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Effect of Vitamins on *In Vitro* Organogenesis of Plant

Peter Abrahamian, Arumugam Kantharajah*

Department of Agricultural Sciences, American University of Beirut, Riad El Solh, Beirut, Lebanon.
Email: a.kantharajah@hotmail.com

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ABSTRACT

Vitamins are necessary compounds synthesized and utilized in plants. In tissue culture media, vitamin addition is not always common; since the amount needed by plants is relatively unknown and varies. Vitamins, in combination with other media constituents, have been shown to have direct and indirect effects on callus growth, somatic growth, rooting, and embryonic development. For example, different studies have shown that thiamine is associated with cytokinin and has a role in inducing callus growth and rooting. Moreover, thiamine was essential in facilitating the production of more secondary metabolites such as proteases in pineapple. Both biotin and riboflavin play a role in callus development as well. Specifically, riboflavin exerts different effects on plant rooting either positively and negatively. Vitamin D known to cause uptake of calcium in animal tissue, exerts a similar effect in plants. In addition, vitamin D causes cell elongation and meristematic cell division. Vitamin C, known for its anti-oxidative properties, has also enhanced shoot growth and rooting.

***Keywords*:** *Vitamin*, *Organogenesis*, *In Vitro*, *Plant Tissue Culture*, *Plant Propagation*

1. Introduction

Plants are a major source of essential vitamins for humans and animals. Their function and synthesis pathways have been extensively studied. Vitamin syntheses in plants are mainly used as essential intermediates in biochemical reactions or as catalysts in various pathways. Vitamins are divided into two main groups, the water-soluble (Ascorbic acid "C"; thiamine "B_1"; riboflavin "B_2"; pyridoxine "B_6"; nicotinic acid; cobalamin "B_{12}"; folic acid; pantothenic acid "B_5"; biotin) and fat-soluble (A, D, E, K) vitamins [1]. According to Bonner [2], working on water-soluble vitamins is of higher interest than fat-soluble vitamins. In tissue culture, some plants can become deficient in vitamin synthesis [3]. Hence, supplementing plant tissue with sub-optimal levels is essential to obtaining vigorous growth. Plant cell requirements for vitamin concentration vary according to the plant species and type of culture.

Thiamine pyrophosphate (TPP) is a derivative of Thiamine (Vit. B1) [1]. Thiamine's physiological functions in plants are diverse and serve as cofactors in enzymatic reactions including pentose phosphate pathway, glycolysis, tricarboxylic acid cycle (TCA), pyruvate dehyrdrogenase complex, transketolase, and pyruvate decarboxylase [4]. Pyruvate decarboxylase has shown to be imperative in energy production in Arabidopsis [5]. Thiamine has also been associated with disease resistance, and expression of PR-1 gene with local acquired resistance, but not systemic acquired resistance (SAR) [6], however, Ahn *et al*. [7] showed induced SAR in Arabidopsis. Under conditions of abiotic stress in Arabidopsis, endogenous thiamine increases dramatically to cope with oxidative stresses by supplying NADH and NADPH [8]. Vitamin C or ascorbate is oxidized by oxygen, hydrogen peroxides, and superoxides into monodehydroascorbate (MDHA) radicals. Ascorbate oxidase is possibly related to cell wall expansion and growth. MDHA, a product of ascorbate oxidase, radicals obtained depolarize the plasma membrane hence causing ion uptake and wall loosening [9]. Other vitamins such as riboflavin, a precursor of FAD and FMN coenzymes, and nicotinic acid, precursor of NAD and NADP, participate in cellular redox reactions. In this review paper, we will provide a basic summary of how these pathways are exhibited, at the macro level, upon vitamin addition to plant tissue culture media.

