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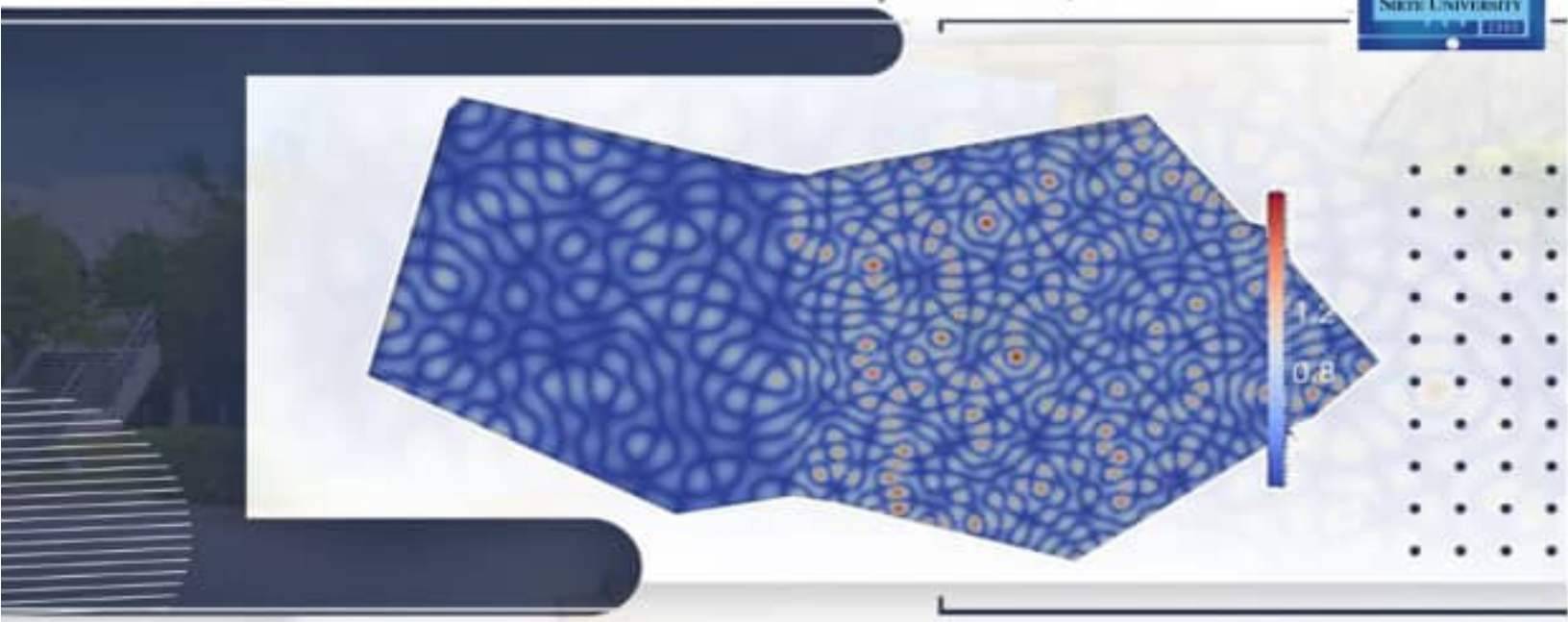

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Micropropagation of *Paulownia elongata* tree through Plant Tissue Culture Technology

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A B S T R A C T

The study was carried out at biotechnology research center laboratories for the purpose of Micropropagation of *Paulownia elongata* tree by using plant tissue culture technique for the purpose of identifying the best plant Micropropagation conditions. The plants were sterilized superficially by immersing them in the Clorox solution, then the sterilized plants were cultured in MS media supplemented with several concentrations of (BA and Kinetin) for the purpose of obtaining the best vegetative growths. Obtained plants were also cultured in MS media supplemented with different concentrations of (IBA and NAA) for the purpose of obtaining the best root growth. Finally plants were moved for the adaptation stage. The results indicated that single-nods cultured in (MS) media supplemented with a concentration of 2 and 2.5 mg / L of (BA) growth regulator resulted in good vegetative growth represented in the number of leaves, while the best treatment of branches growth was using 2 mg / L concentration of the Kinetin (K) growth regulator but For the length of the plant, the treatment of the control achieved the best results. For the root site, the results showed that the culture media (MS) plus 0.2 mg / L of the growth regulator (NAA) resulted in the best root growth. As for the adaptation, the results showed that 85% of the plants could be adapted to the sterile environment of soil with 1: 1 size / volume before transferring them to the greenhouse.

