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Physiological response of cotton seeds treated with thiamethoxam under heat stress[1]

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ABSTRACT - This study aimed to verify the effect of seeds treated with thiamethoxam on the emergence, growth and chlorophyll content of seedlings of two cotton cultivars subjected to abiotic stress. The seeds used were from the DeltaOpal and NuOpal cultivars, each one represented by three lots, previously subjected to determination of moisture content, germination and vigor (low temperature germination). Then, the seeds from each lot, treated with thiamethoxam at a dose of 600 mL of product per 100 kg^{-1} seed or not, were exposed to temperature (18, 25 and 35 °C) and water availability (40, 60 and 80%) stress conditions. The emergence percentage, the root and shoot length, and the chlorophyll content were evaluated. The growth of cotton seedlings from the DeltaOpal and NuOpal cultivars, subjected to low temperature conditions and water availability of 40 to 60%, is favored by seed treatment with thiamethoxam. Seed treatment with thiamethoxam increases chlorophyll content during the development of cotton plants.

Index terms: bioactivator, chlorophyll, *Gossypium hirsutum* L., stress.

Resposta fisiológica de sementes de algodão tratadas com tiametoxam sob estresse térmico

RESUMO – Neste trabalho teve-se por objetivo verificar o efeito do tratamento de sementes com tiametoxam sobre a emergência, o crescimento e o índice de clorofila de plântulas de duas cultivares de algodoeiro, submetidas a estresses abióticos. Foram utilizadas sementes das cultivares DeltaOpal e NuOpal, cada uma representada por três lotes, submetidas previamente a determinação do grau de umidade, germinação e vigor (germinação a baixa temperatura). Em seguida, as sementes de cada lote, submetidas ou não ao tratamento com tiametoxam na dose de 600 mL de produto por 100 kg^{-1} de semente, foram expostas a condições de estresse por temperatura (18, 25 e 35 ºC) e disponibilidade hídrica (40, 60 e 80% de umidade). Foram avaliadas a porcentagem de emergência, o comprimento de raiz e da parte aérea e o índice de clorofila. O crescimento das plântulas de algodoeiro, das cultivares DeltaOpal e NuOpal, submetidas às condições de baixa temperatura e disponibilidade hídrica de 40 e 60%, é favorecido pelo tratamento de sementes com tiametoxam. O tratamento de sementes com tiametoxam aumenta o índice de clorofila durante o desenvolvimento de plantas de algodoeiro.

Termos para indexação: bioativador, clorofila, estresse, *Gossypium hirsutum* L.

Introduction

In order to be successful in a crop, it is important to gather conditions that allow the plant to express all its productive potential. Abiotic stresses such as inappropriate and dry temperatures can significantly reduce crop yield and restrict planting in regions where commercially important species can be grown (Vaz de Melo et al., 2012).

The activation of the germination process of a seed depends directly on water availability (Ávila et al., 2007), temperature (Nascimento, 2013) and oxygen (Brasil, 2009). In order to have high productivity and fiber quality, the cotton tree needs sunny days, average air temperature above 20 ºC and well-distributed rainfalls (Beltrão and Vieira, 2001). However, in conditions in which it is grown in Brazil, there are variations of temperature and humidity during the period of seedling emergence, which are the main stresses that occur during the sowing period and, commonly, the ones that have the highest risks for the normal establishment of the culture (Mattioni et al., 2009). According to Ávila et al. (2007), insufficient water availability in the soil has been considered one of the most common causes of low emergence

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of seedlings in the field. Regarding temperature, both low and high temperatures can enable soaking but not continue with growth of the embryo and the establishment of the seedling, preventing the conclusion of the process (Taiz and Zeiger, 2004). Also, according to Thakur et al. (2010) and Szabados et al. (2011), the stress caused by suboptimal and above optimal temperatures alters cellular homeostasis, causes severe delay in plant growth and development and, in many cases, leads to programmed senescence.

In addition to climate variations, the plants are subject to insects and fungi attack, which can affect the development of the crop. In order to solve these field problems, some insecticides and fungicides are used to control pests and diseases. However, these products may have physiological effects in the plants, changing the physiology of several crops, interfering in the development of the plants in the field (Pereira, 2010). Agrochemical, organic and complex substances can have morphological and physiological effects on the plants and act in the synthesis of hormones, leading to an increase in productivity. They can be called bioactivators (Castro, 2008).