2. Vitamins in Tissue Culture

In tissue culture media, thiamine, nicotinic acid, pyri-

doxine and myo-inositol found in Murashige and Skoog [10] (MS) medium at 0.1 $mg \cdot l^{-1}$, 0.5 $mg \cdot l^{-1}$, 0.5 $mg \cdot l^{-1}$, and 100 $mg \cdot l^{-1}$ respectively are the most commonly used, while the addition of other essential vitamins to media is uncertain. Myo-inositol remains a controversial compound being either classified as a water-soluble plant vitamin or as a sugar alcohol [3]. Earlier studies in pea embryos done by Ray [11] have shown that it is possible to achieve good *in vitro* growth by increasing vitamin C content. This finding cannot be broadly applied as some plants are less receptive to increasing concentrations of vitamin C, indicating more autotrophism than other plants (tomato and oat) [2]. It has also been noticed that adding biotin increased the shoot dry weights of peas, similar to the response observed in *Ricciocarpus* plants treated with pantothenic acid. Unlike other vitamins, thiamine additions to pea embryos *in vitro* affect rooting and shoot growth simultaneously. *In vitro* studies have shown that tomato roots are capable of exhibiting prolonged thiamine dependency. [2].

2.1. Micropropagation

In the presence of 25 $mg \cdot l^{-1}$ of vitamin D_3 micro propagated potato plantlets absorbed Ca^{2+} efficiently [12]. However, vitamin D_3 concentrations higher than 25 $mg \cdot l^{-1}$ *i.e*. 50 $mg \cdot l^{-1}$ did not stimulate higher absorption levels. On the other hand vitamin D_2 suppressed Ca^{2+} uptake. It was concluded that combining both vitamins D_2 and D_3 did not improve calcium absorption hence claiming the superiority of vitamin D_3 for calcium ion uptake [12].

2.2. Callus & Somatic Growth

Gamborg *et al*. [13] cultured soybean root cells unto several media containing different concentrations of thiamine, and to a complete B5 culture media. An initial amount of 53 mg of soybean cell culture was grown in 0 $mg \cdot l^{-1}$ and 10 $mg \cdot l^{-1}$ of thiamine. After 5 days, 138 mg and 203 mg of soybean cells were produced, respectively. Pyridoxine, nicotinic acid and myo-inositol present in the media had no adverse effects on growth individually. Consequently, Gamborg *et al*. [13] concluded the necessity of providing thiamine to the media to sustain growth of soybean root cell.

Eriksson [14] have concluded that nicotinic acid and pyridoxine are essential vitamins accompanying thiamine, when studying the optimum growth of *Haplopappus gracilis* Nutt. on a modified medium of MS [10].

Polikarpochkina *et al*. [15] reported that maize calli decreased in weight from 110 mg/ml to nil after 3 successive passages when thiamine is eliminated. However, the removal of inositol and pyridoxine from the MS [10] medium did not give any significant difference on growth [15]. The change recorded from the first passage until the third passage, when omitting inositol and pyridoxine was 9% and 2.5%, respectively.

Digby and Skoog [16] found a relation between kinetin and thiamine in the normal callus culture of tobacco. It has been shown that high levels of kinetin are needed to induce thiamine synthesis in the absence of any exogenous thiamine added. However, sustaining growth on a low level kinetin media was not possible except if thiamine was added. Whereas, Linsmaier and Skoog [17] maintained tobacco cultures with 1000 μg/l of kinetin and nil thiamine over 17 passages. Dravnieks *et al*. [18] later confirmed that thiamine synthesis was subject to feedback control mechanism thus sensitive to the amount of thiamine in tissue, regardless of kinetin concentration.

Both thiamine and biotin significantly affected callus growth of date palm [19]. Increasing thiamine from 0.1 $mg \cdot l^{-1}$ to 0.5 $mg \cdot l^{-1}$ caused maximum callus growth; furthermore, increasing thiamine to 2 $mg \cdot l^{-1}$ gave reduced callus weights. Moreover, increasing biotin from 0 to 1 $mg \cdot l^{-1}$ gave a maximum callus weight similar to thiamine [19]. On the other hand, an earlier report by Drew and Smith [20] showed that presence of riboflavin reduced callus growth of Papaya. A significant decline in mean callus weight was recorded from 89.32 mg to 0.10 mg per explant, in the absence and presence of riboflavin, respectively [20].

Ascorbic acid, functioning primarily as an antioxidant, is used to prevent browning of tissue [1,3]. However, in tobacco cells, ascorbic acid has been shown to function as a stimulant of mitotic cell division [21].