1 Introduction

Paulownia, a hardwood tree, which belongs to the family Paulowniaceae (Scrophulariaceae), is characterized by a fast-growing and short-rotation plant with large leaves arranged in opposite pairs on the stem. The tree is able to grow under several weather conditions and in different types of soil even poor ones. *Paulownia* tree is a large, fast- vertical growing tree that is considered to be not evergreen tree. Nowadays, Paulownia species are considered the most important forestry crops in the world. There are nine species of Paulownia but the most important of them include *P. kawakamii,P. australis, P. catalpifolia, P. elongata, P. fargesii, P. fortunei, P. albiphloea, and P. tomentosa*(A fahmy and H gendy , 2018) .In fact *paulownia elongate* is considered one of the most important trees producing wood, especially in the last ten years, whereas the global demand on this tree is clearly increased in the global market due to the high quality of wood obtained from *paulownia elongate* tree . Furthermore, the most famous one is *Paulownia tomentosa* originated from China which is a very fast

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growing specie (Oprea, 2007). The length of this tree is 12-15m with large leaves shaped as a heart with the length of 15-40cm. The flower appears at the beginning of the spring with length of 10-30 cm (Atanas Chunchukov and SvetlaYa, 2015). Paulownia wood is good for making paper pulp (Latibari *et al*., 2012). The paper produced from Paulownia trees is of high quality, comparable to Eucalyptus (Feria *et al*.,2013). Due to these qualities, *Paulownia* species are among the most important forestry crops in the world. The *Paulownia* tree is widely used for reclamation of wooded areas, furniture industry, wooden parts of the aircraft (Zhu , 1986) and musical instruments industry (Ayan *et al*., 2003). The tree cultivated in Europe for the purpose of decoration and woodwork (Kaymakci *et al*, 2013). Moreover the tree has been used to improve the properties of soils contaminated with condensate (Miladinova *et al*., 2014) .On the other hand the tree has the potential to grow in several different types of soil and adapt to different types of climate also it has the ability to grow in the tropical climate and regions which have low rainfall rates of 20 inches per year and high temperatures of up to 40C° (Md.Atiqur *et al*., 2013).However *Paulownia* tree adapted easily to environmental different condition. In the first season after culture of the seedling it reaches of 5-6 meters long when suitable conditions are available (Md.Atiqur*et al*., 2013; El-Showk,2003). The trees grow to marketable size in 5-8 years, much faster than other tree species. The plantation does not need re-planting after harvest as new trees grow from the stumps. Paulownia wood is very light, with densities around 0.300 g/cm3 (Akyildiz and Kol, 2010; Kiaei, 2012) .In the age of 3-5 years, the length of the tree may be 15-20 meters, and the urine can be used as feed for the farm animals because it has a good nutritional value that corresponds to wheat straw and alfalfa, and can be used in the production of compost (Lyons, 1993) or as source of the organic matter in soil (Wang and Shogren, 1992), as well as polyuia and phosphorus, are rich in nitrogen and can be used as fertilizer for soil (Md.Atiqur *et al*., 2013). Polonia wood of finest wood, which is characterized by a bright color and has the ability to dry quickly, it has cracks, cracks and does not distort quickly(Atanas Chunchukov and SvetlaYa, 2015).

Using of traditional methods for propagation of Paulownia tree requires large areas and large numbers of workers (Crisanand Petrus, 2016). However, it is very difficult to reproduce Paulownia by traditional methods. However, increasing demand on *Paulownia* in the market has pushed researchers to found appropriate method of propagation, whereas using plant tissue culture technique is one of the most important methods used in the propagation of Paulownia tree, which will be studied in this research. Obtaining to large quantities of plants in a short period is one of the most important challenges facing the scientific researchers. Use of plant biotechnology such as tissue culture technique as tool for propagation of plants normally provides some advantages such as obtaining plants that are genetically identical with the mother plant, large numbers in a short period of time and cultivation of plants in sterile condition.

