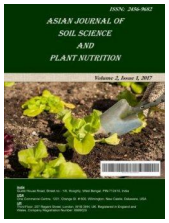

Asian Journal of Soil Science and Plant Nutrition

4(1): 1-5, 2019; Article no.AJSSPN.45415
ISSN: 2456-9682

Studying Impact of Vitamins Application on Growth of Wheat Plantlets Cultured *In vitro*

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Authors' contributions

This work was carried out in collaboration between both authors. Author RMM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KMM managed the analyses of the study and performed the statistical analysis. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJSSPN/2019/45415
Editor(s):
(1) Dr. Paola Angelini, Professor, Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy.
(2) Dr. Tancredo Souza, Professor, Department of Life Sciences, Centre for Functional Ecology, University of Coimbra, Portugal.
Reviewers:
(1) Betül Bürün, Muğla Sıtkı Koçman University, Turkey.
(2) Aida Alfonse Rizkalla, National Research Centre, Egypt.
(3) R. Mahalakshmi, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/27715

Short Research Article

Received 05 September 2018
Accepted 26 November 2018
Published 08 December 2018

ABSTRACT

The present study aimed to investigate physiological and biochemical responses of wheat plantlets to application of concentrations of vitamins. In general, the data point out that addition of low concentrations of vitamins to MS media greatly improved most vegetative growth criteria concerned of wheat plantlets culture *in vitro* via plant tissue culture technique. In the current study root length (7.53 cm), shoots fresh weight (0.480 g), total chlorophyll content (380 µg g^{-1} FW), soluble sugars (6.88 mg glucose g^{-1} DW), and soluble proteins (4.9 mg protein g^{-1} FW) enhancement in *in vitro* of wheat plantlets with application low concentrations of vitamins on the contrast, the highest concentration of the same vitamins, decreased their physiological and biochemical characteristics compared to control.

Keywords: Triticum aestivum; tissue culture; nicotinic acid; thiamine; soluble sugars; physiological and biochemical changes.

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1. INTRODUCTION

Common wheat (*Triticum aestivum*) is a staple of human food, one of the most important sources of carbohydrates in most of the world. Wheat enter into many food industries such as bread, pasta, sweets and others. The remains of the harvest are included in the feed industry. Wheat ranks the among other crops (including rice, maize and potatoes) in terms of area and production at the global level. The total cultivated area of wheat in the world is around 221.12 million hectares with a production of 697.8 million tonnes and productivity of 3160 kg/ha [1]. Despite the interest in growing field crops, especially wheat, there is a significant decline in cultivated areas and this is reflected directly on production. Some plants are able to synthesise the essential requirements of vitamins for their growth.

Some vitamins are required for normal growth and development of plants, they are required by plants as catalysts in various metabolic processes. They may act as limiting factors for cell growth and differentiation when plant cells and tissues are grown *in vitro*. The vitamins most used in the cell and tissue culture media include: thiamine (B_1), nicotinic acid and pyridoxine (B_6). Thiamine is necessarily required by all cells for growth [2]. Vitamins are among the organic nutritional factors required for growth of all living organisms. Application of vitamins mostly has positive effects on plant growth, CO_2 uptake, and protein synthesis [3]. Plants synthesise vitamins endogenously and these are used as catalysts in various metabolic processes. When plant cells and tissues are grown in vitro, some essential vitamins are synthesised but only in suboptimal quantities. Hence, it is necessary to supplement the medium with required vitamins and amino acids to achieve the best growth of the tissue. Plant tissue culture (PTC) is a set of techniques for the aseptic culture of cells, tissues, organs and their components like genes and enzymes under defined physical and chemical conditions *in vitro* and controlled environment. PTC technology also explores conditions that promote cell division and genetic re-programming in *in vitro* conditions and it is considered as an important tool in both basic and applied studies, as well as in commercial application [4]. PTC techniques have become of major industrial importance in the area of plant propagation, disease elimination, plant improvement, and production of secondary metabolites. Our aim in this study is to know the physiological and biochemical changes under exogenous application of different concentrations of vitamins on wheat plantlets.