2.3. Rooting

Vitamin D_3 stimulates rooting of *Phaseolus vulgaris* L. in culture [22]. In a control treatment without any vitamins 43.75% of the roots were longer than 14 mm, while vitamin D_3 addition achieved 78.75%. The effect of vitamin D_3 shown by Boland *et al*. [22] at 10^{-9} M, on root growth was associated with an uptake of calcium ions, an increase in cell elongation in root zone at 0.5 - 1 mm from the apex, and stimulation of mitotic division of meristematic cells.

In vitro rooting of peach rootstock GF677 (*Prunus amygdalus* × *P. persica* Batsch.) was studied by adding different concentrations of riboflavin ranging from 0 to 2.0 $mg \cdot l^{-1}$ [23]. As more riboflavin was added rooting decreased in a linear form until it was completely inhibited. The smallest concentration of 0.5 $mg \cdot l^{-1}$ of riboflavin caused the average number, length, fresh weight and dry weight to decrease sharply [23]. Whereas at 1.5 and 2 $mg \cdot l^{-1}$ of riboflavin chlorotic and necrotic symptoms appeared. Moreover, adventitious root formation in the control was long and thin, while in the treated media, roots were short and thick. Also, callus formation was

inhibited in the rooting MS media, due to the suppressing action of auxin by photo-degradation [23].

On the contrary, riboflavin has been shown to stimulate and help rooting significantly [24-26]. Rooting in apple tissue culture was studied in the presence of riboflavin. In the dark riboflavin stimulated rooting significantly in the presence of auxin (IBA), whereas rooting decreased when the vitamin was omitted and exposed to light [24]. Trindade and Pais [25] showed that *Eucalyptus globulus* Labill. produced 80% rooting ability on a revised De Fossard [27] media containing riboflavin (**Table 1**). On the other hand, 60% rooting was achieved on the same media excluding the latter compound [25]. *Carica papaya* L. rhizogenesis was optimal when 31 μM of riboflavin and 10 μM of IBA were added to the De Fossard [27] media in the dark for 2 days, but losses occurred during media preparation [26]. However, Drew *et al*. [26] found a way to avoid the loss of IBA due to light exposure. The procedure involved injecting riboflavin at 300 μM per ml into 10 ml of media, which is equivalent to the optimum riboflavin level 31 μM, after 1 day of IBA rich medium in the light [26].

Thiamine is another vitamin shown to have significant rooting on pacific yew, an evergreen, *Taxus brevifolia* Peattie [28]. Upon adding thiamine, Chee *et al*. [28] obtained 61.5% of adventitious rooting in *T. brevifolia* Peattie compared to 30% without thiamine. In a literature review on *Eucalyptus* propagation, vitamin E, other than being an antioxidant, affected rooting and speeded up the rooting process upon addition to culture media [29].

Table 1. Effect of vitamins on plant growth and development in *in vitro*.

