

Role of Vitamins in Plant Growth and their Impact on Regeneration of Plants under Invitro Condition

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Abstract: *Vitamins are the essential compounds synthesized and used by plants. In tissue culture media, the addition of vitamins is not that common because the amount needed by the plant is really unknown and it also varies from plant to plant. Vitamins show a direct and indirect effect on the growth of callus, somatic cell growth, rooting, and embryo development. Studies show that thiamine is associated with cytokinin and plays role in inducing root and callus growth. Thiamine also facilitates the production of more secondary metabolites such as proteases in pineapple. Biotin and riboflavin also play role in development of callus. There are many other vitamins which are essential or semi essential for the growth of plants of various types. Vitamin D causes uptake of calcium, cell elongation and meristematic cell division. Vitamin C is known to enhance the shoot and root growth. Nicotinic acid is required by plants for synthesizing the amino acids and also helps in carbohydrate metabolism. In this review we will discuss role of these vitamins in some plants that is associated with different plant species.*

Keywords: *Callus, Vitamin, secondary metabolites, growth and development*

I. INTRODUCTION

Invitro propagation of plants needs several specific hormones, vitamins with appropriate and suitable conditions. If the amount of these hormones or vitamins will change, then the fate of the organ will also be changed. Several vitamins are now commercially available for different plant species alongwith appropriate amounts. Vitamins are essential in obtaining vigorous plant growth by supplementing the media with it. Plant cell requirements for vitamin concentration vary according to the plant species and the type of culture. Vitamin like thiamine and thiamine pyrophosphate (TPP) are known for its important roles in providing nutrition to humans and also helpful in catalysis of enzyme [1]. Thiamine pyrophosphate (TPP) is a derivative of Thiamine (Vitamin B1). Thiamine has various physiological functions in plants and it serves as cofactors in enzymatic reactions including pentose phosphate pathway, glycolysis, tricarboxylic acid (TCA) cycle, pyruvate dehydrogenase complex, transketolase, and pyruvate decarboxylase [4]. Pyruvate decarboxylase is observed to be imperative in energy production in Arabidopsis [5]. Thiamine is also been associated with the resistance of disease, and expression of PR-1 gene with local acquired resistance, but not systemic acquired resistance (SAR) [6]. Under abiotic stress conditions in Arabidopsis, endogenous thiamine increases to cope with oxidative stresses by supplying NADH and NADPH to the plant [7]. Vitamin C is oxidized into monodehydroascorbate radicals by oxygen, hydrogen peroxides and superoxides. Ascorbate oxidase helps in expansion and growth of cell wall. MDHA (product of ascorbate oxidase) the radicals obtained by it, depolarize the plasma membrane due to which uptake of ions is done and cell wall is loosened [8]. Riboflavin and nicotinic acid participate in redox reaction.

A. Vitamins in Tissue Culture

In tissue culture media (MS media) most commonly used, thiamine, nicotinic acid, pyridoxine and myo-inositol is present in the ratio of 0.1:0.5:0.5:100 mg/l [9]. Myo-inositol is a controversial compound, either classified as a water-soluble plant vitamin or as a sugar alcohol [3]. In earlier studies it has been observed that pea embryos achieve a good growth when some amount of vitamin C is added to the medium (Ray et al.) [10]. Also by adding biotin in the medium shoot weight (dry) increases in pea. The result of this experiment was similar to the result of the experiment done on Ricciocarpus plant in which it was treated with pantothenic acid. Similarly, addition of thiamine to pea embryos affects root and shoot growth concurrently. A study showed that tomato roots have capability to exhibit prolonged thiamine dependency [2].

B. Micropropagation

In the presence of 25mg/l of Vitamin D3, micropropagated plants absorbed Ca²⁺ efficiently whereas by increasing its level by 50mg/l no change was found [11]. In the presence of Vitamin D2, Ca²⁺ uptake was found to be suppressed. Also by combining these two vitamins the efficiency did not increased of uptake of Ca²⁺ ions [11].

