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# Stimulative Effects of Ascorbic Acid, Thiamin or Pyridoxine on *Vicia faba* Growth and Some Related Metabolic Activities

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**Abstract:** Soaking of bean seeds in 100 ppm ascorbic acid, thiamin or pyridoxine induced a stimulation effect on germination. Also, soaking of seeds or spraying of bean seedlings (25 day old) with 100 ppm of each of these vitamins, stimulated fresh and dry weight, biosynthesis of photosynthetic pigment fractions and net photosynthetic rate. However, these treatments did not induce a considerable change in dark respiration.

**Key words:** *Vicia faba,* ascorbic acid, thiamin, pyridoxine, photosynthetic pigments net photosynthesis and dark respiration

## Introduction

Vitamins are among the organic nutritional factors required for growth of all living organisms. In autotrophic organisms, although it is well know that vitamins are endogenously synthesized (Arrigoni *et al.,* 1992; De Gara *et al.,* 1993), yet exogenous application of these organic compounds exerted mostly positive effects on plant growth, $CO_2$-uptake and protein synthesis (Mozafar and Oertli 1992; Arrigoni *et al.,* 1997). Also some authors found that the addition of vitamins was necessary for continued growth of algae (Hulburt *et al.,* 1960; Guillard and Ryther, 1962; Provasoli, 1963; Loeblich, 1967; Thomas, 1968; Provasoli, 1971; Herdman and Carr, 1972; Carlucci, 1974; Berland *et al.,* 1978; Swift, 1980). Thus, exogenous addition of such substances to the test organism could lead to growth stimulation through the activation of some enzymatic reactions (Kefeli, 1981; Makled, 1995). Dry seeds of *Vicia faba* are devoid of ascorbic acid and only contain a small amount of dehydroascorbic acid and dehydroascorbic acid reductase (Arrigoni *et al.,* 1992). Moreover, consistent ascorbic acid synthesis starts only after several hours of germination (De Gara *et al.,* 1987).

In this investigation it seemed necessary to study the role of some exogenously added (soaking of seeds or spraying of seedlings) water soluble vitamins (ascorbic acid, thiamin or pyridoxine) in stimulating the growth of *Vida faba* and some related metabolic activities.

## Materials and Methods

Bean seeds were surface-sterilized by dipping in 0.1% $HgC1_2$ for 2 min and allowed to imbibe sterile water for one hour, then incubated in ascorbic acid, thiamin or pyridoxine (100 ppm) for 6 hours at 25°C. Soaking in distilled water for 6 hours was used as control. Seed germination was performed as described by Maftoun and Sepaskhah (1978). Three replicates were prepared each containing twenty vitamin-treated seeds which were incubated (25°C) in Petri-dishes on absorbent pads saturated with 25 $cm^3$ of 1/10 Hoagland solution (Hoagland and Arnon, 1950). Seeds were considered to be germinated after the radical emerged from the testa. The percentage germination was followed during a period of 3 days. To test the effect of exogenously applied vitamins (soaking of seeds or spraying of 25 day old seedlings) on the growth and some related metabolic activities the seeds were sown in plastic pots (10 cm in diameter and 13.5 cm high) each containing one Kg of soil composed of mixed sieved air-dried clay and sand (2:1 by volume). Perforated plastic tubes (1.2 cm in diameter and 15.7 cm long) were inserted into the soil to help the distribution of daily irrigation with water and nutrient solution. The soaked seeds and unsoaked seeds were soil sown and kept by daily irrigation. The plants were harvested 30 days after planting. Fresh and dry weight of leaves, stems and roots were determined. The contents of chlorophylls a and b and carotenoids were determined spectrophotometrically (Metzner *et al.,* 1965). Net photosynthetic rate (oxygen evolution) and dark respiration rate (oxygen consumption) were determined manometrically using disks (diameter 16 mm) of leaf tissue exposed to 25°C, irradiance of 5.9 Wm-2 (40 W GEF lamps) using the Warburg buffer No. 2961 type VL 85 (Umbreit *et al.,* 1959).

## Results and Discussion

Seed soaking in ascorbic acid, thiamin or pyridoxine induced a significant stimulatory effect on percent of germination (Table 1). Arrigoni *et al.* (1992) reported that dry seeds of *Vicia faba* are devoid of ascorbic acid and only contain a small amount of dehydroascorbic acid and dehydroascorbic acid reductase; moreover consistent ascorbic acid synthesis starts only after several hours of germination (De Gara *et al.,* 1987).