Vitamin	Function[1]	Culture Medium	Concentration	Common Name (Species)	Effect[2]	Reference
Thiamine (B1)	Cofactor in carboxylase reactions and amino acid biosynthesis	B5 + 2 mg·l^{-1} 2,4-D	10 mg·l^{-1}	Soybean (*Glycine max* L.)	Stimulate cell growth	Gamborg *et al*. 1968
		MS basal medium + 1.7 gM BAP + 0.2 gM IBA	1.0 μM		Increase embryogenesis	Barwale *et al*. 1986
		MS + 2 mg·l^{-1} 2,4-D	0 mg·l^{-1}	Maize (*Zea mays* L.)	Decrease callus weight	Polikarpochkina *et al*. 1979
		MS (Hormone free MS)	0.5 mg·l^{-1}; 0.5 mg·l^{-1} or 2 mg·l^{-1}	Palm (*Phoenix dactylifera* L.)	Increase callus weight, embryo number; embryo length	Al-Khayri 2001
		MS + 4.2 μM GA	0.3 μM	Pineapple (*Ananas comosus* L.)	Reduce shoot fresh mass	Pérez *et al*. 2004
		Linsmaier and Skoog + 5 mg·l^{-1} 2,4-D + 0.1 mg·l^{-1} BA	0.4 mg·l^{-1}	Turf grass (*Zoysia japonica* Steud.)	Increase embryonic callus	Asano *et al*. 1996
		MS + 1 mg·l^{-1} IBA	0.5 - 2 mg·l^{-1}	Peach (*Prunus amygdalus x persica* Batsch.)	Inhibit rooting and reduces callus	Dimassi *et al*. 2005
Riboflavin (B_2)	Oxidation-reduction reactions (Transfer of electrons)	De Fossard + 1.11 μM BA + 0.1 μM IBA	7.97 μM	*Eucalyptus globulus Labill.*	Stimulate rooting	Trindade and Pais 1997
		De Fossard + 10 μM IBA	31 μM	Papaya (*Carica papaya* L.)	Stimulate rooting	Drew *et al*. 1993
		MS + 3.2 μM IBA	Not Known	Apple (*Malus domestica* Borkh.)	Stimulate rooting	Van der Krieken 1992
Vitamin D_3	-	Shenk-Hildebrandt (Hormone free)	10^{-9} M	Common Bean (*Phaseolus vulgaris* L.)	Stimulate rooting, mitotic division, and calcium absorption	Boland *et al*. 1989
		MS (Hormone free)	25 mg·l^{-1}	Potato (*Solanum tuberosum* L.)	Enhance Calcium absorption	Habib and Donnelly 2003
Biotin	Cofactor of enzymes	MS (Hormone free)	2 mg·l^{-1}; 1 mg·l^{-1}	Palm (*Phoenix dactylifera* L.)	Increase callus weight, embryo number; embryo length	Al-Khayri 2001
Vitamin C (Ascorbate)	Reducing Agent	MS + 10 μM IAA + 10 μM Kinetin	4 - 8× 10^{-4} M	Tobacco (*Nicotiana tabacumn* L.)	Increases shoot number	Joy *et al*. 1988
Nicotinic Acid	Oxidation-reduction reactions	MS basal medium + 1.7 gM BAP + 0.2 gM IBA	32.4 μM	Soybean (*Glycine max* L.)	Increase embryogenesis	Barwale *et al*. 1986

[1]Biochemical pathway in plant cell; [2]Effects reported have been due to mixed interaction between vitamin and hormones in media, unless stated otherwise.

2.4. Embryo & Organ Development

Thiamine and nicotinic acid have been shown to affect embryogenesis [30]. Barwale *et al*. [30] studied the effect of different concentration of both vitamins on 40 immature soybean embryos cultured to a modified MS [10] medium. Thiamine at 1.0 μM, or more, has induced 68% embryogenesis compared to 0.2 μM, the level of salts in MS medium, at 33% of the immature embryos. Also, a concentration of 32.4 μM nicotinic acid induced 76% embryogenesis [30].

Asano *et al*. [31] showed that enhancing embryonic callus of *Zoysia japonica* Steud., a warm season turf grass native to Japan, is obtained by adding thiamine and riboflavin to the media. When thiamine was excluded from the medium 50.3% callus was obtained, on the contrary, 0.4 and 4 $mg{\cdot}l^{-1}$ gave 53 and 60.3% respectively, both insignificantly different. Furthermore, riboflavin was not effective alone, except in the presence of thiamine at 4 $mg{\cdot}l^{-1}$ or higher concentration [31].

Thiamine and biotin have shown to be essential components of tissue culture media for optimizing embryogenesis of date palm (*Pheonix dactylifera* L.) [19]. The effect of thiamine has been shown to be dependent on biotin for maximizing the number of somatic embryos, which was also mentioned by Bonner [2]. The highest number of embryos obtained was with a treatment of 0.5 or 2 $mg{\cdot}l^{-1}$ thiamine and 2 $mg{\cdot}l^{-1}$ biotin. However, the optimum concentration for embryo number was a media containing 0.5 $mg{\cdot}l^{-1}$ thiamine and 2 $mg{\cdot}l^{-1}$ biotin. Embryo elongation also was affected by an interaction between both biotin and thiamin. The maximum embryo length was achieved by 0.5 or 2 $mg{\cdot}l^{-1}$ thiamine and 1 $mg{\cdot}l^{-1}$ biotin [19].

