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# Effect of coconut water from different fruit maturity stages, as natural substitute for synthetic PGR in *in vitro* potato micropropagation

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**Key words:** Micropropagation, potato, coconut water, plant growth regulators (PGRs).

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## Abstract

The present *in vitro* study was conducted to investigate the hormonal efficiency of coconut water on the growth of plantlets of potato variety Desiree. The coconut water was collected from different fruit maturity stages i.e. one, two and six months old coconut fruit and used in three different concentrations i.e. 75ml/L, 150ml/L and 300ml/L each in MS medium instead of synthetic plant growth regulators (PGRs). The results showed that six months old coconut water (T3) significantly increased the number of roots (8.3), root length (4.92cm), shoot length (6.10cm), stem diameter (2.69mm), no of nodes (6.73) and leaf area (0.527cm$^2$) of the potato plantlets at transferable plant stage. However, one month old coconut water (T1) seems to be effective at C3 concentration (300ml/L) while, the two months old coconut water suppressed the growth of the potato plantlets at lower concentrations i.e. 75ml/L and 150ml/L with callus formation and no roots but at C3 (300ml/L) the shoot length increased up to 3.93cm with multiple shoots. It has been concluded that the concentration of plant growth hormones (auxin, cytokinin, gibberellins) present in coconut water changes with fruit maturation which has affected the in-vitro growth of potato plantlets significantly, therefore, it can be used instead of synthetic plant growth regulators (PGRs) in MS media for potato micropropagation.

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## Introduction

Plant tissue culture is a widely used technology in recent years for large scale plant multiplication, viruses elimination, and production of secondary metabolites. Tissue culture media generally contains macro and micronutrients, vitamins, amino acids or nitrogen supplements, carbon source(s), growth hormones and gelling agents (Murashige and Skoog, 1962). In *in-vitro* tissue cultures, different natural extracts like seaweed, Zea mays extract, yeast extract, citrus extract, coconut milk etc were used in culture medium (Cachiţă, 1987). Coconut water is the liquid endosperm of *Cocos nucifera* L used as a supplement in tissue culture medium since 1941 when Overbeek introduced it as growth promotor in culture medium for callus cultures (Tulecke *et al.* 1961).

The most important component of coconut water are phytohormones especially cytokinins, indole-3-acetic acid (IAA) (Yong *et al*, 2009) and gibberellins (Ge *et al.*, 2007). The cytokinin found in coconut water support cell division, and along with other chemical components promotes plant growth. The chemical composition of coconut water is affected by many factors including different varieties that differ in compounds concentration, and also with the stages of fruit maturity (Jackson *et al* 2004). The coconut fruit takes 11 to 12 months to reach full maturity and its composition changes as the nut grows (Jayalekshmy *et al.*, 1986). The sugars concentration gradually increases in the early months of maturation, maximum in eight month old nuts (Kamala and Velayutham 1978) and then decreases at the stage of full maturity (Raissa *et al,* 2007). At full maturity, coconut water represents 15% to 30% of the weight of the nut.

Potato is a leading vegetable and the main cash crop of farmers of Pakistan. Its production in Pakistan is considerably low because of many factors particularly viral diseases. Potato leaf roll virus (PLRV), potato virus X (PVX), potato virus Y (PVY), potato virus S (PVS) and potato virus A (PVA) are reported through out Pakistan which causes severe yield losses in potato (Qureshi *et al.*, 2014). Tissue culture has been successfully applied in vegetatively propagated species like potato for virus elimination (Naik and Karihaloo, 2007). The use of coconut water in tissue culture medium has been reported for various plant species like Banana, Date palm, Olive, Kiwifruit etc (Mamaril *et al.*, 1988; Peixe *et al.*, 2007: Nasib *et al.*, 2008; Vora, and Jasrai 2012; Khierallah and Hussein, 2013). The present study has been designed to compare the hormonal efficiency of coconut water extracted from coconut fruits at different stages of maturity on the *in vitro* growth of potato plantlets and to select the best concentration of coconut water that can be used effectively instead of synthetic growth regulators in potato micropropagation.

## Materials and methods

The experiment was conducted in potato tissue culture laboratory at Hazara Agriculture Research Station, Abbottabad in 2014.