Table 1: Effect of soaking bean seeds in (100 ppm) ascorbic acid, thiamin or pyridoxine treatments on percent of germination, Mean values of 5 replicates + S.E

| Treatment | Percent of Germination | | |
|---|---|---|---|
| Days | 2nd | 3rd | 4th |
| Control | 0 | 75 | 90 |
| Ascorbic acid | 15 | 90 | 100 |
| Thiamin | 20 | 95 | 100 |
| P ridoxine | 10 | 85 | 100 |

Also, Asano *et al.* (1996) concluded that the addition of thiamin to the medium is essential for stimulating embryogenic callus induction from *Zoysia japonica* seeds and 0,4 mg $l^{-1}$, the concentration that is included in the LS basal medium and higher concentrations seem to be necessary.

Generally, it was found that the applied vitamins could stimulate the growth of leaves, stems and roots of the tested plants (Table 2, 3). In accordance with this, El-Zawahry and Hamada (1994) recorded that, soaking of *Solanum melanogena* seeds, before sowing, in ascorbic acid, thiamin or pyridoxine, increased the fresh and dry weights of shoots and roots compared with those of the control plant. Also, Husain *et al.* (1980) working with *Cyperus rotunda,* recorded a promotion in leaf growth, after being treated with 100 mg $l^{-1}$ pyridoxine.

Table 2: The action of soaking seeds in (100 ppm) ascorbic acid, thiamin or pyridoxine treatments on bean growth

| | Fresh weight (g) | | | Dry weight (g) | | |
|---|---|---|---|---|---|---|
| Treatment | Leaf | Stem | Root | Leaf | Stem | Root |
| Control | 21.28 ± 2.50 | 14.91 ± 1.40 | 3.55 ± 0.30 | 3.16 ± 0.20 | 1.79 ± 0.10 | 0.53 ± 0.01 |
| Ascorbic acid | 33.38 ± 2.00 | 28.24 ± 2.1 | 5.66 ± 0.20 | 5.05 ± 0.38 | 3.16 ± 0.19 | 0.84 ± 0.042 |
| Thiamin | 41.14 ± 3.10 | 32.93 ± 2.4 | 7.51 ± 0.10 | 5.86 ± 0.41 | 3.51 ± 0.16 | 1.06 ± 0.12 |
| Pyridoxine | 37.53 ± 2.30 | 32.49 ± 0.7 | 7.71 ± 0.36 | 5.28 ± 0.80 | 3.27 ± 0.20 | 1.03 ± 0.15 |

Table 3: The action of spraying seedlings with (100 ppm) ascorbic acid, thiamin or pyridoxine treatments on growth of bean plants. Mean values of 5 replicates ± S.E

| | Fresh weight (g) | | | Dry weight (g) | | |
|---|---|---|---|---|---|---|
| Treatment | Leaf | Stem | Root | Leaf | Stem | Root |
| Control | 21.28 ± 2.5 | 14.91 ± 1.4 | 3.55 ± 0.30 | 3.16 ± 0.20 | 1.79 ± 0.10 | 0.53 ± 0.010 |
| Ascorbic acid | 29.74 ± 0.65 | 25.44 ± 0.48 | 4.80 ± 0.91 | 3.98 ± 0.38 | 2.55 ± 0.14 | 0.69 ± 0.021 |
| Thiamin | 36.43 ± 0.56 | 27.15 ± 0.90 | 7.21 ± 0.22 | 5.34 ± 0.20 | 3.15 ± 0.18 | 1.02 ± 0.072 |
| Pyridoxine | 40.13 ± 0.68 | 32.15 ± 0.69 | 7.22 ± 0.48 | 5.67 ± 0.11 | 3.64 ± 0.16 | 1.09 ± 0.050 |

Table 4: Effect of spraying seedlings with (100 ppm) ascorbic acid, thiamin or pyridoxine on the biosynthesis of photosynthetically active pigments (mg/g f.w.), net photosynthetic and dark respiration rates (µmol ($O_2$)/g d.w./hour) of bean plants

| | Photosynthetic pigment (mg/g f.w.) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Chi. A | Chl. B | Carot. | Total | Net photosynthesis | Dark respiration |
| Control | 0.962 ± 0.050 | 0.283 ± 0.010 | 0.437 ± 0.026 | 1.682 ± 0.18 | 1029.4 ± 25.7 | 131.5 ± 12 |
| Ascorbic acid | 1.411 ± 0.081 | 0.382 ± 0.021 | 0.621 ± 0.03 | 2.414 ± 0.19 | 1293.6 ± 39 | 128.9 ± 8 |
| Thiamin | 1.306 ± 0.04 | 0.331 ± 0.03 | 0.564 ± 0.01 | 2.201 ± 0.092 | 1178.8 ± 30 | 132.1 ± 11 |
| Pyridoxine | 1.302 ± 0.072 | 0.348 ± 0.042 | 0.585 ± 0.015 | 2.235 ± 0.081 | 1170.1 ± 50 | 127.4 ± 9 |

