Pak. J. Bot., 44: 101-104, Special Issue March 2012.

INFLUENCE OF LEVOTHYROXINE SODIUM ON GROWTH AND UPTAKE OF SOME MINERAL ELEMENTS IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

The aim of this study is to find out whether or not synthetic human thyroxine hormone has any effects on growth and mineral element distribution in plants. Levothyroxine is identical to the hormone thyroxine, which is produced naturally by the human thyroid gland. In human hypothyroidism, the thyroid gland becomes unable to produce normal amounts of thyroxine, and the level of both T_3 and T_4 (thyroid hormones) in the blood decreases. This results in a reduced rate of metabolism, leading to symptoms such as weight gain, intolerance to cold and tiredness. Levothyroxine is given to replace the thyroxine that would normally be produced by the thyroid gland. This restores levels of T_3 and T_4 to their normal state. Once the thyroid gland becomes unable to produce thyroxine, it will generally not return to normal function. When compared to some plant substances, this chemical shows some structural similarities. Therefore in this preliminary study, the effects of levothyroxine (Na form) on growth parameters stem length, leaf, stem and root fresh and dry weights, and leaf area and its influence on uptake of mineral elements such as Fe, Mg and Zn in cotton was studied. As a result, it was observed that some growth parameters increased or decreased related to the applied concentrations and Fe, Mg and Zn values were affected in leaf, stem and root parts of the treated cotton samples.

Introduction

The thyroid hormones, thyroxine 3,5,3′,5′-tetraiodo-L-thyronine (T_4) and triiodothyronine (T_3), are tyrosine-based hormones produced by the thyroid gland and then secreted into the bloodstream (Metzler *et al*., 2001; Richardson, 2009). The major form of thyroid hormone in the blood is thyroxine (T_4) (Richardson, 2009). The synthetic form of T_4 (levothyroxine) and the natural hormone have the same stereochemical configurations, i.e. both adopt an S-configuration at the asymmetric carbon (Fig. 1).

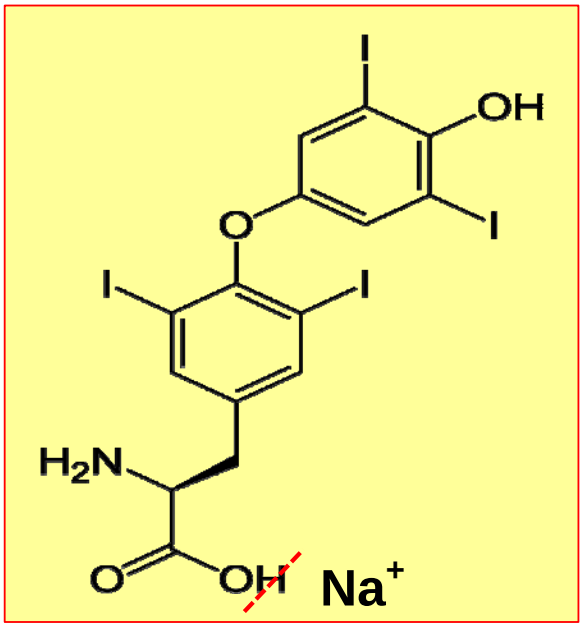

Fig. 1. 3,5,3',5'-Tetraiodo-L-thyronine (levothyroxine) and its sodium form.

Most of the thyroid hormone circulating in the blood is bound to transport proteins. In the blood, levothyroxine has high affinity for the thyroid hormone distributor (THD) proteins (Robbins & Edelhoch, 1986) and these THD proteins avoid greedy, nonspecific partitioning of thyroid hormones into membranes in mammals (Richardson, 2009). Thyroid hormones dissociate from thyroid hormone distributor proteins and due to their hydrophobic nature, they can enter cells by passive diffusion as well as via thyroid hormone transporters (Dietrich *et al*., 2008). Before being translocated into the nucleus within cells, thyroid hormones and specific cytosolic thyroid hormone binding proteins form an association together. Once inside the nucleus, the hormone binds its receptor, and the hormone-receptor complex interacts with specific sequences of DNA in the promoters of responsive genes. The effect of the hormone-receptor complex binding to DNA is to modulate gene expression, either by stimulating or inhibiting transcription of specific genes (Richardson, 2009).

Transthyretin (TTR) is a transport protein in extracellular fluids of vertebrates, where it distributes the two thyroid hormones (Schreiber & Richardson, 1997). A number of proteins related to the transport protein transthyretin (TTR) forms a highly conserved protein family and are found in a wide variety of species including plants (Eneqvist *et al*., 2003). Recently, it has been recognized that both prokaryotic and eukaryotic members of this family are not functionally related to transthyretins. Genomic data support a functional role involving purine catabolism for the transthyretin related proteins (TRP) (Lee *et al*., 2005; Zanotti *et al*., 2006). Purines are major components of nucleic acids and nucleotides and are continuously formed and degraded in biosphere (Nygaard, 1983).

