Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5305 - 5313
Received 15 May 2021; Accepted 20 May 2021.

Effect of Thiamine and Biotin Vitamins on Growth and Yield of Smooth Wheat Triticum aestivum L

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ABSTRACT

Vitamins are organic substances which are present in many forms and are considered as essential for the proper growth of plants. They perform various physiological functions of plants as well as help them in building new tissues. In the present study, the effect of two vitamins (thiamine and biotin) was studied on Triticum aestivum L. (wheat) germination and its growth. The anvil and laboratory experiment was conducted using various concentrations of thiamin and biotin i.e. 1, 5, 10 mg/l. Vegetative characters and chlorophyll content were estimated after treatment. The seed germination rate did not get affected significantly by adding various concentrations of vitamins aquatic extracts in both laboratory and anvil experiments. 10 mg/L concentration of thiamin found to have significant effect on anvils and laboratory experiment. Also, 5mg/L biotin had significant effects, except number of spike and number of grain in spike did not have substantial effect. Mixed aquatic concentration of thiamin and biotin at a 10mg/L also had significant effect on characters of anvils and laboratory experiment, except spike number in plant.

Keywords
Thiamine, Biotin, Triticum aestivum L., Vitamins, Germination

Introduction

A wheat crop is a basic food commodity for poor and average income families (Michail, 2013). It is a major crop in the northern region of Iraq as compared to the central and southern regions which are under flood irrigation (Abdi, 2019). Wheat cultivation is supported by Iraqi government through the provision of affiliation and tax facilities (Mtashar, 2004). Wheat has a good balance of proteins and carbohydrate. It also contains fat, sugar and mineral elements (Hsanin, 2019).

Vitamins are organic substances, which are essential for various physiological functions and plant growth through the participation in many physiological reactions (Zaid, 2011). Vitamin B has been used in the agriculture medium / tissue culture technique for long time. Various tissue culture medium, i.e. White medium, Murashige and Skoog medium were designed for the optimum plant growth *in vitro* (Murashige and Skoog, 1962). Thiamin,also known as vitamin B1 is a member of B complex vitamins. It is water soluble. It has chemical formula $C_{12}H_{17}O_5$ and molecular weight 266.4gm. It is predominantly found in egg white, fish, liver, peanuts, wheat germ, broccoli, etc. (Levy et al., 1992). The active form of thiamin is biphosphate thiamin. It act as a coenzyme in decarboxylation and in the process of ketolin addition in carbohydrate metabolism to form acetyl CoA (Coele et al., 1988). It helps with protein synthesis and the production of neurotransmitters (Calderón-Ospina and Nava-Mesa, 2020).Biotin, also known as vitamin B7, is an imidazole derivative. It has chemical formula C10H16N2O3 and molecular weight 244.31gm. It is present in almost all natural foods such as garlic, oat, barley, corn and sesames (Zaid, 2009). The biotin is especially important because it acts as cofactor for four carboxylase enzymes (Coele et al., 1988) viz. Carboxylase pyrophosphate (first enzyme involved in composition of glucose from other organic molecules and renewal of salicylic acid for citric acid cycle); Acetyl CoA carboxylase (form fatty acids); Propionyl CoA carboxylase (participate

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in citric acid cycle) and Beta-methyl CoA carboxylase (break lysine amino acid and some isopyranoid compounds). In plants, it has crucial role in the citric acid cycle and oleic acid metabolism (Zaid, 2011). It used in culture medium to produce the callus from dates palm tissues (Shimamoto and Hayward, 1975).

In view of the great importance of wheat, the effect of vitamins thiamin and biotin on the growth and yield are need to be studied in detail as these vitamins has opposite action on the growth and production.Application of growth regulators were always a choice for resolving many agricultural problems such as poor growth, less productivity due to environmental conditions (Gamal El-Din et al., 2004). Other compounds involved are vitamins, hormones, micronutrients and different other miscellaneous compounds (Gamal El-Din, 2005). These compounds are known to play an important role due to their specific position in the various pathways, e.g. chlorophyll biosynthesis, plant defense against biotic and abiotic stress, photosynthesis, and enzymatic activities (Samiullah et al., 1988; Belanger et al., 1996; Gamal El-Din et al., 2004; Gamal El-Din, 2005). Biosynthesis of certain compounds in the plant body makes plants resistant to biotic (pathogens and virus) and abiotic stress (salinity, pollution, chilling, frosting, drought, etc.) (Hausladen and Alscher, 1993; Sayed and Gadallah, 2002).