Pérez *et al*. [32] studied the effect of thiamine and other compounds on protease excretion in pineapple culture. Exogenous amounts of thiamine in the range of 0.3 - 1.2 μM had a negative effect on pineapple shoot fresh mass, forming a plateau [32]. On the other hand, thiamine produced a maximum protein content at 0.6 μM, while proteolytic and specific proteolytic activities both at 0.3 μM [32].

Shoot weight of Papaya significantly increased in the presence of both cytokinin and riboflavin, compared to a medium of cytokinin only [20]. While a decrease of shoot weight in the presence of riboflavin and auxin, possibly related to photo-oxidation of auxin, also conveyed in Gorst *et al*. [33] on *Eucalyptus ficifolia* F. Muell, was reported in comparison to a medium containing only auxin [20]. Also Drew *et al*. [34] reported that auxin (Indole-3-butyric acid) concentrations at 10 μM with more or less than, but not, 1 μM of riboflavin caused a small rooting percentage. Moreover, increasing the concentration of riboflavin gradually from 0.1, 1, to 10 μM degraded IBA, in the presence of light [34]. When 10 μM of IBA is used, a complete destruction of IBA occurs after 16 days versus 2 days when riboflavin is absent and present, in light, respectively [34].

Exogenous application of 8×10^{-4} M and $4 - 8 \times 10^{-4}$ M of ascorbate to a shoot-forming media enhanced shoot formation increased by 45% and 450% when using young callus tissue (4 - 12 subcultures) and old callus (>30 subcultures) of tobacco (*Nicotiana tabacum* L), respectively, after 35 days in culture [35]. In the non-shoot forming media, containing gibberellic acid, shoot-growth of the young callus was significant at 4×10^{-4} M and almost negligible for the old callus [35]. The former phenomenon indicates an inhibitory action by ascorbate on gibberellic acid. In addition, ascorbic acid reduced the shoot-forming period [35].

Roest and Bokelmann [36] have shown that a high number of adventitious shoot formation and transferable shoots of *Chrysanthemum* was obtained when vitamins were kept in the complete MS [10] medium. Whereas, a medium where vitamins were eliminated suppressed shoot formation although all other minerals were retained [36].

On the contrary, omitting vitamins (thiamine, pyridoxine, nicotinic acid, folic acid, and biotin) from a Bourgin and Nitsch [37] media in vitro did not affect 16 cultivars, except one, of *Begonia x hiemalis* shoot and root formation [38]. Also, Soczek and Hempel [39] studied the shoot multiplication of three *Gerbera* cultivars in the presence and absence of thiamine, pyridoxine, nicotinic acid and other compounds. It was concluded that reducing the concentration, to half or quarter of the Murashige *et al*. [40] medium, or removing the vitamins, did not have any significance on growth over three passages (each 4 weeks), except in the case of one cultivar requiring nicotinic acid [39].

3. Conclusions

Vitamins in culture media should be further studied in order to justify their addition. For instance, little is known about vitamin E (α-tocopherol), a phenol anti-oxidant, presence in culture media. In the last few decades, little interest has been observed in studying certain vitamins, such as biotin and pantothenic acid. Plant species and cultivars require different amount of vitamins, while other do need any at all. For instance, after several passages, thiamine is essential to soybean, rice, and tobacco cultures but non-essential to peanut cells, which contain high thiamine concentration [41]. The physiological and morphological output varies between plants when using the same vitamins. According to our desired outcome culture media remain open to modifications, especially

the common Murashige and Skoog [10]. Although significant vitamins such as thiamine impose their application in culture media; others are poorly applied such as ascorbic acid.

Scientific knowledge on plant propagation was not the only significant outcome; however, some experiments offered economic solutions. In order to reduce costs, Drew *et al*. [27,34] suggested adding riboflavin, which degrades auxin, to the tissue culture media rather than transferring the tissue to a hormone (*i.e.* auxin) free media. In the future, studying the effect on a wider range of vitamins and plant simultaneously is needed for an enhanced feasibility outcome.