Within a plant, the apoplast is the free diffusional space outside the plasma membrane. The apoplast plays a major role in a diverse range of processes in plants, including intercellular signaling, plant-microbe interactions and both water and nutrient transport. Mineral nutrients must pass the cell wall before they can be absorbed and because of physico-chemical properties of cell walls, mineral nutrients sometimes do not cross the cell wall. As a result they accumulate next to or attach to

cell-wall components, which might have a negative effect on both nutrient acquisition (Thornton & Macklon, 1989; Ae & Otani, 1997) and tolerance against toxicity (Horst, 1995). Due to the predominance of free carboxyl groups on the galacturanic acids of the pectins in the middle lamella and primary wall, cell walls posses negative charges, movement of ions in cell wall is characterized by electrostatic interactions leading to an accumulation of cations in the apparent free space in a nonmetabolic step (Marschner, 1995). The model is useful especially in understanding of uptake phenomena such as the apparent synergism between Ca^{2+} and $H_2PO^-_4$ (Franklin, 1969) or differences in the uptake of Zn^{2+} in ionic or chelated form (Marschner, 1995). An accumulation of cations and a repulsion of anions in the root apoplast are noticed due to negative charges on the root cell wall (Clarkson, 1993). This is particularly clear for di- and polyvalent ions (Haynes, 1980). Binding of certain metal cations such as Cu (Thorton & Macklon, 1989), Mn (Bacic *et al*., 1993), B (Matoh *et al*., 1997), Fe (Zhang *et al*., 1991a) or Zn (Zhang *et al*., 1991b), to cell wall components may be quite specific. For example, Cu may be bound in nonionic form to nitrogen containing groups of cell wall proteins (Harrison *et al*., 1979; Van Cutsem & Gillet, 1982) while B is bound to diols and polyols, particularly cis-diols (Goldbach, 1997). In this context rhamnogalacturonan II is of special significance (O'Neill *et al*., 1996; Kobayashi *et al*., 1996). This binding causes an accumulation of the relevant nutrient in the cell wall. It has been shown that the cell wall components of roots are involved in obtaining sparingly soluble Fe phosphates for groundnut in low fertility soil (Ae *et al*., 1996). It has been implied that this effect is due to a binding of Fe to root cell wall components and a subsequent release of phosphate. Unstirred layers are of principal importance for all transport processes across barriers such as the apoplast or the plasmalemma. As a consequence, it stands as a barrier itself determining not only transport rate but also the diffusion across the unstirred layers (Zimmermann *et al*., 1992).

Thyroid hormones play an important role in many physiological processes such as embryonic development, cell differentiation, metabolism, and the regulation of cell proliferation in all vertebrates (Yamanaka *et al*., 2007). Although there has been no study reported related with levothyroxine sodium and its effects on plants, influence of levothyroxine sodium on growth and uptake of some mineral elements in cotton was studied in this research for investigating plant (cotton was used as a model organism here) responses. This preliminary study showed that physiological processes are altered in cotton resulting changes in plant growth and uptake and accumulation of microelements (Fe, Mg, and Zn) grown with levothyroxine sodium.

Materials and Methods

Plant material: Cotton is one of the most important fiber crops in the global textile industry and thus great deal of scientific research has been carried out in cotton anatomy, physiology, biochemistry, genetics and biotechnology (Kumbhar *et al*., 2008; Malik *et al*., 2009; Mumtaz *et al*., 2010; Nawab *et al*., 2011). In this study, cotton var. Nazilli 84S, which was obtained from Nazilli Cotton Research Institute Aydın-Turkey was used to investigate the effects of levothyroxine sodium on growth and uptake of some mineral elements in cotton.

Growing seeds: The surface of cotton seeds were soaked by immersion in ethyl alcohol (50%) for 1 minute followed by immersion in deionized water for 5 minutes. They were then transferred into small vessels containing sterilized compost for germination. During the germination period (2 weeks), the seeds were moisturized with deionized water. When the shoot lengths of the young plantlets reached 3-4 cm, they were transferred into standard plastic pots containing sterilized compost and maintained under growth-room conditions. The plants were grown under fluorescent tubes giving an irradiance of 5000 lx (day/night-16/8 respectively), and a temperature of 23 ± 2°C and relative humidity 45-50%. Each of the experimental groups of eight replicates was watered with levothyroxine sodium solutions at two-day intervals for 80 days.