*Experimental Materials*

Fresh coconut fruits at different maturity ages i.e. one month old, two months and six month old were collected from the garden of Dewa Academy Karachi, Pakistan. After removing the mesocarp, coconut water was extracted aseptically by making hole in the endosperm, the vials were marked as treatments T1(one month fruit), T2(two months old) and T3 (six months old). The potato plantlets with 5-6 nodes were selected as explants and nodal segments were used for inoculation.

## Methods

MS Media (Murashige & Skoog, 1962) containing 1 $mgl^{-1}$Ca-pentothenate, 100$mgl^{-1}$ Myoinsitol and 30$gl^{-1}$ sucrose with no growth hormones was prepared and coconut water for each treatment at three different concentrations (75, 150 and 300 ml $l^{-1}$ ) was used instead of synthetic plant growth regulators. The MS medium with no coconut water served as control. pH of the media was adjusted to 5.8 with either 0.1 N KOH or 0.1 N HCl before sterilization. For every treatment there were 10 replicates under each concentration. All the cultures were kept in the growth chamber at 22 $^{0}$C temperature and 16 h

photoperiod. Data were recorded after one week, 15 days, and at transferable plant stage (30 days) in terms of root/shoot emergence, number of roots, shoot length, number of nodes and number of leaves, stem diameter, leaf area and fresh weight of the plants. The data was analyzed by using computer software statistics 8.1 and Least significance difference test (LSD) at 95% level of significance was used to assess significant difference between the control and treated groups.

**Results**

The data revealed that the coconut water from each maturity stage showed great variation on the *in-vitro* growth of potato plantlets as compared to control.

**Table 1.** Plantlets showing root and shoot emergence on 2nd and 8th day of culturing.

| No. of Days | | No. of Plantlets | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | T1 | | T2 | | T3 | | Control (To) | |
| | Coconut water concentration (ml l-1) | Root Emergence | Shoot Emergence | Root Emergence | Shoot Emergence | Root Emergence | Shoot Emergence | Root Emergence | Shoot Emergence |
| 2nd Day | 75 | 0/10 | 10/10 | 1/10 | 10/10 | 3/10 | 10/10 | 0/10 | 7/10 |
| | 150 | 0/10 | 10/10 | 1/10 | 10/10 | 3/10 | 10/10 | 0/10 | 5/10 |
| | 300 | 0/10 | 10/10 | 0/10 | 10/10 | 3/10 | 10/10 | 0/10 | 10/10 |
| 8th Day | 75 | 6/10 | 10/10 | 0/10 | 10/10 | 7/10 | 10/10 | 3/10 | 10/10 |
| | 150 | 0/10 | 10/10 | 2/10 | 10/10 | 3/10 | 10/10 | 0/10 | 6/10 |
| | 300 | 1/10 | 10/10 | 2/10 | 10/10 | 8/10 | 10/10 | 1/10 | 10/10 |

*Root/Shoot Emergence*

On 2nd day of culturing shoot emergence was observed in all the three treatments (T1, T2, T3) as compared to control (To) (Table 1), however only 3 plantlets of treatment T3 showed roots at all the three concentrations (Fig. 1). While on 8th day it was noted that plantlets on MS media with 75 ml l-1 and 300 ml l-1 of 6 months old coconut water (T3) resulted in comparatively maximum root emergence i.e 7 and 8 plantlets, respectively (Table 1).

**Table 2.** Comparison of the effect of different treatments at different concentrations of coconut water on various growth parameters of in-vitro grown potato plantlets after 15 days.

| Conc: | No of Roots | | | | No. of leaves | | | | No. of Nodes | | | | Shoot Length | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mll-1 | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control |
| 75 | 2 a | 0.6cd | 1.7ab | 0.4cd | 4.5abcd | 5.2abc | 6.7ab | 2.6cd | 2.3abcd | 2.6abc | 3ab | 2cd | 2.62abc | 1.8cdef | 2.74ab | 0.92f |
| 150 | 0.3cd | 0.4cd | 1.1abc | 0.5cd | 6.6abc | 3.5d | 6.1abc | 2.6bcd | 2.8abc | 1.6d | 2.8abc | 2.1bcd | 2.42abcd | 1.04f | 2.09bcde | 1.28ef |
| 300 | 0.1 d | 0.2cd | 1bcd | 0.6cd | 6.1a | 5.2abc | 5.9abc | 3.6abcd | 3.2a | 2.8abc | 2.9abc | 2.3abcd | 3.02a | 1.67def | 2.33abcd | 1.79cdef |
| Mean | 0.8ab | 0.4b | 1.266a | 0.5b | 5.733ab | 4.633ab | 6.233a | 2.933b | 2.766ab | 2.33ab | 2.9a | 2.13b | 2.686a | 1.503b | 2.386a | 1.33b |

Mean values followed by different letters along a column or row are significantly different at P ≤ 0.05.