An ample amount of articles has been published on the effect of vitamins on seed germination and its growth (Reda and Gamal El-Din, 2005; Batifoulier et al., 2006; El-Shahawyet al., 2008; Datta et al., 2011). Application of various concentrations of micronutrients, thiamine and riboflavin showed increased growth and yield of flax cv. Giza plant (El-Shahawy et al., 2008). However, very few reports were available on effect of vitamins on germination and growth of wheat. Henceforth, in the present study, we evaluated the effect of thiamin and biotin vitamins in germination and growth of wheat seedling. Also, physiological effect on vegetative characters of wheat plant were studied.

Methodology

The study was conducted in green house of Ibn- Alhaitham education college, Baghdad University.

Experimental design

The effect of various concentrations (1,5,10 mg/l) of thiamin and biotin was studied individually and in combination on seed germination and its growth. All the experiments were performed in triplicate. For each treatment, 25 seeds were used.

Table 1: Experimental design

Sr. No.	Treatment	Concentrations (mg/l)	No of plantlets
1	Thiamin	1mg/l	05
2		5 mg/l	05
3		10mg/l	05
4		Control (No treatment)	05
5	Biotin	1mg/l	05

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6		5 mg/l	05
7		10mg/l	05
8		Control (No treatment)	05
9	Thiamin and biotin	1mg/l	05
10		5 mg/l	05
11		10mg/l	05
12		Control (No treatment)	05

Study the effect of vitamins on the seed germination

Briefly, about 25 wheat seeds were taken in a petridish containing whatmann filter paper (No.1). The aquatic extract (100 ml) of individual vitamin was added in each petridish except control. In the control treatment 100 ml of distilled water was added. The petridish was covered by the glass lid and incubated at 20°C for two weeks.

After two weeks in the seed germination experiment, germination rate (%), plumule length (cm), radicle length (cm), dry weight of plumule and radicle (mg) were taken. The percentage germination was calculated by following formula (Saied, 1984)

Germination rate (%) = (Number of germinated seeds / number of cultivated seeds)*100

For dry weight calculation, plumule or radical (100mg) was taken in the preweighted aluminium foil followed by oven drying for 72hrs at 70°C. After complete drying of plumule or radical, the weight was recorded again by electric weighing balance (Hasan et al., 2018).

Study the effect of vitamins in the anvils experiment

Agriculture anvils (20cm diameter x 20cm height) were selected randomly. Muddle soil and pitmoss (1:1) were used to fill the pots. Around 25 seeds were sown per pot. After seed germination, plants were separated randomly (5 plants in each group). Each group received different vitamin treatment.

The pots were filled with clean muddle soil and pitmos (1:1). In each pot around 25 seed were seeded. After germination, the plants were separated into 5 groups where each group contains 5 plantlets. About 100ml of desire concentration of vitamin (as mentioned in Table 1) was given to the designated set of plants. Control plants received the same quantity of distilled water. Irrigation was given by hand sprinkle. Following characters measured after 14 days.

1. Germination rate (%)= Number of seedling / Number of cultivated seeds x 100
2. Plant length (cm)
3. Leaf space (cm^2) =0.95 x leaf length x width
4. Chlorophyl content (mg/g)
   About 2 gram of wet plant leaves weighed and 80% ethyl alcohol (20ml) was added. The leaves were crushed with mortar and pestle followed by centrifugation at 3000 rpm for 5 minute. The supernatant was separated and volume was make upto 20 ml using 80% ethyl

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alcohol. Absorbance was measured at 645nm and 663nm by using spectrophotometer. Then the chlorophyll content was calculated by equation:

5. Chlorophyll content = $[20.2(A_{645})+8.02(A_{663})] \times V/ (1000 \times W)$
   Where, A= Absorbance of extract chlorophyll at 645nm and 663nm; V= Final volume for ethyl alcohol (80%); W= Plant tissue wet weight in gram
6. Number of spike in plant
7. Number of grain in spike

Data Analysis

The experiment was conducted by Completely Randomize Design (C.R.D) by using an analysis of variance test (ANOVA), difference tested between accounts intermediate at probability level 0.05 by using polynomial Dankn test (Alrawi et al., 2000).

Results

In the present study, two types of experiments were carried out i.e. lab experiment and anvils experiment.