Dosage and application: For an adult human, dose equates to 100 µg daily for the average-sized woman (60kg) and 125 µg daily for the average sized man (75kg) (Vaidya & Pearce, 2008). In this study, approximately between 1/200 and 1/25 of daily doses (for man) were applied to cotton seedlings. While control plants were watered only with deionized distilled water, the experimental groups were watered with 30 ml 1.25, 2.5, 5 and 10 µg levothyroxine sodium solution at two-day intervals.

Analytical techniques: After an 80-day application period, leaf, stem and root samples of seedlings were dried in an oven at 80^{o}C over the course of two days. Then samples were exposed to the "wet ashing" method (Kacar, 1972). Samples were analyzed using Thermo Elemental SOLAAR M6 Series flame atomic absorption spectrometer (FAAS) instrument. Atomic absorption measurements were carried out using an air-acetylene flame. Single-element hallow cathode lamps were used at the wavelengths of 248.3, 279.5 and 213.9 nm for Fe, Mn and Zn respectively. A deuterium hollow cathode lamp was used for background correction in all determinations. For the quantification of magnesium, the emission mode of the instrument was selected in such a way that best results were obtained using the 285.2 nm magnesium line.

Statistical methods: At the end of the experiment the research data were analyzed statistically by using SPSS 13.0 for windows**.** The standard error values of the means were calculated to compare the site categories. A paired *t*-test was performed to determine any significant changes between the groups, and an F-test (ANOVA) was performed on data. According to the results of variance analysis and Tukey test, the mean difference was significant at $p < 0.05$ level.

Results and Discussion

The stem length and stem, leaf and root growth rates were analyzed in cotton (*Gossypium hirsutum* L.) seedlings in response to different levothyroxine sodium concentrations. Data showed that stem lengths decreased

with an increase of levothyroxine sodium concentrations compared to the control seedlings, but a slight increase at 2.5µg levothyroxine sodium was found relative to the control (Table 1). Levothyroxine sodium had an inhibitory effect on stem, leaf, and root growth of cotton. The changes in stem, leaf and root fresh- and dry-weights of cotton seedlings exhibited an inverse relationship with levothyroxine sodium concentration. It was found that following a slight increase in stem, leaf and root fresh- and dry weights, a decrease was observed for each (stem, leaf and root fresh and dry weights). Also, a slight increase at 2.5µg levothyroxine sodium was obtained for leaf area of cotton seedlings (Table 1). In cotton seedlings grown under different levothyroxine sodium levels, the concentrations of some microelements were examined in stems, leaves, and roots at 1.25, 2.5, 5 and 10 µg of levothyroxine sodium exposure. It is clear from the results that microelement composition in stems, leaves and roots was altered by levothyroxine sodium exposure. The microelement concentrations in cotton seedlings plant tissues are shown in Table 2. There existed differences in the uptake and accumulation of some microelements in roots, stems, and leaves of cotton seedlings under levothyroxine stress. The concentrations of microelements (Fe, Mg and Zn) were reduced by the presence of levothyroxine sodium (at 1.25µg and 2.5µg levels) in roots but following the decrease, slight increments were observed at 5µg and 10µg levothyroxine sodium levels. Stem and leaf concentrations of Fe, Mg and Zn showed an initial increase following levothyroxine sodium treatment, with the greatest increments observed at 1.25µg 2.5µg levels of levothyroxine sodium whereas a decrease following the increment was observed at 5µg and 10µg of levothyroxine sodium levels for each micronutrient Fe, Mg, and Zn (Table 2).

Table 1. Some growth parameters of levothyroxine sodium treated cotton in different concentrations.

	Control	1.25µg	2.5µg	5µg	10µg
Stem length (cm)	26.250 ± 1.207*	23.250 ± 0.752*	28.320 ± 0.928*	26.633 ± 0.801*	23.467 ± 1.180*
Leaf fresh weight (g)	1.182 ± 0.033*	1.127 ± 0.029*	1.268 ± 0.058*	1.163 ± 0.036*	0.911 ± 0.069*
Leaf dry weight (g)	0.206 ± 0.009	0.192 ± 0.009	0.227 ± 0.017	0.200 ± 0.015	0.168 ± 0.018
Stem fresh weight (g)	2.669 ± 0.101*	2.351 ± 0.145*	2.906 ± 0.153*	2.560 ± 0.085*	1.679 ± 0.136*
Stem dry weight (g)	0.613 ± 0.041*	0.469 ± 0.024*	0.940 ± 0.090*	0.704 ± 0.060*	0.551 ± 0.106*
Root fresh weight (g)	0.981 ± 0.052	1.003 ± 0.079	1.104 ± 0.080	0.917 ± 0.082	0.844 ± 0.067
Root dry weight (g)	0.171 ± 0.021*	0.291 ± 0.023*	0.348 ± 0.044*	0.241 ± 0.028*	0.157 ± 0.014*
Leaf area (cm^2)	81.756 ± 4.000	80.135 ± 1.505	86.995 ± 1.706	80.629 ± 2.559	74.045 ± 2.460

*According to the results of variance analysis and tukey test, the mean difference is significant at 0.05 levels.