*No. of Leaves*

The data collected after 15 days (Table.2) showed no significance difference in mean number of leaves in any treatment of coconut water however, the maximum leaves (6.7) were recorded for treatment T3 at 75 ml l-1 concentration while the control showed significantly least number of leaves (Table.2). After one month (Table 3) the T3 potato plantlets showed the highest mean number of leaves (7.8) followed by T1 (6.9). No significant difference was observed for mean leaves number between treatment T2 and control. Regarding concentration effect the 300 ml l-1 concentration of every treatment enhanced the number of leaves (Table 3). Data recorded after 40 days also showed a similar trend for number of leaves where the treatment T3 showed significantly maximum leaves (8.66) (Table 4).

*No. of Nodes*

Statistical analysis after 15 days revealed no significant difference between treatments and control for no. of nodes. The concentration effect varied in

each treatment and the 300 ml $l^{-1}$ concentration of treatment T1 showed the greatest no. of nodes (3.2) (Table 2). Results after 30 days showed that the highest mean no. of nodes (5.9) were observed in treatment T3 at 300 ml $l^{-1}$ concentration (Table 3). Data at the time of transplantation showed similar trend for mean number of nodes among treatments (Table 4) .

**Table 3.** Comparison of the effect of different treatments at different concentrations of coconut water on various growth parameters of in-vitro grown potato plantlets after one month.

| Conc: | No of Roots | | | | No. of leaves | | | | No. of Nodes | | | | Shoot Length | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ml $l^{-1}$ | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control |
| 75 | 3.7a | 1.8bcd | 3.6a | 0.8d | 6.9ab | 6.2bc | 8.1a | 4.4c | 5.2abc | 3.4de | 5.6ab | 3.2de | 4.21abc | 2.59de | 5.11a | 1.58e |
| 150 | 1.2cd | 0.7d | 2.5abc | 0.5d | 6.8ab | 4.7c | 6.9ab | 4.7c | 4.6abcd | 2.3e | 4.8abcd | 3.4de | 3.54cd | 1.68e | 3.72bcd | 1.62e |
| 300 | 1d | 0.8d | 3.2ab | 0.8d | 7.2ab | 5.9bc | 8.6a | 5.5bc | 4.6abcd | 3.6cde | 5.9a | 4bcde | 4.79ab | 2.84de | 4.83ab | 1.832e |
| Mean | 1.96b | 1.1c | 3.1a | 0.7c | 6.96a | 5.6b | 7.86a | 4.86b | 4.8a | 3.1b | 5.43a | 3.53b | 4.18a | 2.37b | 4.553a | 1.677b |

Mean values followed by different letters along a column or row are significantly different at $P \leq 0.05$.

*Shoot Length*

Statistical analysis showed that no significant difference was observed between treatments and also between concentrations of each treatment for shoot length while the control has lowest mean shoot length i.e. 0.9 cm after 15 days of culturing (Table.2). At the time of transplantation the maximum shoot length was recorded at 300 ml $l^{-1}$ concentration of all the treatments (Table 4) as compared to control.