REFERENCES

[1] R. H. Horton, "Coenzymes and Vitamins," *Principles of Biochemistry*, Pearson Education International, Upper Saddle River, 2006.

[2] J. Bonner, "The Role of Vitamins in Plant Development," *Botanical Review*, Vol. 3, No. 12, 1937, pp. 616-640. doi:10.1007/BF02872294

[3] E. F. George, M. A. Hall and G.-J. De Klerk, "The Components of Plant Tissue Culture Media II," *Plant Propagation by Tissue Culture*, Springer, Dordrecht, 2008, pp. 115-119.

[4] A. Goyer, "Thiamine in Plants: Aspects of Its Metabolism and Functions," *Phytochemistry*, Vol. 71, No. 14-15, 2010, pp. 1615-1624. doi:10.1016/j.phytochem.2010.06.022

[5] O. Kursteiner, I. Dupuis and C. Kuhlemeier, "The Pyruvate Decarboxylase1 Gene of Arabidopsis Is Required During Anoxia but Not Other Environmental Stresses," *Plant Physiology*, Vol. 132, No. 2, 2003, pp. 968-978. doi:10.1104/pp.102.016907

[6] J. Malamy, P. S. Casas, J. Hennig, A. L. Guo and D. F. Klessig, "Dissection of the Salicylic Acid Signaling Pathway in Tobacco," *Molecular Plant—Microbe Interaction*, Vol. 9, No. 6, 1996, pp. 474-482. doi:10.1094/MPMI-9-0474

[7] I. P. Ahn, S. Kim and Y. H. Lee, "Vitamin B1 Functions as an Activator of Plant Disease Resistance," *Plant Physiology*, Vol. 138, No. 3, 2005, pp. 1505-1515. doi:10.1104/pp.104.058693

[8] M. Tunc-Ozdemir, "Thiamin Confers Enhanced Tolerance to Oxidative Stress in Arabidopsis," *Plant Physiology*, Vol. 151, No. 1, 2009, pp. 421-432. doi:10.1104/pp.109.140046

[9] J. A. Gonzalez-Reyes, F. J. Alcain, J. A. Caler, A. Serrano, F. Cordoba and P. Navas, "Stimulation of Onion Root Elongation by Ascorbate and Ascorbate Free Radical in *Allium cepa* L," *Protoplasma*, Vol. 184, 1995, pp. 31-35. doi:10.1007/BF01276898

[10] T. Murashige and F. Skoog, "A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures," *Physiologia Plantarum*, Vol. 15, No. 3, 1962, pp. 473-497. doi:10.1111/j.1399-3054.1962.tb08052.x

[11] S. Ray, "On the Nature of the Precursor of the Vitamin C in the Vegetable Kingdom. I. Vitamin C in the Growing Pea Seedling," *Biochemical Journal*, Vol. 28, No. 3, 1934, pp. 996-1003.

[12] A. Habib and D. J. Donnelly, "Vitamin Ds Improve Calcium Uptake in Micropropagated Potato Plantlets," *Acta Horticulturae*, Vol. 619, 2003, pp. 263-269.

[13] O. Gamborg, R. Miller and K. Ojima, "Nutrient Requirements of Suspension Cultures of Soybean Root Cells," *Experimental Cell Research*, Vol. 50, No. 1, 1968, pp. 151-158. doi:10.1016/0014-4827(68)90403-5

[14] T. Eriksson, "Studies on the Growth Requirements and Growth Measurements of Cell Cultures of *Haplopappus gracilis*," *Physiologia Plantarum*, Vol. 18, No. 4, 1965, pp. 976-993. doi:10.1111/j.1399-3054.1965.tb06994.x

[15] R. T. Polikarpochkina, K. Z. Gamburg and E. E. Khavin, "Cell-Suspension Culture of Maize (*Zea mays* L.)," *Zeitschrift fr Pflanzenphysiologie*, Vol. 95, No. 1, 1979, pp. 57-67.