Thiamethoxam, registered and widely used for a great number of crops in Brazil, is believed to have a bioactivation effect, able to increase the agricultural production and promote gains in vigor, germination and grain yield, besides protecting the plants from stress conditions when applied in seeds treatment (Elbert, 2008), providing better chances of achieving their genetic potential for productivity (Castro, 2009).

Knowing the risks of stress by temperature and humidity that a cotton crop is subjected to and the potential benefits that the seeds treatment with thiamethoxam may provide, the objective of this paper was to verify the effect of seeds treatment with this bioactivator on the emergence, growth and chlorophyll content of seedlings from two cultivars of cotton subjected to abiotic stress.

Material and Methods

The experiment was carried out in the Seeds Testing Laboratory of the Post-Graduate program in Seed Science and Technology, at the Federal University of Pelotas (UFPel) and in a low density polypropylene greenhouse belonging to "Embrapa Clima Temperado". Seeds from two cotton cultivars were used (DeltaOpal and NuOpal), each one represented by three lots.

The seeds were treated with the commercial product containing 35 grams of thiamethoxam active ingredient per liter of product, composing two treatments: 1: untreated seeds, and 2: 600 mL of product kg of seed^{-1}. The syrup (product + water) was applied with the help of a graduated pipette, at the bottom of a transparent plastic bag and distributed through the walls of the bag. The solution volume was 1.3 L. 100 kg^{-1} of seeds.

Before submitting it to different stresses, we proceeded to the characterization of the quality of the lots by the following tests:

a) *water content***:** it was determined with two subsamples of 5 g in an oven, at 105 ºC ± 3 ºC for 24 hours (Brasil, 2009);

b) *germination*: done with four replications of 200 seeds, one of the replications being formed by two rolls of 100 seeds each, amounting to 800 seeds for each cultivar, sowed in moistened paper towels with the amount of water equivalent to 2.5 times its dry mass, remaining in a germinator at 25 °C. The counting was done at four and twelve days after sowing and the results expressed in average percentage of normal seedlings for each lot (Brasil, 2009);

c) *germination at low temperature***:** the seeds were disposed on moistened paper towels in a proportion equivalent to 2.5 times its dry matter and, right after, taken to a germination chamber at 18 ºC, for seven days, being considered vigorous, by the end of this period, the normal seedlings that reached 4 cm in length (Dias and Alvarenga, 1999).

Water and temperature stresses were determined by taking into account the possibility of their occurrence at the moment of sowing, germination of seeds and emergence of seedlings in normal situations for the regions where cotton is grown in Brazil.

Sowing was done in polypropylene plastic trays containing sand at a 2 cm depth. The water retention capacity was calculated according to the methodology proposed by Brasil (2009), with water availability of 40, 60 and 80% of water retention capacity in the substrate. They were placed in a germination chamber, in the temperatures of 18, 25 and 35 ºC.

A temperature of 25 ºC and water retention capacity in the substrate of 60% (Brasil, 2009) were adopted as the optimal conditions for seed germination.

For each treatment, four replications of 50 seeds were used, in order to avoid interference of pathogenic fungi, the seeds were treated, in all of the tests, with fungicide Thiran 200 g .L^{-1}, in a dosage equivalent to 200 mL for 100 kg of seeds^{-1}. After 12 days of sowing, the following evaluations were done:

a) *seedling emergence*: all seedlings emerged in trays were counted, adopting as a criterion the complete expansion of cotyledonary leaves, and the results were expressed in percentage; b) *root and aerial part length*: carried out with the same seedlings from emergence, measuring the main root and the aerial part length of 15 seedlings per replication, with the help of a graduated ruler. The result was expressed in centimeters per seedling.

At the same time, another experiment was done at the "Embrapa Clima Temperado" greenhouse to determine the chlorophyll content. Seeds of both cultivars were sown in vases and, seven days after emergence, readings were done of the newly mature leaves of four plants per vase, chosen at random considering each plant a replication. The determinations were done in four leaf points, with a distance of 2 cm from the margin, through a CCM-200-Opti-scienc equipment, between 8 and 10 a.m. in regular intervals of seven days for 70 days.

