

In Vitro Organogenesis and Vitamins

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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

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 "B1"; riboflavin "B2"; pyridoxine "B6"; nicotinic acid; cobalamin "B12"; folic acid; pantothenic acid "B5"; biotin) and fat-soluble (A, D, E, K) vitamins. According to Bonner, working on water soluble vitamins is of higher interest than fat-soluble vitamins. In tissue culture, some plants can become deficient in vitamin synthesis. 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). 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 dehydrogenase complex, transketolase, and pyruvate decarboxylase. Pyruvate decarboxylase has shown to be imperative in energy production in Arabidopsis. Thiamine has also been associated with disease resistance, and expression of PR-1 gene with local acquired resistance, but not systemic acquired resistance (SAR), however, Ahn et al. 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. 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. 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.

Vitamins in Tissue Culture

In tissue culture media, thiamine, nicotinic acid, pyridoxine and myo-inositol found in Murashige and Skoog (MS) medium at 0.1 mg·l⁻¹, 0.5 mg·l⁻¹, 0.5 mg·l⁻¹, and 100 mg·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. Earlier studies in pea embryos done by Ray 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). 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.

Micropropagation:

In the presence of 25 mg·l⁻¹ of vitamin D3 micro propagated potato plantlets absorbed Ca²⁺ efficiently. However, vitamin D3 concentrations higher than 25 mg·l⁻¹ i.e. 50 mg·l⁻¹ did not stimulate higher absorption levels. On the other hand vitamin D2 suppressed Ca²⁺ uptake. It was concluded that combining both vitamins D2 and D3 did not improve calcium absorption hence claiming the superiority of vitamin D3 for calcium ion uptake.

Callus & Somatic Growth:

Gamborg *et al.* cultured soybean root cells into 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·l⁻¹ and 10 mg·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.* concluded the necessity of providing thiamine to the media to sustain growth of soybean root cell.

Eriksson 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.

Polikarpochkina *et al.* 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 medium did not give any significant difference on growth. 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 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 maintained tobacco cultures with 1000 µg/l of kinetin and nil thiamine over 17 passages. Dravnieks *et al.* 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. Increasing thiamine from 0.1 mg·l⁻¹ to 0.5 mg·l⁻¹ caused maximum callus growth; furthermore, increasing thiamine to 2 mg·l⁻¹ gave reduced callus weights. Moreover, increasing biotin from 0 to 1 mg·l⁻¹ gave a maximum callus weight similar to thiamine. On the other hand, an earlier report by Drew and Smith 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.

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

Rooting:

Vitamin D3 stimulates rooting of *Phaseolus vulgaris* L. in culture. In a control treatment without any vitamins 43.75% of the roots were longer than 14 mm, while vitamin D3 addition achieved 78.75%. The effect of vitamin D3 shown by Boland *et al.* 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·l⁻¹. As more riboflavin was added rooting decreased in a linear form until it was completely inhibited. The smallest concentration of 0.5 mg·l⁻¹ of riboflavin caused the average number,

length, fresh weight and dry weight to decrease sharply. Whereas at 1.5 and 2 mg·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. On the contrary, riboflavin has been shown to stimulate and help rooting significantly. 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. Trindade and Pais showed that *Eucalyptus globulus* Labill. produced 80% rooting ability on a revised De Fossard media containing riboflavin. On the other hand, 60% rooting was achieved on the same media excluding the latter compound. *Carica papaya* L. rhizogenesis was optimal when 31 µM of riboflavin and 10 µM of IBA were added to the De Fossard media in the dark for 2 days, but losses occurred during media preparation. However, Drew et al. 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. Thiamine is another vitamin shown to have significant rooting on pacific yew, an evergreen, *Taxus brevifolia* Peattie. Upon adding thiamine, Chee et al. 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.

Embryo & Organ Development:

Thiamine and nicotinic acid have been shown to affect embryogenesis. Barwale et al. studied the effect of different concentration of both vitamins on 40 immature soybean embryos cultured to a modified MS 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.

Asano et al. 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·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·l⁻¹ or higher concentration.

Thiamine and biotin have shown to be essential components of tissue culture media for optimizing embryogenesis of date palm (*Phoenix dactylifera* L.). 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. The highest number of embryos obtained was with a treatment of 0.5 or 2 mg·l⁻¹ thiamine and 2 mg·l⁻¹ biotin. However, the optimum concentration for embryo number was a media containing 0.5 mg·l⁻¹ thiamine and 2 mg·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·l⁻¹ thiamine and 1 mg·l⁻¹ biotin.

Pérez et al. 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. 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.

Shoot weight of Papaya significantly increased in the presence of both cytokinin and riboflavin, compared to a medium of cytokinin only. 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. on *Eucalyptus ficifolia* F. Muell, was reported in comparison to a medium containing only auxin. Also Drew et al. 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. 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. Exogenous application of 8 × 10⁻⁴ M and 4 - 8 × 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. 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. The former phenomenon indicates an inhibitory action by ascorbate on gibberellic acid. In addition, ascorbic acid reduced the shoot-forming period.

Roest and Bokelman 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 medium. Whereas, a medium where vitamins were eliminated suppressed shoot formation although all other minerals were retained.

On the contrary, omitting vitamins (thiamine, pyridoxine, nicotinic acid, folic acid, and biotin) from a Bourgin and Nitsch media in vitro did not affect 16 cultivars, except one, of *Begonia x hiemalis* shoot and root formation. Also, Soczek and Hempel 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.* 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.

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 antioxidant, 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. 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. 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.* 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.

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