[16] J. Digby and F. Skoog, "Cytokinin Activation of Thiamine Biosynthesis in Tobacco Callus Cultures," *Plant Physiology*, Vol. 41, No. 4, 1966, pp. 647-652. doi:10.1104/pp.41.4.647

[17] E. M. Linsmaier-Bednar and F. Skoog, "Thiamine Requirement in Relation to Cytokinin in 'normal' and 'mutant' Strains of Tobacco Callus," *Planta*, Vol. 72, No. 2, 1966, pp. 146-154. doi:10.1007/BF00387478

[18] D. E. Dravnieks, F. Skoog and R. H. Burris, "Cytokinin Activation of De Novo Thiamine Biosynthesis in Tobacco Callus Cultures," *Plant Physiology*, Vol. 44, No. 6, 1969, pp. 866-870. doi:10.1104/pp.44.6.866

[19] J. M. Al-Khayri, "Optimization of Biotin and Thiamine Requirements for Somatic Embryogenesis of Date Palm (*Phoenix dactylifera* L.)," *In Vitro Cellular & Developmental Biology—Plant*, Vol. 37, No. 4, 2001, pp. 453-456. doi:10.1007/s11627-001-0079-x

[20] R. A. Drew and N. G. Smith, "Growth of Apical and Lateral Buds of Papaya (*Carica papaya* L.) as Affected by Nutritional and Hormonal Factors," *Journal of Horticultural Science*, Vol. 61, No. 1, 1986, pp. 535-543.

[21] M. C. De Pinto, D. Francis and L. Gara, "The Redox State of the Ascorbate-dehydroascorbate Pair as a Specific Sensor of Cell Division in Tobacco BY-2 Cells," *Protoplasma*, Vol. 209, No. 1-2, 1999, pp. 90-97. doi:10.1007/BF01415704

[22] R. Boland, L. Talmon, M. Vega and B. Mujica, "Cytohistological Studies on the Action of Vitamin D3 and Stigmasterol on Phaseolus Vulgaris Roots Growing in Vitro," *Plant Science*, Vol. 59, No. 2, 1989, pp. 183-190. doi:10.1016/0168-9452(89)90136-2

[23] K. Dimassi, C. Antonopoulou, I. Therios, C. Chatzissavvidis and V. Tsirakoglou, "Inhibitory Effects of Riboflavin (Vitamin B) on the *in Vitro* Rooting and Nutrient Concentration of Explants of Peach Rootstock GF 677 (×)," *Scientia Horticulturae*, Vol. 106, No. 2, 2005, pp. 268-272.

[24] W. M. Van der Krieken, H. Breteler, M. H. M. Visser and W. Jordi, "Effect of light and riboflavin on indolebutyric acid-induced root formation on apple *in vitro*," *Physiologia Plantarum*, Vol. 85, No. 4, 1992, pp. 589-594. doi:10.1034/j.1399-3054.1992.850405.x

[25] H. Trindade and M. S. Pais, "*In Vitro* Studies on Eucalyptus Globulus Rooting Ability," *In Vitro Cellular & Developmental Biology—Plant*, Vol. 33, No. 1, 1997, pp. 1-5. doi:10.1007/s11627-997-0032-8

[26] R. A. Drew, J. A. McComb and J. A. Considine, "Rhizogenesis and Root Growth of *Carica Papaya* L. *in Vitro* in Relation to Auxin Sensitive Phases and Use of Riboflavin," *Plant Cell*, *Tissue and Organ Culture*, Vol. 33, No. 1, 1993, pp. 1-7. doi:10.1007/BF01997591

[27] R. A. Fossard, A. Myint and E. C. M. Lee, "A Broad Spectrum Tissue Culture Experiment with Tobacco (*Nicotiana tabacum* L.) Pith Tissue Callus," *Physiologia Plantarum*, Vol. 31, No. 2, 1974, pp. 125-130. doi:10.1111/j.1399-3054.1974.tb03116.x

[28] P. P. Chee, "Stimulation of Adventitious Rooting of *Taxus* Species by Thiamine," *Plant Cell Reports*, Vol. 14, No. 12, 1995, pp. 753-757. doi:10.1007/BF00232916

[29] J. J. Le Roux and J. Van Staden, "Micropropagation and Tissue Culture of Eucalyptus—A Review," *Tree Physiology*, Vol. 9, No. 4, 1991, pp. 435-477.