The initial quality data of seeds were analyzed under an entirely randomized design (DIC), while the emergence results, root length and aerial part length were analyzed under DIC with a 3x3x2 factorial design (temperature, water availability and treatment). All analyses were done with four replications, and the means were compared by the Tukey test at 5% probability. For the experiment done in a greenhouse, a completely randomized design with four replicates was used. The data was submitted to a variance analysis and, when significant at 5%, expressed through the polynomial regression analysis.

Results and Discussion

From the initial quality results of the cotton seeds, it can be observed that there are no significant differences between the lots regarding germination test for cultivar DeltaOpal (Table 1). Regarding germination at low temperature, lots 3 and 4 had a better performance. The contents of water in the cultivars evaluated presented variations of 0.3 and 0.4 p.p. for cultivar DeltaOpal and NuOpal, respectively (Table 1).

Table 1. Characterization of the initial quality of cotton seeds without treatment.

Cultivar	Lots	WC (%)	GT (%)	GTALT (%)
DeltaOpal	1	8.6	88a	71b
	2	8.4	87a	77b
	3	8.3	91a	86a
NuOpal	4	8.3	91a	75a
	5	7.9	93a	68b
	6	8.1	86b	54b*

*Means inside each column followed by the same letter do not differ from each other by the Tukey test at 5% probability. Water content (WC), Germination test (GT), Germination test at low temperature (GTALT).

Analyzing the germination tests results, it was seen that it was inferior when the seeds were submitted to low temperature. In rice seeds, Mertz et al. (2009) observed that germination at low temperature (13 ºC) harmed germination due to physiological alterations caused by cold. This test can be used to make decisions as to the storage or sowing potential of seed lots (Freitas et al., 2002).

The emergence, root length and aerial part tests presented interaction among the factors. Regarding the emergence test (Table 2), done under different temperatures and water availability, it can be observed that the cotton seeds from the DeltaOpal cultivar, subjected or not to the thiamethozam treatment, presented higher seedling emergence at a temperature of 35 ºC and water availability of 60%. The exposition of seeds to a temperature of 18 ºC, combined with the high water availability (80%), influenced negatively the emergence of seedlings, whether they have been submitted to treatment or not. Almeida et al. (2014), saw a positive effect of the treatment of rice seeds with thiamethoxam/lambda cyhalothrin, with higher percentage of normal seedlings, even at low temperatures (10 and 13 ºC). According to Vaz de Melo et al. (2012), the seeds generally die in a few days above the maximum temperature and they do not germinate in a reasonable timeframe below the minimum temperature.

Regarding the NuOpal cultivar (Table 3), untreated seeds presented emergence of seedlings compromised by the highest level of water availability and under a lower temperature. A similar result was shown in lot 4 at a temperature of 25 ºC. This explanation agrees with the information obtained for the DeltaOpal cultivar, in which the high water availability, associated to the low temperature, is not favorable to the emergence of cotton seedlings. John and Christiansen (1976), affirmed that the occurrence of cold after the issuing of the radicle and in the first stages of cotton development affects the initial growth and causes leakage of metabolites through the membrane, interference in the production of ATP, reduction of the protein synthesis and nucleic acid, and alterations in the size and format of the first leaves.

For the root length test, the treatment of seeds increased significantly its size in all lots subjected to low temperature stress conditions (18 ºC) and water availability of 40 and 60%, for cultivar DeltaOpal (Table 4). The use of thiamethoxam becomes important because it reduces the time of crop establishment in the field, reducing the negative effects caused by the lack of humidity in the soil or temperature extremes, making the plants develop faster and expressing their vigor better (Cataneo, 2008).

At 25 ºC, there were no differences in the root growth between treated and untreated seeds, except for lot 1, with 40% of water availability, in which treatment impaired the development of roots and lot 3, with 80% water availability, where treatment of seeds stimulated the development of roots. At extreme temperature conditions (18 and 35 ºC), the tendency was for the 80% of water availability to influence negatively the root growth of cotton seedlings, subjected or not to the treatment with thiamethoxam. Generally, for 25 ºC, the water availability of 60% was the most favorable to root development of cotton seedlings, whether the seeds were treated or not (Table 4).

