in this category is very significant as well as the function of cytokinins at this period is necessary to interrupt apical controls in shoots as well as to talk into the subsidiary meristem raise into a bud.

Stage 2: Multiplication

3.2 Interpretation of BA as well as NAA during Bud Propagation

Figure 3 tells the interpretation of different concentrations of BA, NAA as well as interactions of these and categories of these shoots depend on the satisfactory quantity of buds, satisfactory quantity of leaves as well assizes of new buds at proliferation period. Important individual were found between the lateral as well as terminals shoots in which lateral shoots formed upper quantities of new bud, leaves as well as sizes of new buds. It is anticipated that cytokinins support the foundation of forested tissues bordering to the vascular tissues of the shoot as well as stem,

Figure 3. Ex vitro Microplants established in pots after 1.50–2.50 months of transfer.

thus will create stress-free the transfer of water as well as nutrients in the plant that causes shoot opening[22]. It was observed, by utilizing BA I little concentrations (01.50 mg × l^{-1}) run for growing the maximum effects in bud's quantity (4.40 as well as 4.20 buds/explant), quantity of leaves (15.40 as well as 4.20 leaves/explant) as well as size of new shorts (5.345 as well as 4.91 cm) for lateral as well as terminal shoots correspondingly. These effects comply with those described[23] in their homework on the significance of cytokinins in bud proliferation. Beside this, the NAA (0.20 milligram × l^{-1}) usage provided the maximum quantity of newly grown buds (03.40, 03.0) buds/explant, regarding the leaves quantity as well as size of new buds, the concentration of NAA (0.10 mg × l^{-1}) was pointedly greater upon the other various concentrated medium for lateral as well as terminal shoots progress. This possibly will result because of the action of auxins on plant cell wall development. Taking the interface between BA, NAA as well as kinds of shoots in consideration, it may be stated without any doubt that for average quantity of buds, quantity of leaves as well as the size of new buds for both lateral as well as terminal shoots, the action of 1.50 milligram × l^{-1} BA as well as 0.10 milligram × l^{-1} of NAA exerted effects in upper values (05.0, 04.20 buds/explant, 17.20, 13.40 leaves/ explant as well as 7.70, 5.46 cm by the given sequence) after being compared with regulator. The consequence of interface between cytokinins as well as auxins in vegetative multiplication as well as increasing progress sizes can be understood by the rise of cytokinins character in the attendance of auxins as stated that cytokinins movement is usually make active in the action of auxins[23], that is; a higher amount of shoots will have a chance to produce as well as start to produce buds[24]. These results comply with those reported[25], who highlighted the significance of the counter actions between auxins as well as cytokinins in vegetative proliferation procedures.

Stage 3: Rooting

At rooting period Micro shoots were shifted from proliferation Broth as well as located in half strong point MS macro- as well as micro-elements improved with different IBA, NAA concentrations as well as IAA (0–2.0 mg × l^{-1}). It displayed dissimilar responses to rooting after 1–1.50 months of culture (Figures 4 as well as 5). On half strong point MS Broth improved by 00.5, 1.0 milligram l^{-1} NAA as well as IBA the significant percentage of rooting (100%) was found, respectively.

Beside this, in case of IAA, the maximum percent of rooting of Cestrum buds grown in half strong point MS (90%) were found as concentration of 1.0 mg × l^{-1} IAA. Hormones existed in plants (Endogenous) might have been associated in promoting plants to root[26], up to the hormonal steadiness stretched its optimum level to force the roots to raise as well as improve in the existence of exogenous hormones, since growing auxin concentration enhances root creation on bud bases[27]. There is a role of IBA, IAA as well as NAA concentrations on the mean quantity of roots each bud as well as average root size. In half strength MS medium it can be noted that IBA as well as IAA exerts an important effect on root quantities each bud as well as root size. At 1.0 mg l^{-1} concentration, IBA as well as IAA provided the maximum amount roots (013.20, 6.80 roots/explant) as well as root size (8.51, 5.82 cm), individually. Taking the effect of NAA in consideration, it is clear that the maximum prices for root numbers each bud (7 roots/explant) as well as root size (5.52 cm), separately, were obtained at the concentration of 0.50 milligram l^{-1} NAA in half strength MS Broth.