2. MATERIALS AND METHODS

Fresh wheat seeds were purchased from a local market in Al Bayda – Libya. Taxonomist at the Department of Botany Herbarium, Faculty of Science, and Omar Al-Mukhtar University further identified the samples. Rabha [5] has described the method of sterilisation of seeds and preparation of culture media in details. Sterilised seeds *in vitro* were selected and transferred onto MS and on MS augmented with one of the two levels of nicotinic acid (2.5 and 5 mgL^{-1}) and/or with 5 and 10 mg L^{-1} thiamine as follow:-

A_1= control [MS Including vitamin concentration (0.018 mgL^{-1} of nicotinic acid and 0.090 mgL^{-1} of thiamine)] $A_{2=}$ MS + 2.5 mgL^{-1} of nicotinic acid; $A_{3=}$ MS + 5 mgL^{-1} of nicotinic acid; $A_{4=}$ MS + 5 mgL^{-1} of thiamine; $A_{5=}$ MS + 10 mgL^{-1} of thiamine.

2.1 Estimation of Photosynthetic Pigment (Total Chlorophyll) Content

Leaf samples (2 g) harvested from control and treated plantlets were homogenised in acetone 80% (v/v) following [6] method. Extract was centrifuged at 5,000 rpm for 15 min and absorbance was recorded at 646 and 663 nm for chlorophyll (a and b) estimation. Pigment content was calculated according to the following formulae as reported by Lichtenthaler and Wellburn [7]:

Chlorophyll a µg g^{-1} FW =12.7xO.D $_{663}$-2.69XO.D_{645}

Chlorophyll b µg g^{-1} FW =22.9xO.D.$_{645}$-4.68XO.D_{663}

Total chlorophyll µg g^{-1} FW =20.2xO.D.$_{645}$+18.2 O.D_{633}

2.2 Extraction and Estimation of Soluble Sugars

Soluble sugars in control and treated plantlets were extracted following Angelov [8] method. Soluble sugars were expressed as mg glucose / g DW.

2.3 Estimation of Total Soluble Proteins

Soluble protein was determined by using Folin - Ciocalteau reagent according to Bradford [9] assay.

2.4 Statistical Analysis

The test of least significant using difference (L.S.D) at the level of 0.05% significance was used to examine differences among treatment means and interactions. Data were statistically analysed using SPSS software package.

3. RESULTS AND DISCUSSION

Vitamins -treated plants (low concentrations) showed a clear increase in lengths and weights in both shoots and root than the control plants is presented in Table 1. Moreover, all treatments (high concentrations) caused increases in plantlets lengths and weights of wheat plantlets as compared with the control. The increase in plantlets lengths because of the vitamin addition treatments ranged between 7.4 and 7.53 cm with A_2 (MS + 2.5 mg L^{-1} of nicotinic acid) for shoot and root respectively. While it was in the best result in the plant weights fresh and dry (g) treatment A_2 (MS + 2.5 mg L^{-1} of nicotinic acid) and A_4 (MS + 5 mg L^{-1} of thiamine). The growth and development of wheat plantlets has been greatly improved by adding vitamins to the nutritional medium *in vitro*. The results of our study agreed with [10] found that the plant species were affected in the composition of its members under the application of vitamins, which will have a significant impact on the yield of the crop. In this study, we found that the growth of wheat plantlets was significantly enhanced when cultivating on MS medium supplemented with vitamins compared with control (Table 1). Moradi and Otroshy [11] showed such results efficient germination can be achieved using optimal media with added plant growth regulators which have shown to have important roles in the regulation of seed of soybeans germination. In addition, These results are also consistent with the results published by Foda [12] on the increase in plant height under vitamin effects. The results also corresponded with [13] who concluded that, vegetative growth, plant chemical constituents, and fresh and dry weights were increased as a result from the application of thiamine and gibberellins. In this test, our results were similar to those of several authors [14,15] which had the highest increases in plant height when plants were exposed to nicotinic acid. Also El-Shawy et al. [16] found that treated plants with vitamins significantly increased vegetative growth in wheat plantlets as compared with untreated control plants. The exogenous application of vitamins (low concentrations) to wheat plantlets cultured media significantly enhanced the biosynthesis production of total chlorophyll, soluble sugars and soluble proteins content in shoots and roots compared to non treat plantlets. In this experiment there have been various and significant changes in content of total chlorophyll, soluble sugars, and soluble proteins under effects of different types of media. The present data illustrated in Table 2 showed that the highest frequency of total chlorophyll ,soluble sugars and soluble proteins content observed for wheat grown in MS + 2.5 mgL^{-1} of nicotinic acid (A_2). Chlorophyll *a,* chlorophyll *b* and total chlorophyll are main photosynthetic pigments and play important role in photosynthesis. The changes in the amount of pigments were evaluated as the changes in photosynthesis. Also soluble sugar accumulation may be due to efficiency photosynthesis and further transformation of starch to sugars [17]. The positive impact of the vitamins on the production and metabolism of carbohydrates is quite well known in plants [18].