In general the most commonly used culture media in plant Micropropagation side is Murashige and Skoog media (MS), which usually depend on addition of micro and macro elements to culture media supplemented with growth regulators .However (MS, DKW, N6 and QL) culture medias are used for Micropropagation of Paulownia tree, with different concentrations of growth regulators (BA and IBA) which, added to the dietary medium (Chunchukov and Yancheva, 2014) , whereas single nodes, leaves or roots are usually used in the Micropropagation (Ozaslan *et al*., 2005). In fact there are attempts to adapt Paulownia tree in Libya in order to spread the idea of planting the tree within the country due to economic benefits of Paulownia tree which may contribute the national economy situation. The aim of this study was obtaining a suitable method to propagate Paulownia tree and obtain large quantities in order to obtain a successful procedure for culture Paulownia tree.

Furthermore Paulownia tree has the potential to grow in sandy and clay soils and grows under different climatic conditions such as high temperatures with low rainfall (Md.Atiqur *et al*., 2013). The process of settling the tree in Libya and obtaining success cultivation inside the country through use of plant tissue culture technology is considered as an important task.

2 Materials and Methods

This experiment was carried out at Plant Tissue culture Laboratory of the Libyan center for biotechnology research, Tripoli, Libya in order to investigate the impact of the plant regulators BA(Benz ail adenine) , Kin (Kinetin) , NAA(Naphtalin acetic acid) and IBA (Indol butyric acid)on Micropropagation of *paulownia elongata* tree using Murashige and Skoog culture media.

Sources of plants

Plant samples and single-nods of *Paulownia* tree were collected from the *Paulownia* plants growing in Qasr ben Ghasheer area (Abu Aisha project) while the experiment started by using single-nod parts of *Paulownia* tree to establish tissue culture free of contamination.

Culture media

Preparation of free contamination media

The first stage of the experiment was conducted for the purpose of obtaining a primary media free of contamination to obtain sufficient number of plants for the next stages of the stud whereas the Murashige and Skoog (MS) was prepared with 3% sucrose and 0.7% agar at pH of 5.7 to 5.8 . While about 200 ml of prepared MS were placed in special jars. Plant samples were sterilized by sodium hypochlorite with a concentration of 2.5% .Use of MS media supplemented with different plant regulators.

The second stage started with preparation of MS media supplemented with plant regulator for obtaining the best-cultured media to be used in Micropropagation of Paulownia tree. A nutrition media (MS) was prepared by using MS media supplemented with benzyl adenine growth regulator (BA) and the Kin growth regulator at concentrations of 0.5, 1, 1.5, 2 and 2.5 mg / L for both growth regulators.

Rooting stage:

Rooting stage was conducted through preparation of culture media (MS) was prepared with half the concentration of salts with addition of growth regulators (IBA and NAA) at concentrations of (0.1, 0.2, 0.5 mg / L) for NAA growth regulator and (0.1,0.2, 0.5 and 1 mg / L) for the growth regulator IBA in order to induce the cultured plants to produce roots.

Adaptation stage:

Adaptation stage was started with transferee the obtained plants from tissue culture conditions to natural conditions by taking the plants out of the jars and washing the roots to remove the residues of the culture media then transferee the plants into pots with sterile environment of soil with a 1: 1 or 1: 2 size / volume. The pots were covered with a plastic cover with a limited number of holes to maintain humidity. The number of holes in the plastic cover was gradually increased to reduce the moisture before removing the cover completely so that the plants were adapted to the outside. When the plants reached the appropriate size, they were transferred to the greenhouse with keeping observation time to time to monitor the plants growth.

Experimental design

The experiment was designed using full random design RCD and the results were analyzed by ANOVA system. Was used at level 0.05 and the means were compared using Duncan test.

3 Results

The results of the first stage of this experiment showed that after 60 days of cultivation the growth rate of the single nods of paulownia plants which were cultured in (MS) media free of growth regulators was 70%. A sufficient number of developing plants were obtained free of pathogens (Fig. 1) then obtained plants were transferred to MS media supplemented with different plant regulators.