Table 2. Mineral element (Fe, Mg and Zn) distributions of leaf stem and root samples of levothyroxine sodium treated cotton in different concentrations.

		Control	1.25µg	2.5µg	5µg	10µg
Fe (mg/kg)	Leaf	269.797±17.970*	329.730±19.399*	349.202±11.674*	316.275±22.063*	235.369±12.459*
	Stem	38.260±2.258	39.292±2.327	37.686±2.220	31.548±2.531	29.133±2.394
	Root	757.341±78.110*	534.436±50.028*	396.522±22.188*	413.653±22.698*	493.405±30.773*
Mg (mg/kg)	Leaf	2198.882±110.802	2882.922±201.918	2398.738±171.723	2236.109±264.072	2473.387±187.085
	Stem	166.910±6.775*	176.804±1.969*	219.608±9.640*	70.280±2.015*	60.780±5.395*
	Root	5309.681±157.875*	4742.371±195.084*	3804.631±175.379*	4218.178±233.357*	5231.796±127.452*
Zn (mg/kg)	Leaf	101.506±4.052*	103.097±5.070*	138.098±11.682*	110.219±3.510*	103.656±4.150*
	Stem	22.921±1.364*	31.836±3.012*	14.075±2.109*	17.382±1.855*	19.136±1.872*
	Root	53.017±4.099*	46.455±2.810*	29.791±2.482*	44.873±3.165*	33.511±1.269*

*According to the results of variance analysis and tukey test, the mean difference is significant at 0.05 levels.

The growth and uptake and accumulation of microelements are altered in cotton grown with levothyroxine sodium. Levothyroxine toxicity could result from complex levothyroxine sodium interactions with apoplastic, plasma membrane, and symplastic targets. Levothyroxine sodium is taken up by cotton through the root system and does not simply pass through cell wall but can also be fixed to cell-wall components resulting in accumulation on the cell wall. It may be of significance for both nutrient acquisition and tolerance against levothyroxine toxicity. The data implies that levothyroxine sodium may interfere directly with uptake and translocation of divalent cations because levothyroxine sodium may change root morphology by either replacement of Fe, Mg, and Zn with itself at nonspecific binding sites, or direct interference with uptake processes. The restricted uptake and translocation of these divalent cations may result in accumulation of these divalent cations in the root apoplast. Therefore, the concentrations of Fe, Mg and Zn were reduced at 1.25µg and 2.5µg levels because of interference of uptake and translocation by levothyroxine sodium in roots but following the decrease, slight increments were observed at 5 and 10µg levothyroxine sodium levels because of accumulation of Fe, Mg, and Zn in the root apoplast. Meanwhile, the data showed that following the slight increment there was a reduction for each micronutrient (Fe, Mg, and Zn in this study) with increasing levothyroxine sodium levels in stems and leaves. The same pattern was also observed for each growth parameter (fresh and dry weights, length and leaf area) in stems and leaves. Increasing the levothyroxine sodium concentration seems first to stimulate growth and then to exert a toxic effect. Low concentration of levothyroxine sodium may stimulate growth by relieving proton stress at the root surface. Displacement of excess protons from the plasmalemma surface by levothyroxine sodium could result in this growth-stimulating effect of levothyroxine

sodium. However, high level of levothyroxine sodium exerts a toxic effect. At high levothyroxine sodium levels (5µg and 10µg), a sufficient gradient could be created in the root apoplast and therefore, levothyroxine sodium can pass plasma membrane easily via passive diffusion because of its hydrophobic nature. After being translocated into the cytosol and then the nucleus, levothyroxine sodium could modulate the expression of genes such as transthyretin related protein genes which are involved in the purine catabolic pathway in plants. Because of altered metabolic pathways, secondary stresses such as oxidative damage linked to the production of reactive oxygen intermediates could occur resulting in mutation, protein destruction peroxidation of lipids and finally these lead to reduction of growth and development in cotton seedlings at high levels of levothyroxine sodium treatments (5µg and 10µg).

Acknowledgement

This study was funded by Marmara University, Commission of Scientific Research Project under grant FEN-D-070211-0016.

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(Received for publication 12 February 2011)