**Table 4.** Comparison of the effect of different treatments at different concentrations of coconut water on various growth parameters of in-vitro grown potato plantlets at transferable plant stage.

| Conc: | No of Roots | | | | Shoot Length (cm) | | | | No. of Leaves | | | | No. of Nodes | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mll$^{-1}$ | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control |
| 75 | 8ab | 3.1def | 9.3a | 0.8ef | 5.28bc | 3.58cde | 6.45ab | 2.38de | 6.5bcde | 7.6bcd | 8.2abc | 4.4e | 6.3a | 5.8ab | 6.4a | 3.2c |
| 150 | 4.6bcd | 0.3f | 6.7abc | 0.5f | 5.33bc | 1.83e | 5.07bc | 2.32de | 7.8abc | 6.1cde | 7.9abc | 4.7e | 6.2a | 4.1bc | 6.3a | 3.4c |
| 300 | 4cde | 0.5f | 8.9a | 0.8ef | 7.13a | 3.93cd | 6.79ab | 2.73de | 7.4bcd | 8.4ab | 9.9a | 5.5de | 6.2a | 6.2a | 7.5a | 4bc |
| Mean | 5.53b | 1.3c | 8.3a | 0.7c | 5.913a | 3.113b | 6.103a | 2.476b | 7.23b | 7.36b | 8.66a | 4.86c | 6.23ab | 5.36b | 6.733a | 3.533c |

Mean values followed by different letters along a column or row are significantly different at $P \leq 0.05$.

*Root Length*

Root length was recorded at the time of transplantation of the potato plantlets. The analysis of the data showed that treatment T3 (coconut water at 4 months maturity) increased the root length significantly at 300 ml $l^{-1}$ concentration while the minimum root length was observed in treatment T2 and control (Table 5) (Fig. 2).

*Stem Diameter*

The results showed that stem diameter was greatest in treatment T3 (2.697mm) in which coconut water from the fruit at six month maturity stage was used however treatment T1 in which one month fruit maturity stage coconut water was used showed the maximum stem diameter (2.934mm) at concentration 300 ml $l^{-1}$. The minimum stem diameter was observed in treatment T2 and control (Table.5).

*Leaf Area*

Analysis of the data showed that leaf area was influenced significantly by the treatments as compared to control. The maximum leaf area was showed by treatment T1 (0.655 $cm^2$) followed by treatment T3 (0.527 $cm^2$). Whereas regarding concentrations no significant effect was recorded on leaf area within each treatment (Table 5).

*Fresh Plant Weight*

The statistical analysis revealed that the difference in mean plants fresh weight was non-significant among treatments except control which differed significantly.

The maximum plant weight (474.73 mg) was observed in treatment T3 (Table.5).

**Discussion**

The coconut water contains several organic compounds and mineral nutrients as well as phytohormones: Auxin, Cytokinins, Gibberellins and Abscisic acid (Yong *et al.* 2009; Mullukattil, 2013). In the present study the results revealed that shoots were developed just after 2 days in all the three treatments of coconut water. It might be due to the presence of cytokinins (George & Sherrington 1984) that play major role in cell division, formation and activity of shoot meristem (Kwapata *et al.*, 1999 ; Razdan, 2003; Yong *at al.*, 2009).

**Table 5.** Comparison of the effect of different treatments at different concentrations of coconut water on various growth parameters of in-vitro grown potato plantlets at transferable plant stage.

| Conc: | Root length (cm) | | | | Stem Diameter (mm) | | | | Fresh Plant Weight (mg) | | | | Leaf Area ($cm^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ml[1] | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control | T1 | T2 | T3 | control |
| 75 | 5.09a | 1.7c | 4.43a | 0.77c | 1.906cd | 2.19bcd | 2.904ab | 1.998cd | 348.6bcd | 321.9bcd | 403abcd | 231.4d | 0.8175a | 0.1925d | 0.567abc | 0.4103bcd |
| 150 | 2.31bc | 0.71c | 4.22ab | 0.87c | 2.56abc | 1.824d | 2.268abcd | 2.066cd | 383.1abcd | 316.1bcd | 454.abc | 227.1d | 0.473bcd | 0.193d | 0.474bcd | 0.236d |
| 300 | 2.26bc | 0.51c | 6.11a | 1.2c | 2.934a | 2.522abcd | 2.92a | 2.353abcd | 421.4abcd | 508.3ab | 566.5a | 275.7cd | 0.674ab | 0.309cd | 0.5395abc | 0.1932d |
| Mean | 3.22b | 0.973c | 4.92a | 0.946c | 2.4673ab | 2.1786b | 2.6973a | 2.139b | 384.36a | 382.1a | 474.73a | 244.73a | 0.655a | 0.2315b | 0.527a | 0.2799b |

Mean values followed by different letters along a column or row are significantly different at $P \leq 0.05$.