C. Callus & Somatic Growth

Eriksson [12] have concluded that pyridoxine and nicotinic acid are essential vitamins assist thiamine (when studying the optimum growth of *Haplopappus gracilis* on a modified MS medium) [9]. Decreased weight of maize calli was observed by addition of thiamine from 110mg/ml to 0mg/ml but by removing inositol and pyridoxine from MS medium does not show as an important factor in growth (Polikarpochkina et al.) [13]. According to Digby and Skoog [14], in callus culture of tobacco, high amount of kinetin is needed to induce thiamine synthesis whereas growth was not sustained on a low amount kinetin medium unless thiamine was added to the medium. Linsmaier and Skoog [15] maintained cultures (tobacco plant) with 1000:0 µg/l of kinetin and thiamine respectively over 17 different passages. Synthesis of thiamine is subjected to feedback control mechanism so the plant is sensitive to thiamine in tissue. In date palm thiamine and biotin affected callus growth. By increasing the amount of thiamine from 0.1 to 0.5mg/litre and biotin from 0 to 1mg/litre, the maximum growth of callus was achieved but by increasing the amount to 2mg/litre the callus weights were reduced (Dravnieks *et al.*) [16]. Ascorbic acid which acts as an antioxidant, prevents tissue browning in papaya and in tobacco plant it acts in stimulating the mitotic cell division (Drew and Smith). Gamborg *et al.* [17], cultured root cells of soybean into several media that contains different concentrations of thiamine and to a complete B5 culture media. Soyabean cell culture weighing 53 mg was grown in no amount of thiamine in 1 litre and another as 10 mg per litre. After 5 days, 138 mg soyabean cells were produced in the media containing no amount of thiamine and 203 mg of soybean cells were produced in the media containing thiamine. Consequently, he concluded that the necessity of providing thiamine to the media helps in sustaining growth of soybean root cell.

D. Rooting

In vitro rooting of peach plant GF677 (*Prunus amygdalus* × *P. persica* Batsch.) was studied by the addition of riboflavin in different concentrations ranging from 0 to 2.0 mg/litre [18]. The more the riboflavin was added, rooting decreased until it was completely inhibited. Addition of vitamin D3 stimulates rooting of *Phaseolus vulgaris* L. by 78% than the media devoid of vitamin D3. It gave 14mm longer roots than the control, it also helped in stimulation of mitotic cells and cell elongation (Boland et. al.) [19]. 0.5 mg/l of riboflavin caused fresh and dry weight to decrease sharply whereas 1.5 mg/l concentration showed chlorotic symptoms and 2mg/l showed necrotic symptoms. Riboflavin has shown to stimulate and help rooting [20]. Root formation in apple tissue culture was studied under the presence of riboflavin. Under dark conditions riboflavin in the presence of auxin helped in inducing rooting whereas in absence of riboflavin and the plant in exposure to light, the root formation decreased. Thiamine is another vitamin which has rooting capacity on *Taxus brevifolia* Peattie [21]. According to an experiment, on addition of thiamine, Chee et al obtained 61.5% of adventitious rooting in *T. brevifolia* compared to 30% without thiamine. Vitamin E (an antioxidant) when added to culture media affected rooting process and increased the rooting process [22].