Table 1. Impact of application of vitamins on growth of wheat plantlets cultured *in vitro* (It was harvested after 35 days)

Parameters	Length(cm)		Fresh weight(g)		Dry weight(g)	
Culture media composition	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
A_1= control	5.78b	4.50b	0.37b	0.31b	0.018b	0.016b
$A_{2=}$ MS + 2.5 mgL^{-1} of nicotinic acid	7.4a	7.53a	0.48a	0.26ab	0.034a	0.024 ab
A3= MS + 5 mgL-1 of nicotinic acid	4.82c	3.50c	0.31b	0.21 b	0.012b	0.019b
A4= MS + 5 mgL-1 of thiamine	5.87b	4.20b	0.42a	0.36a	0.032a	0.030a
A5= MS + 10 mgL-1 of thiamine	4.20c	1.53d	0.25c	0.31b	0.014b	0.012b

Means in the same column that have the same letter are not significantly different at P < 0.05

Table 2. Impact of application of on total chlorophyll content, soluble sugars and proteins content of wheat plantlets cultured *in vitro* (It was harvested after 35 days)

Parameters Culture media composition	Total chlorophyll contact (µg g-1 FW)	Soluble sugars (mg g^{-1} DW)	Soluble proteins (mg g^{-1}FW)
A_1= control	286b	5.81b	4.0ab
$A_{2=}$ MS + 2.5 mgL^{-1} of nicotinic acid	380a	6.88a	4.9a
A3= MS + 5 mgL-1 of nicotinic acid	217c	3.67c	3.8ab
A4= MS + 5 mgL-1 of thiamine	316a	6.71a	4.7a
A5= MS + 10 mgL-1 of thiamine	197c	3.75d	2.8c

Means in the same column that have the same letter are not significantly different at P < 0.05

In other studies, explained that the thiamine has been shown to play a large and effective role in pentose phosphate cycle [19], improving photosynthesis pigments and enhanced protein content in wheat *cicer arietinum* [20], and *Brassica Campestris* [21]. Therefore, for one specific plant species, precursor concentration should be carefully adjusted, compared and optimised. As to the concentration, too high concentration treatment can result in decrease of biomass accumulation and even the content of secondary metabolites can be improved, however, the total yield will still not be maximised.

4. CONCLUSION

In our study summary, we found that the addition of vitamins as dietary supplements to wheat plantlets treated with low concentrations showed a clear increase in lengths and weights in both shoot and roots relative to control. The content of total chlorophyll, soluble sugars, and protein content was also improved. Moreover, all high concentration coefficients led to a decrease in the above measurements compared to those control, and our results were consistent with previous studies in this regard.

At higher concentration, the reaction speed is usually higher and affects on physiological and biochemical characteristics than that when precursor concentration is lower. In some cases, high concentration of intermediates can even be toxic for cells. Originally, in cells, these intermediates are all maintained in very low concentration, and converted to down-stream compounds quickly.

ACKNOWLEDGEMENTS

All thanks to the University of Omar Mukhtar and all workers in the Plant Physiology Laboratory 3 Department of Botany Faculty of Science.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27715