The maximum root emergence and root number was observed in treatment T3 where 6 months old fruit coconut water was used. This may be due to the presence of adequate quantity of auxin known to be involved in adventitious rooting (Wiesman *et al.*, 1988) and changes in auxin level might occurs with increase in age of coconut fruit results in altering the auxin-cytokinin balance that causes the plants of treatment T3 to rapid root initiation. Agampodi & Jayawardena 2007 reported 0.088 mg $L^{-1}$ of IAA in coconut water which plays a major role in formation of main root, lateral and adventitious root initiation. Heloir *et al.*, 1996 discussed that the physiological changes of rooting are correlated with changes in auxin concentration and the high endogenous auxin concentration is normally associated with a high rooting rate at the beginning of the rooting process (Blažková *et al.*, 1997; Caboni *et al.*, 1997). Number of roots may also be influenced by the presence of GA3 in coconut water as reported by Farhatullah *et al.,* 2007 that 0.248 $mgL^{-1}$ GA3 doubled the root number per plantlet in potato as compared to control.

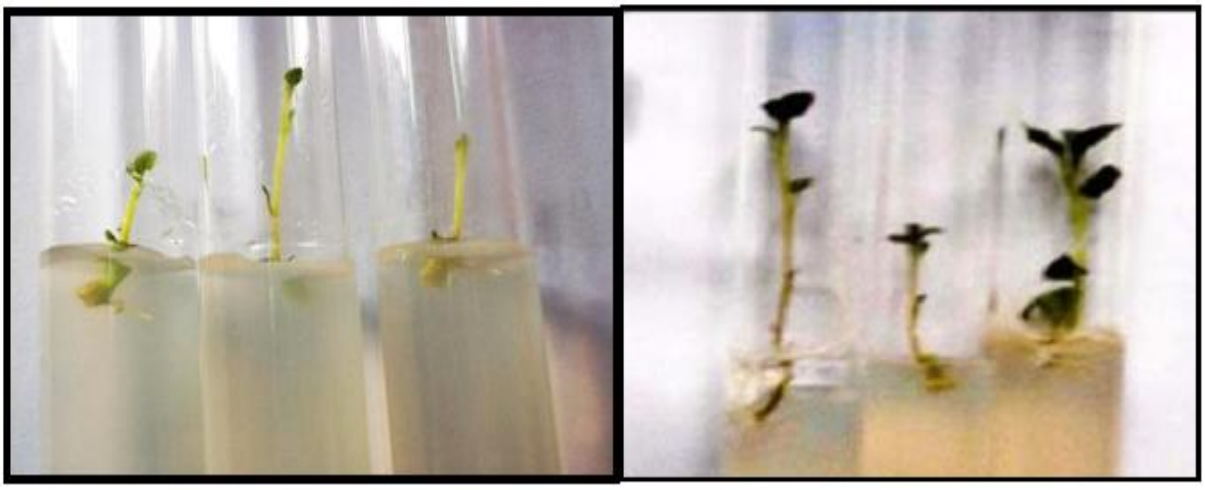

**Fig. 1.** Root/Shoot Emergence on 2nd day of culturing (left); plantlets showing root/shoot development after 15 days (right).

The numbers of leaves and nodes were also higher in treatment T3. Leaves arise from buds and it was reported by Afshin *et al.,* 2011 that cytokinins are usually known to make promotion of buds formation in many *in- vitro* cultured organs and shoot length and number of node of *Matthiola incana* increased with increasing kinetin concentration. Yong *at al.*, 2009 discussed that kinetin (0.31nM) is present in mature young green coconut water.


**Fig. 2.** *In vitro* grown potato plantlets showing root/shoot development at various coconut water treatments T1 (a), T2 (b), T3 (c) and control (d) at plant transferable stage.

The statistical analysis of the data revealed that among the treatments the highest shoot length was observed in treatments T1 & T3. The plantlets of T1 showing maximum shoot length have no roots and instead callus was formed (Fig.2). It was reported that when cytokinin and auxin are present in equal levels, the parenchyma cells form an undifferentiated callus, while more cytokinin induces growth of shoot buds (Chen, *et al.,* 1985). Shoot length can also be effected by the presence of Gibberellin in coconut water because it was also reported by Yong *et al*., 2009 that young green coconut water contain Gibberellin-1 (16.7nM), Gibberellin-3 (37.8 nM), and in-vitro addition of GA3 (0.5 mg.L-1) combined with low cytokinin concentration was effective in the shoot growth (Farhatullah *et al*., 2007). Similarly, in treatment T2 all the plantlets showed stunted growth with multiple shoots and callus formation (Fig.2). Afshin *et al.,* 2011 found that cytokinin is responsible for multiple shoot formation.