Results of lab experiment

Table 2.Explains effect on adding thiamin and biotin in lab experiment for wheat plant

Vitamin	Con. (mg/l)	Germination rate (%)	Plumule length (cm)	Radical length (cm)	Plumule dry weight (mg)	Radical dry weight (mg)
Thiamine	1	97^{a}	15.31^{b}	12.00^{c}	9.31^{c}	7.95^{c}
	5	94^{a}	15.22^{b}	12.23^{c}	11.00^{b}	8.81^{b}
	10	95^{a}	17.34^{a}	14.22^{a}	12.97^{a}	9.92^{a}
	Control	98^{a}	13.51^{c}	9.56^{b}	8.20^{d}	6.98^{d}
Biotin	1	90^{a}	15.24^{b}	13.12^{b}	9.81^{c}	7.85^{c}
	5	97^{a}	17.22^{a}	14.98^{a}	12.24^{a}	9.95^{a}
	10	100^{a}	15.00^{b}	12.62^{b}	10.22^{b}	8.73^{b}
	Control	93^{a}	13.51^{c}	9.54^{c}	8.21^{d}	6.98^{d}
Thiamine + Biotin	1	98^{a}	15.00^{b}	12.45^{b}	9.22^{c}	8.91^{b}
	5	91^{a}	15.32^{b}	12.22^{b}	10.12^{b}	7.65^{c}
	10	100^{a}	18.35^{a}	14.91^{a}	12.95^{a}	9.89^{a}
	Control	97^{a}	13.50^{c}	9.56^{c}	8.21^{d}	6.99^{d}

Means sharing the same superscript letters under the same column do not differ significantly at $P<0.05$.

Effect of Thiamin

Theresults of thiamin showed that, the rate of germination in all concentrations was not significantly affected, it affected significantly in concentration 10mg/l on other characters, here length of plumule was affected significantly [result- 17.34, control- 13.51]. The radical length

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was affected significantly [result- 14.22, control- 9.56]. The plumule dry weight was also showing significant results [result- 12.97, control- 8.20]. The radical dry weight was having significant results [result- 9.92, control- 6.98]. The thiamin plays role in activation and regulation of wheat growth.

Effecct of Biotin

Results showed the biotin in all concentrations don't affect significantly the rate of germination but it showed significant effect at concentration 5mg/l on other characters, where it affected length of plumule significantly [result-17.22, control- 13.51]. It also affected significantly the radical length [result- 14.98, control-9.54]. It affected significantly the plumule dry weight at same concentration [result- 12.24, control- 8.21]. It also affected significantly on radical dry weight [result- 9.95, control- 6.98].

Effect of thiamin and biotin on seed germination

The results showed mixture for thiamin and biotin in all concentration do not affect significantly the germination rate , but it affected significantly in concentration 10mg/l on other characters where it affected the plumule length significantly, [result-18.35, control-13.50] it also affected significantly the radical length, [result- 14.51, control- 9.56]. It affected significantly the dry weight of plumule [result- 12.95, control- 8.21]. It also affected significantly the radical dry weight [result-9.89, control- 6.99].

Results of anvils experiment

Table 3.Explains effect adding thiamin and biotin in field experiments for wheat seedling

Vitamin	Con. (mg/l)	Germination rate (%)	Plant height (cm)	Leaf space (cm^2)	Whole chlorophyll (mg/g)	Spikesin plant	Grains in spike
Thiamine	1	98^a	62.32^b	18.32^b	6.765^b	2.000^b	15.000^b
	5	100^a	60.22^b	17.23^b	6.989^b	2.000^b	16.000^b
	10	92^a	75.35^a	25.12^a	8.463^a	4.000^a	22.000^a
	Control	95^a	58.22^b	15.22^b	5.831^c	2.000^b	15.000^b
Biotin	1	100^a	63.46^b	17.00^b	6.223^c	2.000^a	17.000^a
	5	94^a	76.12^a	24.45^a	8.986^a	2.000^a	15.000^a
	10	91^a	60.16^b	17.35^b	7.665^b	2.000^a	16.000^a
	Control	100^a	58.21^b	15.22^b	5.830^d	1.000^a	16.000^a
Thiamine + Biotin	1	93^a	62.24^b	15.56^b	6.342^b	2.000^a	17.000^b
	5	97^a	62.00^b	18.34^b	6.232^b	2.000^a	22.000^a
	10	100^a	75.31^a	25.45^a	8.854^a	2.000^a	15.000^b
	Control	98^a	58.20^b	15.20^b	5.831^c	2.000^a	15.000^b

Mean sharing the same superscript letters under the same column do not differ significantly at $P < 0.05$.