E. Embryo & Organ Development

Embryogenesis is shown to be affected by the addition of thiamine and nicotinic acid (Barwale et al.) [23]. 40 immature soybean embryos were taken in which these two vitamins were added in MS media in different concentrations. Nicotinic acid and thiamine at very low concentration (32.4 µM and 0.1µM respectively) has induced 76% and 68% embryogenesis induction respectively. According to Asano et al. [24] enhancement of embryonic callus of *Zoysia japonica* Steud., a Japanese grass, is obtained by adding thiamine and riboflavin to the media. On the exclusion of thiamine from medium, 50% callus growth was obtained and in its presence 60% growth was obtained. Biotin and thiamine have proved to be essential in tissue culturing for optimizing embryogenesis of *Pheonix dactylifera* L. [25]. According to Bonner [2], thiamine and biotin are equally responsible for maximizing the number of somatic embryos and also the embryo elongation. Maximum length of embryo was achieved by 2:1 ratio of thiamine: biotin. A study done by Perez et al. [26] showed that, thiamine affects other compounds on protease excretion in pineapple culture. Thiamine shows a negative effect on pineapple shoot formation but it helped in producing a high amount of protein. The weight of shoot of papaya increased in the presence of cytokinin and riboflavin whereas, in presence of auxin and riboflavin, it decreased the shoot weight (Drew and Smith) [27]. According to Roest and Bokelmann [28], high number of adventitious shoots was formed when vitamins were kept in MS medium whereas in media with absence of vitamins the shoot formation is suppressed but the minerals are retained. Removal of vitamins such as pyridoxine, nicotinic acid, folic acid, thiamine and biotin from Nitsch media [29] in vitro did not affect any of the 16 plants except *Begonia x hiemalis* rooting and shooting [30]. Also, the shoot multiplication of three *Gerbera* plants in presence as well as absence of thiamine, pyridoxine, nicotinic acid and other compounds reducing or removing of vitamins from MS medium did not show any change in growth over three passages of 4 weeks each [31].

An experiment done on *Eucalyptus ficifolia* showed that the medium containing only auxin decreased dry weight of shoot (Gorst et al.) [32]. Concentration of riboflavin not less than $1\mu\text{M}$ caused small change in rooting. By increasing its concentration up to $10\mu\text{M}$, degraded IBA (in presence of light). Complete destruction of IBA occurs when IBA is used. In case of $1\mu\text{M}$ of riboflavin, rooting remained up to 16 days whereas it remained only for 2 days in the case of $10\mu\text{M}$ of riboflavin was added (Drew et al.) [33]. Tobacco (*Nicotiana tabacum* L), ascorbate was added to a shoot inducing media, shoot formation increased from 45% to 450% (after 35 days in culture), when firstly young callus tissues were used and later old callus tissue were used. The medium which contains gibberellic acid (shoot inhibiting medium), shoot growth from young callus was remarkable at 4×10^{-4} M and almost zero for the old callus. This phenomenon shows that the inhibiting effect of ascorbate on gibberellic acid and also ascorbic acid reduced the period of shoot induction [34].

Table 1: Name of vitamins and their effects on plant growth and development.

| Vitamin | Name of Plant | Concentration | Culture Media | Function | Effect |
|----------------|-------------------------------|---|--|---|--|
| B1(thiamine) | Soybean Maize Pineapple | 10mg/l ,0.1 μM 0mg/l 0.3 μM | B5+MSmedium MS medium MS medium | Cofactor in carboxylase reactions and amino acid biosynthesis | Stimulate cell growth Decrease callus growth Reduce shoot fresh mass |
| B2(riboflavin) | Eucalyptus Papaya Apple | 7.97 μM 31 μM Not known | DeFossard+IBA DeFossard+IBA MSmedium+IBA | Oxidation-reduction reactions | Stimulate rooting Stimulate rooting Stimulate rooting |
| Vitamin C | Tobacco | $4-8 \times 10^{-4}$ M | MS medium+ IAA+ Kinetin | Reducing agent | Increases shoot number |
| Biotin | Palm | 2mg/l, 1mg/l | MS medium | Cofactor of enzymes | Increase callus weight, increase |
| Nicotinic acid | Soybean | 32.4 μM | MS+ BAP+IBA | Oxidation-reduction | Increases embryogenesis |

II. CONCLUSION

As we have seen in this review paper that there are a lot of vitamins which supports the growth of plants but some of them such as biotin, pantothenic acid, vitamin C and many more vitamins are there whose main abilities or the main factors involved in plant growth are still unknown [35]. The vitamins used for plant growth have different physiological and morphological effects on the plant. Thiamine is proved to be essential in the growth of soybean and rice but it is not beneficial for the growth of peanuts [36]. Addition of riboflavin with auxin is more beneficial than keeping the plant in auxin free medium because riboflavin degrades auxin. This helps in cost reduction (Drew *et. al*) [33]. The culture medium can be modified according to our needs but the most common used is MS medium. It concludes there are many vitamins whose actual value is unknown and work is to be done to explore the effects of those vitamins on variety of plants.

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