Root length of the potato plantlets was highly affected by treatment T3 and only by C1 concentration (6.25 ml/L) of treatment T1 (Table.5). The effect may be observed due to the presence of auxin, because IAA plays an important role in the regulation of root growth (Torrey, 1976 ; Overvoorde, 2010).

The treatment T3 also increased the stem diameter of the potato plantlets. It was reported by Naeem *et al*., 2004 that when IAA was applied on *Lens culinaris Medik*, it showed an expansion in stem diameter, while cytokinin plays important role in cell division in lateral meristems, and causes the stem and root thickness.

Coconut water also affected the leaf area and fresh weight of the plantlets as compared to control. Abdullahil, 2011 reported that 50 ml/l of coconut water in media significantly increased shoot and root fresh and dry weight, leaf width and leaf area of

*Calanthe* hybrids. In our studies MS media was supplemented with different concentration of coconut water and the plantlets of treatment T3 (six month old coconut water) showed excellent organogenesis at the concentration of 75ml/L and 300ml/L. The plantlets of treatment T2 showed stunted shoot growth as compare to T1&T3 but the callus formation was comparatively high in T2 which increased the fresh weight of the plantlets.

It has been concluded that the hormonal efficiency of coconut water changes with the maturity of coconut fruits and the coconut water from the 6 months old coconut fruit enhanced overall potato plantlets growth and development and can be used instead of synthetic plant growth regulator (PGR) in tissue culture media. However, further research is needed for the quantification of various hormones related to different stages of fruit maturity

**References**

**Abdullahil MB, Shin YK, Elshmari T, Lee EJ, Paek KY.** 2011. Effect of light quality, sucrose and coconut water concentration on the micro-propagation of Calanthe hybrids ('Bukduseong' × 'Hyesung' and 'Chunkwang' × 'Hyesung'). Australian Journal of Crop Sciences **5,** 1247-1254.

**Afshin AH, Kaviani B, Tarang A, Zanjani SBI.** 2011. Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics Journal 4, 236-238.

**Agampodi VA, Jayawardena B.** 2007. Identification and characterization of plant growth regulators present in coconut (*Cocos nucifera*) water using HPLC (High Performance Liquid Chromatography), Department of Chemistry, University of Kelaniya. 1- 118 P.

**Awua AK, Doe ED, Agyare R.** 2011. Exploring the influence of sterilisation and storage on some physicochemical properties of coconut (*Cocos nucifera* L.) water. BMC Research Notes. **4,** 451.

**Blažková A, Sotta B, Tranvan H, Maldiney R, Bonnet M, Einhorn J, Kerhoas L, Miginiac E.** 1997. Auxin metabolism and rooting in young and mature clones of *Sequoia sempervirens*. Physiologia Plantarum **99,** 73-80. http://dx.doi.org/10.1111/j.13993054.1997.tb03433.x

**Caboni E, Tonelli MG, Lauri P, Iacovacci P, Kevers C, Damiano C, Gaspar T.** 1997. Biochemical aspects of almond micro cuttings related to in vitro rooting ability. Biology Plant **39,** 91-97

**Cachiţă Cosma D.** 1987. Metode in vitro la plantele de cultură", Editura CERES, Bucureşti, 50-132 P.

**Chen CM, Ertl JR, Leisner SM, Chang CC.** 1985. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiol **78,** 510–513.

**Farhatullah, Abbas Z, Abbas SJ.** 2007. In -vitro effects of gibberellic acid on morphogenesis of potato explant. International Journal of Agriculture & Biology **1,** 181–182.

**Ge L, Peh CYC, Yong JWH, Tan SN, Hua L, Ong ES.** 2007. Analyses of gibberellins by capillary electrophoresis-mass spectrometry combined with solid-phase extraction. Journal of Chromatography A. **1159,** 242–249.