Table 5: Effect of spraying seedlings with (100 ppm) ascorbic acid, thiamin or pyridoxine on the biosynthesis of photosynthetically active pigments (mg/g f.w.), net photosynthetic and dark respiration rates (µmol ($O_2$)/g d.w./hour) of bean plants Mean values of 5 replicates ± S.E

| | Photosynthetic pigment (mg/g f.w.) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Chi. A | Chl. B | Carot. | Total | Net photosynthesis | Dark respiration |
| Control | 0.962 ± 0.50 | 0.283 ± 0.10 | 0.437 ± 0.026 | 1.682 ± 0.18 | 1029.4 ± 25.7 | 131.5 ± 12 |
| Ascorbic acid | 1.224 ± 0.026 | 0.339 ± 0.005 | 0.590 ± 0.039 | 2.153 ± 0.03 | 1152.4 ± 43.5 | 139.6 ± 8 |
| Thiamin | 1.209 ± 0.010 | 0.317 ± 0.008 | 0.537 ± 0.028 | 2.063 ± 0.02 | 1141.7 ± 39 | 136.1 ± 12.5 |
| Pyridoxine | 0.992 ± 0.008 | 0.294 ± 0.002 | 0.477 ± 0.026 | 1.763 ± 0.028 | 1127.5 ± 50 | 133.2 ± 6 leaves and |

This promotion in growth depends on the endogenous thiamin supply or on the thiamin synthesis in its transport to different plant organs (Proebsting *et al.,* 1990; Mozafar and Oertli, 1992; 1993). In this respect, Kefeli (1981) working with *Chlorella vulgaris* and *Ankistrodesmus falcatus* recorded that the exogenously added thiamin and nicotinamide caused a considerable increase in algal growth. Also, Desouky (1995) working with *Chlorella vulgaris* showed that the addition of ascorbic acid, thiamin or pyridoxine up to 300 ppm exhibited a stimulation in algal growth. Arrigoni *et al.* (1997) observed that, when ascorbic acid content is lower, a decrease in growth of *Lupines albus* occurs and when ascorbic acid content is raised, the growth is stimulated. The growth responses to ascorbic acid variations are due to the fact that ascorbic acid stimulates both cell division and cell expansion. Large amounts of ascorbic acid are utilized during primary root meristem cell division and that when the ascorbic acid content of actively proliferating cells is experimentally lowered, the cell cycle is arrested in the G1 phase (Liso *et al.,* 1984); the addition of ascorbic acid restores DNA synthesis and cells enter the S phase (Liso *et al.,* 1988). Asano *et al.* (1996) showed that, the addition of 4 mg $l^{-1}$ thiamin to the medium yielded a significantly increased embryogenic callus rate of up to 28.4% per total calli induced. It is known that thiamin, as a functional coenzyme thiamin pyrophosphate, plays an integral role in the regulation of the carbon metabolism in plants. Bender (1985) mentioned that pyridoxine having the pyridine ring represents a precursor for the essential enzyme pyridoxal-phosphate, which is utilized in all phases of amino acids metabolism.

The photosynthetic pigments were stimulated by the different treatments of the applied vitamins, which consequently led to enhanced photosynthesis and growth (Table 4, 5). In this respect, Zidan (1991) working with *Triticum aestivum,* sprayed with thiamin, nicotinic acid or pyridoxine, obtain the same trend. Also, Makled (1995) working with *Chlorella vulgaris* and *Ankistrodesmus falacatus,* reported that the addition of thiamin, nicotinic acid or pyridoxine, showed a considerable increase in algal growth rate as well as in the contents of the photosynthetic pigments. In accordance with this, it was found that chloroplasts isolated from leaves, which were previously sprayed with ascorbic acid showed higher contents of chlorophylls than those isolated from untreated leaves (Choudhury *et al.,* 1992). It has been known that ascorbic acid is an essential antioxidant of the stromal compartment (Foyer, 1993; Foyer *et al.,* 1994). It is involved in the protection and regulation of photosynthesis. It is, for example, an essential co. factor for the synthesis of the energy quencher, zeaxanthin, in the thylakoid lumen (Hager, 1969; Pfundel and Bilger, 1994). Similarly, foliar application with thiamin helped some bleached plants to re synthesis chlorophyll and consequently exerted positive effect on plant growth and productivity (Oertli, 1987). As regards the rate of respiration (dark oxygen-uptake), remained more or less unchanged or even slightly increased by vitamins-treatments (Table 4, 5). These results are in accordance with those obtained by Desouky (1995), who recorded that the rate of respiration remained more or less unaffected by exogenously addition of (100, 200 and 300 ppm) ascorbic acid, thiamin or pyridoxine to *Chlorella vulgaris* cultures. From the preceding results and discussion, it can be concluded that treatments of bean seeds

or seedlings with ascorbic acid, thiamin or pyridoxine could stimulate the growth via the enhancement of the biosynthesis of photosynthetic pigments and photosynthetic rate and stability of dark respiration.

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