Study impact of application of vitamins on growth the wheat plantlets cultured in vitro.

Abstract

In present study aimed to investigate physiological and biochemical responses of wheat plantlets to application different concentrations water-soluble 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 length (7.53 cm) for root, fresh (0.480 g) and dry weights (0.034 g) for shoots , 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 contrast, the high concentration of the same vitamins, decreased their amount compared to control.

Keywords: Technology; Plantlets; Thiamin; Nicotinic acid; soluble sugars; Triticum aestivum and Water-soluble vitamins.

Introduction

Common wheat (Triticum aestivum) is a staple of human food, one of the most important sources of carbohydrates in most of the world. And 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 first 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 **MOA. (2013).** Despite the interest in growing field crops, especially wheat, there is a significant decline in cultivated areas and this is reflected directly on production. Plants need nutritional supplements to improve their growth and productivity, the vitamins have tangible and rapid effects on the growth of plants, and this has been demonstrated by modern research and applied to a certain **(Torres 1989).** Vitamins are among the organic nutritional factors required for growth of all living organisms. Application of vitamins mostly positive effects on plant growth, CO_2 uptake, and protein synthesis **(Arrigoni et al., 1997).** Plants synthesize 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 synthesized 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 an important tool in both basic and applied studies, as well as in commercial application (**Thorpe 1990).** 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 water-soluble vitamins on wheat plantlets.

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 (2016)** has described the method of sterilization of seeds and preparation of culture media in detail. Sterilized 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} thiamin as follow:-

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

Estimation of photosynthetic pigment (total chlorophyll) content

Leaf samples (2 g) harvested from control and treated plantlets were homogenized in acetone 80% (v/v) following **Arnon (1949)** 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 (1983):**

Chlorophyll a = 12.25 A_{663} – 2.79 A_{645}

Chlorophyll b= 21.21 A_{646} – 5.1 A_{663}

Total Chlorophyll = 20.2 A_{645}+ 8.02 A_{663}

Extraction and estimation of soluble sugars

Soluble sugars in control and treated plantlets were extracted following **Angelov et al., (1993)** method. Soluble sugars were expressed as mg glucose / g DW.

Estimation of total soluble proteins

Soluble protein was determined by using Folin - Ciocalteau reagent according to **Bradford (1976) assay.**

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 analyzed using SPSS software package.

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 addition vitamin 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 thiamin). 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 **Abrahamian and Kantharajah (2011)** ~~the findings of~~ ~~the~~ study 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 (**El-Tohamy and El- Gready, 2008**). 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 (2012)** showed such results when soybeans were processed when germination was developed. In addition, These results are also consistent with the results published by **Foda** (1987) on the increase in plant height under vitamin effects. The results also corresponded with **Yusuf and Talat (2003)** ~~show that visible increases in~~ vegetative growth, plant chemical constituents, ~~increased growth,~~ and fresh and dry weights result from the application of thiamine and gibberellins. In this test, our results were similar to those of **Deyab (1989)** and **Noctor, et al (2006)** which had the highest increases in plant height when plants were exposed to nicotinic acid.also **El-Shawy et al. (2008)** found that treated plant 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 its 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 (**Hare et al. 1998**). The positive impact of the vitamins on the production and metabolism of carbohydrates is quite well known in plants (**Vardhini et al. 2011**).

In other studies, **Amin et al., (2008),** they explained that the thiamine has been shown to play a large and effective role in pentose phosphate cycle, improving photosynthesis pigments and enhanced protein content in wheat Cicer arietinum (**Beltagi, 2008**), and Brassica Campestris (**Khan et al., 2010**).

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.

Table 1. Impact of application of water-soluble 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 thiamin	5.87b	4.20b	0.42a	0.36a	0.032a	0.030a
A5= MS + 10 mgL-1 of thiamin	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 water-soluble vitamins on total chlorophyll content, soluble sugars and proteins content of wheat plantlets cultured in vitro(It was harvested after 35 days).

Parameters	Total chlorophyll contact	**Soluble sugars**	**Soluble proteins**
Culturemedia composition	(µg g-1 FW)	**(mg g^{-1} DW)**	**(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 thiamin	316a	6.71a	4.7a
A5= MS + 10 mgL-1 of thiamin	197c	3.75d	2.8c

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

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