[30] U. B. Barwale, H. R. Kerns and J. M. Widholm, "Plant Regeneration from Callus Cultures of Several Soybean Genotypes via Embryogenesis and Organogenesis," *Planta*, Vol. 167, No. 4, 1986, pp. 473-481. doi:10.1007/BF00391223

[31] Y. Asano, H. Katsumoto, C. Inokuma, S. Kaneko, Y. Ito and A. Fujiie, "Cytokinin and Thiamine Requirements and Stimulative Effects of Riboflavin and Alpha-Ketoglutaric Acid on Embryogenic Callus Induction from the Seeds of *Zoysia japonica* Steud," *Journal of Plant Physiology*, Vol. 149, No. 3-4, 1996, pp. 413-417.

[32] A. Pérez, L. Nápoles, C. Carvajal, M. Hernandez and J. C. Lorenzo, "Effect of Sucrose, Inorganic Salts, Inositol, and Thiamine on Protease Excretion during Pineapple Culture in Temporary Immersion Bioreactors," *In Vitro Cellular & Developmental Biology—Plant*, Vol. 40, No. 3, 2004, pp. 311-316. doi:10.1079/IVP2004529

[33] J. R. Gorst, M. Slaytor and R. A. De Fossard, "The Effect of Indole-3-Butyric Acid and Riboflavin on the Morphogenesis of Adventitious Roots of *Eucalyptus ficifolia* F. Muell. Grown *in Vitro*," *Journal of Experimental Botany*, Vol. 34, No. 11, 1983, pp. 1503-1515. doi:10.1093/jxb/34.11.1503

[34] R. A. Drew, B. W. Simpson and W. J. Osborne, "Degradation of Exogenous Indole-3-Butyric Acid and Riboflavin and Their Influence on Rooting Response of Papaya *in Vitro*," *Plant Cell*, *Tissue and Organ Culture*, Vol. 26, No. 1, 1991, pp. 29-34. doi:10.1007/BF00116606

[35] R. W. Joy, K. R. Patel and T. A. Thorpe, "Ascorbic Acid Enhancement of Organogenesis in Tobacco Callus," *Plant Cell*, *Tissue and Organ Culture*, Vol. 13, No. 3, 1988, pp. 219-228. doi:10.1007/BF00043670

[36] S. Roest and G. Bokelmann, "Vegetative Propagation of Chrysanthemum morifolium Ram. *in Vitro*," *Scientia Horticulturae*, Vol. 3, No. 4, 1975, pp. 317-330. doi:10.1016/0304-4238(75)90046-1

[37] J. P. Bourgin and J. P. Nitsch, "Obtention de Nicotiana Haploïdes à Partir d'Étamines Cultivées in Vitro. (Production of Haploid *Nicotiana* from Excised Stamen)," *Annales de Physiologie Végétale*, Vol. 9, 1967, pp. 377-382.

[38] T. Welander, "*In Vitro* Organogenesis in Explants from Different Cultivars of Begonia x Hiemalis," *Physiologia Plantarum*, Vol. 41, No. 2, 1977, pp. 142-145. doi:10.1111/j.1399-3054.1977.tb05546.x

[39] U. Soczek and M. Hempel, "The Influence of Some Organic Medium Compounds on Multiplication of Gerbera *in Vitro*," *Acta Horticulturae*, Vol. 226, 1988, pp. 643-646.

[40] T. Murashige, M. Serpa and J. B. Jones, "Clonal Multiplication of Gerbera through Tissue Culture," *Horticulture Science*, Vol. 9, No. 3, 1974, pp. 175-180.

[41] K. Ohira, M. Ikeda and K. Ojima, "Thiamine Requirements of Various Plant Cells in Suspension Culture," *Plant Cell Physiology*, Vol. 17, No. 3, 1976, pp. 583-588.