Auxins have a system in the rooting method was proved by these results, though they helps in adventitious root initiation in the bases of grownbuds[28]. These results agrees with those founder[15] who concluded that decreasing the MS salts level in the broth to half, facilitates rooting of a lot of tree species; Lowering the salts level in the Broth means lowering the nitrogen level of the Broth to the half (1/2) or to the quarter (1/4) will outcome in lower nitrogen level inside the buds, which may cause the carbohydrate percentage level higher that is increase if

the nitrogen level is increased as well as this consequently may results in increasing root primordial percentage as well as root records[29].

Stage 4: Acclimatization

Figure 6 showed that the tiny plants (Micro plants) of *Cestrum Nocturtum* were warily removed from rooting media as well as shifted in a Broth consists of peat moss as well as soil collected from river (1:1) and kept in the greenhouse in small plastic pots. These plants were finally hard-bitten by reducing the moisture gradually. The survival percentage of plants reached 90% after four weeks from transplanting. This procedure for vegetative micro propagation agrees with the protocol that was established by lots of investigates for fruit plants like apples[30], peaches[31], as well as chestnut[32] that were transferred on an open air field.

4. Conclusion

The study was conducted to measure the micro propagation of *Cestrum nocturnum* by utilizing solo nodes as well as shoot tips collected by cutting it from smooth cuttings utilizing MS salts, sucrose and agar as well as distinguish concentrations of plant progress regulators. The outcomes unveil that utilize of mercuric chloride for 7.50 minutes much more effectively preventing contamination. At initiation period from lateral shoot explants on MS Broth supplemented with BA with greatest concentration of NAA; maximum effect were achieved. At rooting stage, the maximum percentage of rooting (100%) were found after the treatment with IBA it also gave the maximum amount of roots as well as the lengthiest roots respectively on half strength MS Broth. Plantlets grown were shifted to pots as well as acclimatized with 90% achievement. Micro propagation is an important technology and techniques of culturing tissues has found huge applications in several fields of plant science, together with basic plant physiology, pharmaceuticals and natural compounds production from plant, plant pathology studies, germ plasm conservation techniques, breeding, recovery of transgenic plant, as well as proliferation. Thus, improved procedures for virus free plant regeneration, salinity tolerance, herbicide resistance, disease resistance, frost resistance, incorporation of high protein content and genetically engineer plants for desirable property is desired with the advancement of other branches of Sciences. Advances in plant tissue culture will help in rapid expansion and sustainable utilization of medicinal plants for upcoming generations.

5. References

1. Western Garden Book. 2007 Ed. Hortus Third Cornell University.
2. Jawale C, Kirdak R, Dama L. Larvicidal activity of Cestrum nocturnumon Aedes aegypti. Bangladesh Journal of Pharmacol. 2010 Mar; 5(1):39–40.
3. Patil CD, Patil SV, Salunke BK, Salunkhe RB, Bioefficacy of Plumbago zeylanica and Cestrum nocturnum plant extracts against Aedes aegypti and nontarget fish Poecilia reticulata. Parasitol Res. 2010 Nov; 108(5):1253–63.
4. Perez-Saad H, Buznego MT. Behavioral and antiepileptic effects of acute administration of the extract of the plant Cestrum nocturnum Lin (lady of the night). Epilepsy Behav. 2008 Apr; 12(3):366–72.
5. Huang LG, Zhang XC, Xiao H, Ye HY, Zeng J. Analgesic effect of Cestrum nocturnum L. extract on mice. Chin J Clin Rehab. 2006 Sep; 10(35):172–4.
6. Catterjee SK, Bhattacharjee I, Chandra G. Bactericidal activities of some common herbs in India. Pharm Biol. 2007; 45(5):350–4.
7. Zeng J, Li FZ, Ye HY. Study of the inhibitory effect of Cestrum nocturnum, L. n–butl alcohol extract on central nerve system. Journal of Gannan Medical University. 2003; 23(3):237–9.
8. Mello JRB. Calcinosis-calcinogenic plants (Review). Toxicon. 2003; 41(1):1–12.
9. Sahai M, Singh M, Singh AK, Hara N, Fujimoto Y. Cesternosides A and B, novel glucosides from the leaves of Cestrum nocturnum. J Chem Res Synop. 1994; 1:22–3.
10. Mimaki Y, Ntonifor NN, Ngufor CA, Kimbi HK, Oben BO. Traditional use of mosquito repellent to protect human against mosquito and other insect bite sin rural community of Cameroon. East Afr Med J. 2006 Oct; 83(10):553–8.
11. Jawale CS, Dama LB. Haematological changes in the fresh water fish, exposed to sub-lethal concentration of Piscicidal compounds. National Journal of Life Sciences. 2010; 7(1):82–4.
12. Raj RSDP, Morais SM, Gopalakrishnan K. In vitro propagation and cell suspension culture of Callistemon citrinus L. Indian Journal of Science and Technology. 2010 Jan; 3(1):67–9. Doi no: 10.17485/ijst/2010/v3i1/29648.
13. Karthikeyan S, Prasad R, Mahendran TS, Rajagopal K, Ravendran V. Direct regeneration and in vitro flowering of Scopariadulcis L. Indian Journal of Science and Technology. 2009 May; 2(5):55–7. Doi no: 10.17485/ijst/2009/v2i5/29467.
14. Indira Iyer R, Jayaraman G, Ramesh A. In vitro responses and production of phytochemicals of potential medicinal