Table 2. Emergence (%) of cotton seedlings, cultivar DeltaOpal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	US			TS		
		Temperature ºC			Temperature ºC		
		18	25	35	18	25	35
1	40	77 Aa	79 Ab	80 Ab	83 ABb	79 Bb	88 Ab
	60	82 Ba	86 ABa	89 Aa	90 Aa	93 Aa	94 Aa
	80	59 Bb	59 Bc	84 Ab	75 Bc	62 Cc	87 Ab
2	40	81 Ba	88 Aa	83 ABb	85 ABa	83 Bb	90 Ab
	60	80 Ba	88 Aa	90 Aa	86 Ba	90 ABa	94 Aa
	80	62 Bb	79 Ab	80 Ab	79 Bb	84 ABb	86 Ab
3	40	79 Ba	75 Bb	88 Aa	87 Ba	79 Cb	94 Aa
	60	83 Aa	88 Aa	89 Aa	91 Aa	91 Aa	91 Aa
	80	60 Bb	82 Aab	79 Ab	74 Bb	80 ABb	85 Ab*

*Means followed by the same uppercase letter in the rows and lowercase letters in the column do not differ significantly from each other by the Tukey test at 5% probability.

Table 3. Emergence (%) of cotton seedlings, cultivar NuOpal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	US			TS		
		Temperature ºC			Temperature ºC		
		18	25	35	18	25	35
1	40	77 Aa	78 Aa	78 Ab	84 Aab	60 Bc	88 Aab
	60	80 Ba	84 ABa	87 Aa	90 Aa	90 Aa	92 Aa
	80	60 Bb	66 Bb	80 Ab	79 Bb	79 Bb	87 Ab
2	40	81 Aa	80 Aa	81 Ab	88 Ab	84 Ab	88 Aa
	60	86 Aa	85 Aa	90 Aa	92 Aa	93 Aa	93 Aa
	80	64 Bb	80 Aa	83 Ab	83 Ab	83 Ab	88 Aa
3	40	80 Aa	77 Aa	78 Aa	88 Ab	75 Ba	77 Ba
	60	85 Aa	79 Ba	77 Ba	91 Aa	77 Ba	78 Ba
	80	60 Bb	78 Aa	76 Aa	78 Ac	78 Aa	75 Aa*

*Means followed by the same uppercase letter and lowercase lettres in the columns do not differ significantly from each other by the Tukey test at 5% probability.

Table 4. Comparison of the root length (cm) of cotton seedlings, cultivar Delta Opal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	18 °C		25 °C		35 °C	
		US	TS	US	TS	US	TS
1	40	3.1 Ba	4.9 Aa	5.8 Ab	3.3 Bb	4.4 Aa	5.2 Ab
	60	3.1 Ba	5.1 Aa	7.5 Aa	7.7 Aa	3.9 Bab	6.5 Aa
	80	2.3 Aa	2.7 Ab	3.4 Ac	3.7 Aa	2.8 Ab	3.3 Ac
2	40	2.9 Ba	5.1 Aa	3.2 Aa	3.7 Aa	4.4 Bb	6.7 Aa
	60	3.0 Ba	5.3 Aa	3.5 Aa	3.4 Aa	5.7 Aa	5.7 Aa
	80	2.1 Aa	2.7 Ab	3.8 Aa	4.2 Aa	2.7 Ac	3.2 Ab
3	40	3.2 Ba	4.7 Aa	3.0 Ab	2.2 Bc	4.1 Ba	5.4 Ab
	60	3.2 Ba	5.2 Aa	5.3 Aa	6.0 Aa	4.1 Ba	6.7 Aa
	80	2.2 Ab	2.5 Ab	3.5 Bb	4.6 Ab	2.8 Ab	3.3 Ac*

*Means followed by the same uppercase letter in the rows and lowercase lettres in the columns do not differ significantly from each other by the Tukey test at 5% probability.

Regarding the root growth of cultivar NuOpal (Table 5), it can be observed that under extreme temperature conditions, 18 and 35 ºC, considered stressful conditions for the cultivation of cotton, there were significant alterations in the root length after treatment with thiamethoxam for water availability conditions of 40 and 60%. This effect thiamethoxam has of increasing root length corroborates the rooting effect seen by Tavares et al. (2007) in soybeans crops, by Lauxen et al. (2010) in cotton, and by Almeida et al. (2009, 2011) in carrots and rice seeds.