Figure (1) plants in MS media free of Contamination

Number of leaves:

In the second phase of the experiment, obtained plants were cultured in MS media supplemented with different concentrations of plant regulators which are (BA) and Kin at concentrations of 0.5, 1, 1.5, 2 and 2.5 mg / L. The results in terms of number of leaves parameter (Fig2) showed that a significant differences among the used treatments were found in case of number of leaves parameter , while the treatment of 2 mg BA was the best treatment compared with other used treatments followed by 2.5 mg BA and finally treatment of 2mg Kin whereas,

the 2mg BA , 2.5BA and 2mg Kin treatments recorded average of number of leaves of 19, 18.4 and 18.4, respectively .Our results were in complete agreement with those Donia *et al*., (2014) who found that use of modified MS media supplemented with PAB growth regulator produced vigorous and well-developed plants from paulownia tree which proved that use of plant tissue culture technology was very successful for Micropropagation of paulownia tree.

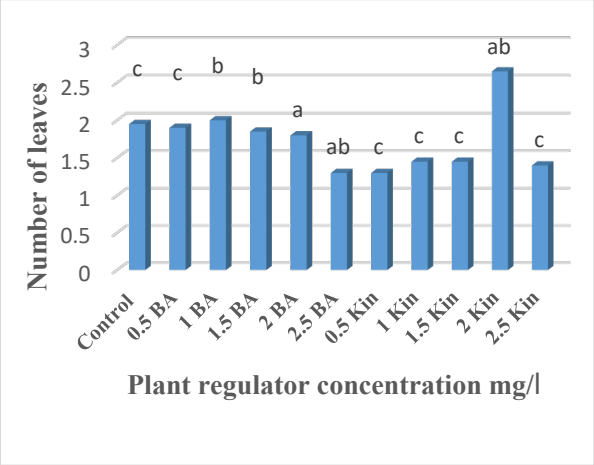

Figure (2) effect of plant regulator (BA, Kin) on plant

(0.5 mg/ l) and IBA (0.01 mg l-1l) and cultivation in big vessels resulted in high proliferation and induced the development of uniform plants as a prerequisite for effective rooting and quality material production. Successful *in vitro* rooting was achieved when plants were cultivated on MS medium with IBA (0.1 mg/ l). High average efficiency of adaptation (96%) was obtained.

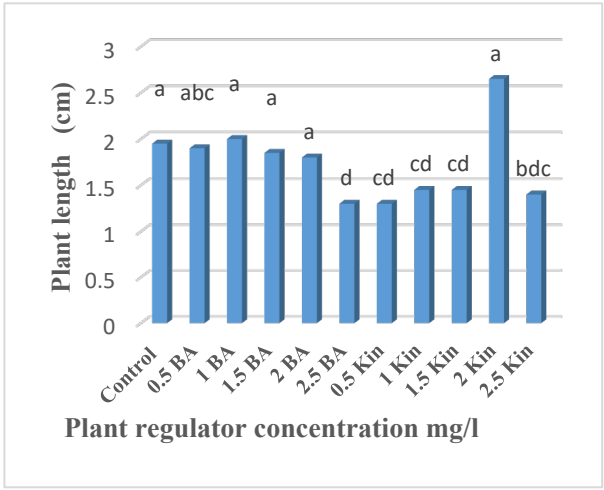

Figure (3) effect of plant regulator BA, Kin on plant height

4 Discussion

This should explore the significance of the results of the work, not repeat them.

Plant height:

The results (Figure3) showed that in order to determine the best concentration of the growth regulator for plant growth and plant height parameters the results of this study showed that the performance of BA growth regulator in most of its coefficients was better than Kin growth regulator .The results proved that treatments of 1, 1.5, 2 mg /l BA and treatment of 2 mg/l Kin were significantly higher than other used treatments which explain that use of BA and Kin at concentration of 2 mg/l is successful for Micropropagation of paulownia tree .The result of this study proved that use of BA plant regulator is the best choice for paulownia In vitro Micropropagation. Our results agreed with Atanas and Svetla, (2015) who reported that application of the propagation medium with MS salts and addition of BAP

Number of branches

In terms of number of branches parameter, the results showed that the treatment of 2mg/l Kin gave the highest average number of branches Fig (4). The concentration 2 mg / l of Kin reach the value of 2.65 branches which was the best treatment among the others in case of number of brunches factor. Although the treatment of 2mg/l gave the best results but BA treatments gave acceptable results specially treatment of 1mg/l BA which explain that BA growth regulator was able to induce the obtained plants for better growth. The results proved that (BA) is the best choice for Micropropagation of Paulownia tree .Our results were in complete agreement with Osvaldo and Jose. (2006) and Lobna, (2008) who found that the vegetative total increased with addition of (BA) at concentration of 2 mg / L.