**Heloir MC, Kevers C, Hausman JF, Gaspar T.** 1996. Changes in the concentrations of auxin and polyamines during rooting of in-vitro-propagated walnut shoots. Tree Physiol. **16,** 515-519.

**Jackson JC, Gordon A, Wizzard G, McCook K, Rolle R.** 2004. Changes in chemical composition of coconut (*Cocos nucifera* L.) water during maturation of the fruit. Journal of the Science of Food and Agriculture **84,** 1049–1052. http://dx.doi.org/10.1002/jsfa.1783

**Jayalekshmy A, Arumughan C, Narayanan CS, Mathew AG.** 1986. Changes in the chemical

composition of coconut water during maturation. Journal of Food Science and Technology **23** , 203 207.

**Kamala Devi CB, Velayutham M.** 1978. Changes in the chemical composition of nut water and kernel during development of coconut. In: Proc First Annual Symp Plantation Crops, PLACROSYM I, Nelliat EV (ed), Indian Society for Plantation Crops, Kasaragod, Kerala, 20–23 March, 340–346.

**Khierallah SMH, Hussein NH.** 2013. The role of coconut water and casein hydrolysate in somatic embryogenesis of date palm and genetic stability detection using RAPD markers. Research in Biotechnology **4,** 20-28

**Kwapata M B, Kalengamaliro F, Bakuwa J, Manyela S.** 1999. In-vitro rooting and axillary shoots proliferation of faidherbia albida (del.) A. Chev. Under varying levels of plant growth regulators. African Crop Science Journal **7,** 303-311.

**Mamaril JC, Paner ET, Trinidad LC, Palacpac ES, Cruz A.** 1988. Enhancement of seedling growth with extracts from coconut water. Philippines Journal of Crop Science **13,** 1-7.

**Mullukattil RL.** 2013. The Chemical Composition of Tender Coconut (Cocos Nucifera L.) Water and Coconut Meat and Their Biological Effect in Human Body. International Journal of Green and Herbal Chemistry **2,** 723-729.

**Murashige T, Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum **15,** 473-497.

**Naeem M, Bhatti I, Ahmad RH, Ashraf MY.** 2004. Effect of some growth hormones (ga3, iaa and kinetin) on the morphology and early or delayed initiation of bud of lentil (*Lens Culinaris Medik).* Pakistan Journal of Botany **36,** 801-809.

**Naik PS, Karihaloo JL.** 2007. Micropropagation for production of quality potato seed in Asia- Pacific Consortium on Agricultural Biotechnology, New Delhi, India. 47 P.

**Nasib A, Kashif A, Saifullah K.** 2008. An optimized and improved method for the in vitro propagation of kiwifruit (*Actinidia deliciosa*) using coconut water. Pakistan Journal of Botany **40,** 2355-2360.

**Overvoorde P, Fukaki H, Beeckman T.** 2010. Auxin control of root development. Cold Spring Harb. Perspect. Biol. **2,** a001537.

**Peixe A, Raposo A, Lourenc o R, Cardoso H, Macedo E.** 2007. Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. Scientia Horticulture. **113,** 1–78.

**Qureshi MA, Gul Z, Ali S, Khan AR.** 2014. Comparative growth response of potato plantlets developed on liquid vs solidified (MS) medium using tissue culture technology. International Journal of Recent Scientific Research. **5,** 736-739.

**Razdan MK.** 2003. Introduction to Plant Tissue. 2nd Edition. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi. 27-29.

**Tulecke W.** 1961. The biochemical composition of coconut water coconut milk as related to its use in plant tissue culture. Contributions from Boyce Thompson Institute **21,** 115 -128

**Vora NC, Jasrai YT.** 2012. Natural and low-cost substitutes of synthetic PGR for micropropagation of banana . *CIB* Tech Journal of Biotechnology **2, 9**-13

**Wiesman Z, Riov J, Epstein E.** 1988. Comparison of movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean cuttings. Physiologia Plantarum **74,** 556-560. http://dx.doi.org/10.1111/j.13993054.1988.tb02018.x

**Yong JWH, Ge L, Ng YF, Tan SN.** 2009. The chemical composition and biological properties of coconut (Cocos nucifera L.) water. Molecules. **14,** 5144-5164. http://dx.doi.org/10.3390/molecules14125144