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Thiamin

Results showed that thiamin in all concentrations do not significantly affect on germination rate but it affected significantly in concentration 10mg/l on other characters where it significantly affected the plant height [result- 75.35, control- 58.22] and also it significantly affected on leaf space [result-25.12 cm2, control-15.22 cm2]. It affected significantly the quantity of chlorophyll result- 8.463mg/g, control- 5.831 mg/g]. It also affected significantly the number of spikes [result- 4 spikes, control- 2]. Its effect on numbers of grain in spike was also significant [result- 22 grains, control- 15 grains.

Biotin

Results showed that the biotin in all concentrations do not significantly affect the germination rate. It affected significantly the plant height at 5mg/l concentration [result-76.12cm, control- 58.21cm]. Biotin also affected the leaf space significantly at same concentration [result- 24.45 cm2, control- 15.22 cm2]. It significantly affected the quantity of chlorophyll at 5mg/l concentration [result- 8.986 mg/g, control- 5.830mg/g]. It also affected significantly the number of spikes at all concentrations [result-2.000, control- 1.000]. The number of grains in a spike were significantly affected by 1mg/l concentration of biotin [result- 17.000, control- 16.000].

Thiamin and biotin

Results showed that the biotin and thiamin at 10mg/l concentration significantly affected the rate of germination. They significantly affected the plant height [result- 75.31 cm, control- 58.20 cm]. The leaf space was also significantly affected [result- 25.45cm2, control- 15.20cm2]. They affected significantly the quantity of chlorophyll [result- 8.854 mg/g, control- 5.831mg/g].However, its effect on number of spike was not significant at any concentration. The numbers of grains in spike were significantly affected at 5mg/l concentration [result- 22 grains, control- 15 grain].

Discussions

Glucose is oxidised to produce metabolic form of energy, ATP (Zaid, 2009). This pathway is essential resource of NADH. Thiamin pyrophosphate is a derivate of thiamin, this coenzyme removes carboxyl groups from pyruvic acid leaving acetyl group that gives lipoic acid which transform acetyl group into coenzyme A that enters in creb's cycle. Where,the biotin has important role in construction process and food regulation inside plant and essential in breathing (Zaid, 2009). It participates in interactions including transfer of carboxyl group in fatty acid synthesis. So it affected the lab characters for wheat seedling. These results were corresponding with results of Alazawy, 2010 In their study they found the mixture of thiamin and biotin in concentrations 1,5,10 mg/l significantly affecting on seedling growth for dates palm Phoenix dacctylifera. Leonardi et al., in 2005 found that the thiamin significantly affected the metabolic activity of wheat seedling (Leonardi et al., 2005).

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Where most of these vitamins transfer to coenzymes so they participate in catalytic processes. Vitamins are generally organic compounds working in a little quantity but they are important in regulating metabolic processes (Bolander, 2006).

The biotin in all concentrations do not affect significantly on germination rate but it affected significantly in concentration 5mg/l on other treatments, as it affected significantly on plant height as it reached 76.12 cm compare with control treatment that reached 58.21 cm, also biotin affected on leaf space significantly as it reached 25.12 cm2 compared with control treatment that reached 15.22 cm2, but it affected significantly on quantity of chlorophyll as it reached 8.986 mg/g compared with control that reached 5.830mg/g. This contradicts with study by Alazawy, in 2010, the biotin concentration 10mg/l lead to appearing significantly different in its effect on growth and reveal dates palm seedling Phoenix dactylifera, but its effect on numbers of spikes and number of grains in spike do not affect significantly, which may be due to weak concentration used for this vitamins so significant effects not appear.

Various studies by researchers explained the role of thiamin and biotin in cell division and callus growth for dates palm in vitro (Al-Khayri, 2001; EL-Shiaty et al., 2004). So, studies reveals the role of these vitamins on growth of corn, chickpeas and legumes (Bender, 1999; Eduardo et al., 2019). So, the effect of these vitamins has been studied on germination and growth of wheat seedling..

Conclusion(Times New Roman, bold, 12)

The present study clearly demonstrated that vitamins, biotin and thiamin, when analysed in lab experiment and anvils experiment using various concentrations are having significant effect on growth of wheat seedling.

Limitations and Future Studies

In the present study, the effect of vitamins was analyzed in details. However, the biochemical estimation (quantitatively) of some key or important compound (secondary metabolites) after the vitamins treatment might give a better understanding of the effect on germination and plant growth. This will be the future approach to evaluate the effect of vitamins specifically in the biosynthesis of secondary metabolites.

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