According to our results the treatments of 2mg BA, 2.5mg BA and 2 mg Kin were significantly different among the other treatments even control treatment in case of number of leaves factor whereas, the treatments 2mg

BA gave the highest number of leaves followed by 2.5mg BA and 2mg Kin treatments.

The treatments of 1, 1.5 and 2 mg BA gave the best results compared with other treatments in case of plant height parameter but in number of branches factor treatment of 2mg Kin was significantly higher than the others followed by 1 and 2mg BA treatments, In general plant growth regulator BA and Kin have positive effect on paulownia elongate plant specially treatments of 2mg BA and 2mg Kin . Our results clearly proved that use of plant growth regulators

BA and Kin at concentrations 2mg/l were successful for in vitro Micropropagation of paulownia through use of plant tissue culture technology

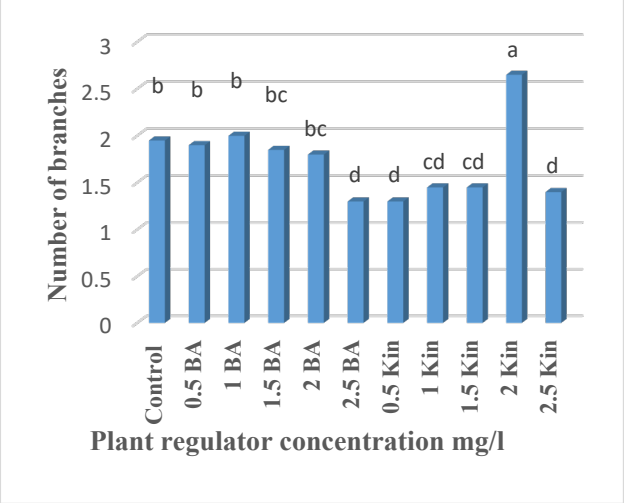

Figure (4) effect of plan growth regulators on number of brunches of *paulownia elongata***.**

successful but not compared with NAA treatments which mean that the plants were able to produce roots in MS media without addition of hormones even in low numbers. Our results were agreed with Atanas and Svetla,(2015) who reported that application of the propagation medium with MS salts

and addition of BAP (0.5 mg/ l) and IBA (0.01 mg l-1l) and cultivation in big vessels resulted in high proliferation and induced the development of uniform plants as a prerequisite for effective rooting. Our results were agreed with Fahmy and Gendy, (2018) who reported that the rooting percentage was highly improved by addition of NAA to the medium without significant differences among different concentrations since all concentrations gave 100% rooting percentage.

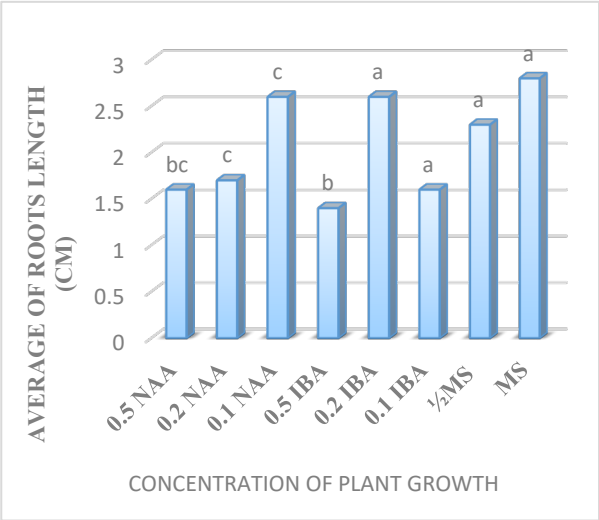

Figure (5) effect of plant regulator (NAA, BA) on number of roots

Number of roots:

The results of root induction stage in case of number of root parameter fig (5) the results showed that all NAA (0.1, 0.2 and 0,5mg/l) treatments were significantly higher than the other used treatments except treatment of 0.5mg/l IBA ,while the NAA growth regulator treatments reached average of 3.2, 3.9 and 3.4 respectively whereas the treatment of 0.5mg/l IBA reached average of 3.5 number of leaves. Our results proved that addition of NAA hormone was successfully induced the paulownia plants to produce roots which extremely needed for successful micro propagation of paulownia tree .Moreover control treatment (MS) gave acceptable average of number of roots this treatment seems to be

Root length:

In terms of root length Fig (6), the results showed that the best results were achieved in the control treatment (MS) free of growth regulators with an average value of 2.8 cm .Our results proved that (MS) free of growth regulators treatment seems to be the most effective dietary media followed by the treatment of IBA 0.2 mg / L which recorded 2.6 cm and finally treatment of 0.1 mg/l NAA which reached 2.6 cm. Our results were in agreement with those Al-Tinawi *et al*., (2009) who reported that the effect of growth regulators (IBA and NAA) is not dissimilar in promoting root lengths and this is almost consistent with the results obtained. . In the same line Zayova *et al*., (2014) reported that the highest rooting

percentage (100%) and the maximum number of roots per plant were recorded on a ½ MS medium supplemented with 0.5 m / l IBA.

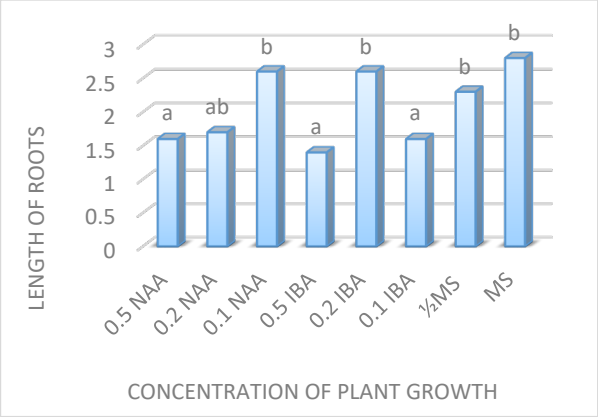

Figure (6) effect of plant regulator on average of roots length of *paulownia elongate* plant

Acclimatization stage:

The results of adaptation stage (Fig8) showed that 85% of the plants were able to reach the state of complete adaptation stage in the sterile environment of soil with peat moss in average of (1: 2, 1: 1 size / volume). After 6 weeks of adaptation some adapted plants were not able to resist and grow well due to that the number of their roots was not enough for growing normally.However the rest of adapted plants continued growing well and have good resistance to natural conditions .In general our result in this stage proved that adaptation of *paulownia* plants which obtained through plant tissue culture technology was successful. This was in the seam line with what has been found by Ozaslanetal (2005). Our obtained results were agreed also with Atanas and Svetla, (2015) who reported that Ex Vitro adaptation of the rooted plants was performed in a growth chamber with a gradual decrease of the atmospheric humidity and the average survival rate 96% was achieved. Our results were in same lineas Crisan and Petrus. (2016) who found an excellent adaptation for propagated rooted plants.

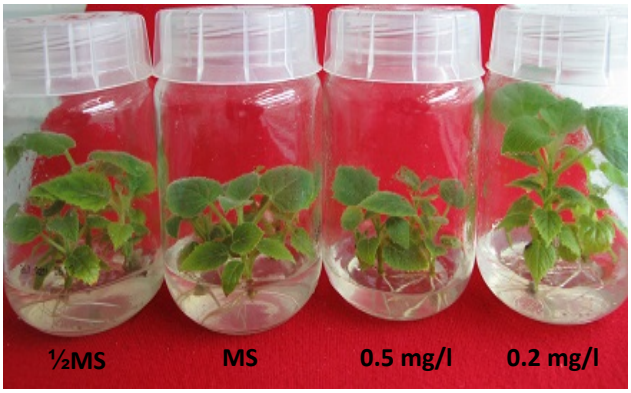

Figure (7) ***paulownia elongate*** plants at age of seven weeks grown in MS, 1/2 MS media and MS media supplemented with IBA and NAA

Figure (8) adaptation of plants ***paulownia elongate***

5 Conclusions

Recently *paulownia* tree considered as one of the most important trees in commercial side due to its high quality wood. Thereby obtaining large numbers of this tree is extremely needed, whereas plant tissue technology is one the propagation methods helps to obtain an sufficient numbers of this tree. The result of proved that Micropropagation of *paulownia* tree was really successful and large numbers of this tree might be obtained. However, the results reported that use of MS media supplemented with BA and Kin growth regulator at concentration of 2mg/l was clearly successful for Micropropagation of *paulownia* tree even without use of rooting hormones such as IBA and NAA. The results showed that present of an acceptable ability of obtained plants to adaptation conditions.

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

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