value in Nutmeg, Myristica Fragrans Houtt. Indian Journal of Science and Technology. 2009 Apr; 2(4):65–70. Doi no: 10.17485/ijst/2009/v2i4/29434.

15. Jawale CS, Vinchurkar AS, Dama LB, Pawar. Cestrum nocturnum (L) a prospective piscicide for control of predatory fish Channa punctatus (bloch.). Trends in Fisheries Research. 2012; 1(1):14–7.
16. Backhouse NC, Delporte R, Salinas P, Pinto A, Aravena S, Cassels BK. Antiinflammatory and antipyretic activities of Cuscuta chilensis, Cestrum parqui and Psorela glandulosa. Int J Pharm.1996; 34(1):53–7.
17. Hartmann HT, Kester DE, Davies FT, Geneve RL. Plant propagation, principles and practices. 7th ed. Prentice Hall; 2002.
18. Jansky S, Stern KR, Bidlack JE. Introductory plant biology. Bon: The McGraw-Hill Companies Inc; 2004.
19. Hirabayashi T, Moriguchi T, KozakiI, Ya-mamoto Y, Matsuzaki S. In vitro propagation of pears shoots tips. Bull Fruit Tree Res Stn Ser A.1987; 14:9–16.
20. Druart Ph, Gruselle R. Plum (Prunus domestica). Biotechnology in Agriculture and Forestry, Trees II. Bajaj, Y.P.S. (Ed.) NewYork: Springer Berlin Heidelberg; 1986; 5:42–58.
21. Singh S, Ray BK, Bhattacharryya S, Deka PC. In vitro propagation of C. reticulata Blanco and C. limon Burm. Hort Science. 1994; 29(3):214–6.
22. Mohammed AA, Al–Younis MA. Fundamentals of plant physiology (In Arabic). Third part. College of Agriculture. Iraq: Baghdad Univ; 1991.
23. Brookner N. Studies on the in vitro propagation of Mussaenda erythrophlla "Rosea". (Trinidad and Tobago: St. Augustine; 1991. p. 181.
24. Thanhran TK. Control morphogenesis in in vitro culture. Ann Rev Plant Physiol. 1981 Jun; 32:291–311.
25. Roy PK, Mamun ANK, Ahmad G. In vitro plantlets regeneration of rose. Plant Tissue Culture. 2004 Dec; 14(2):149–54.
26. Peak KY, Chandler SF, Thorpe TA. In vitro propagation of Chinese cabbage from seedling shoot tip. J Amer Soc Hort Sci. 1987; 112(5):841–5.
27. George EF, Shermington PD. Plant propagation by tissue culture. Exegetics Ltd; Eversley England. 1984. p. 307–8.
28. Saleh MS. Physiology of plant growth regulators. (In Arabic). Salahaddin. Univ Ministry of Higher Education and Scientific Research; IRAQ.1990
29. Gawel NJ, Robacker CD. Corly WL. In vitro propagation of Miscanthus sinesis. Hort. Science. 1990 Oct; 25(10):1291–3.
30. Soni M, Thakur M, Modgil M. In vitro shoot multiplication of Merton I. 793, a clonal apple rootstock, suitable for replantation. Indian Journal of Science and Technology. 2011 Jul; 10(3):362–8.
31. Reeves DW, Horton BD, Couvillon GA. Effect of media and media pHon in vitro propagation of Nemaguard peach rootstock. Hort Science. 1983 Dec; 21(4):353–7.
32. Preece JE, Sutter EG. Acclimatization of micro propagation plants to greenhouse and field. Micropropagation Technology and Application; 1991. p. 71–93.