Table 5. Comparison of the root length (cm) of cotton seedlings, cultivar Nu Opal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	18 °C		25 °C		35 °C	
		US	TS	US	TS	US	TS
1	40	3.3 Ba	5.1 Aa	3.7 Aa	3.5 Ab	4.8 Ba	5.8 Ab
	60	3.0 Ba	5.6 Aa	3.2 Ba	4.8 Aa	4.0 Ba	7.2 Aa
	80	2.1 Ab	2.7 Ab	3.4 Aa	2.7 Bb	2.7 Ab	3.4 Ac
2	40	3.2 Ba	4.7 Aa	2.8 Ab	3.0 Ab	4.4 Ba	5.5 Aa
	60	3.1 Ba	5.2 Aa	4.2 Ba	6.9 Aa	3.8 Ba	6.5 Aa
	80	2.3 Aa	3.0 Ab	2.7 Ab	2.1 Ab	2.7 Ab	3.2 Ab
3	40	3.4 Ba	5.0 Aa	3.0 Bb	4.3 Aa	4.5 Aa	5.4 Ab
	60	3.4 Ba	5.3 Aa	6.0 Aa	3.7 Bb	4.1 Ba	6.7 Aa
	80	2.2 Ab	2.7 Ab	4.3 Ab	3.5 Ab	2.8 Ab	3.2 Ac*

*Means followed by the same uppercase letter in the rows and lowercase lettres in the columns do not differ significantly from each other by the Tukey test at 5% probability.

At 18 and 35 ºC, the higher water availability (80%) affected root development, presenting to be inferior to the others, whether seed-treated or not (Table 5). Mattioni et al. (2009) also saw smaller root growth of cotton seedlings from cultivar FMT 701 under higher water availability (80%) in relation to the other humidity conditions (40 and 60%). According to Beltrão and Vieira (2001), water stress, both by deficiency and excess, reduces plant height, total fitomass, and the ration root/aerial part, plant photosynthesis, as well as the oxidative respiratory process.

The treatment of seeds with thiamethoxam did not have any effect on the growth of the aerial part of cotton seedlings under temperatures of 25 and 35 ºC, regardless of the water availability and the physiological potential of the lots (Table 6). However, the treatment was effective for all the lots at a temperature of 18 ºC associated with water availability of 60%. It can also be observed that the combination of soaked soil (80%) and low temperature impairs the development of the aerial part of cotton seedlings of seeds treated with thiamethoxam, corroborating Marcos-Filho's findings (2005) that the excess of water causes problems due to aeration restrictions and to possible damage during soaking.

Table 6. Comparison of the aerial part length (cm) of cotton seedlings, cultivar DeltaOpal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	18 °C		25 °C		35 °C	
		US	TS	US	TS	US	TS
1	40	5.1 Aa	6.5 Aa	8.4 Ab	5.1 Bb	7.1 Aa	7.9 Aa
	60	4.9 Ba	7.5 Aa	11.0 Aa	11.0 Aa	7.1 Aa	8.2 Aa
	80	3.5 Aa	3.6 Ab	4.9 Ac	5.4 Ab	3.9 Ab	5.7 Ab
2	40	4.8 Ba	6.4 Aa	4.2 Ab	4.8 Aa	7.2 Aa	8.1 Aa
	60	5.0 Ba	7.7 Aa	8.2 Aa	5.8 Ba	7.0 Aa	7.4 Aa
	80	3.8 Aa	3.6 Ab	5.8 Ab	5.5 Aa	3.8 Aa	5.3 Aa
3	40	4.9 Aa	6.4 Aa	3.5 Ab	2.9 Ab	7.2 Aa	7.9 Aa
	60	5.0 Ba	7.7 Aa	9.0 Aa	7.5 Aa	7.2 Aa	7.6 Aa
	80	3.7 Aa	3.4 Ab	4.4 Ab	5.7 Aab	3.7 Ab	5.7 Aa*

*Means followed by the same uppercase letter in the rows and lowercase lettres in the columns do not differ significantly from each other by the Tukey test at 5% probability.

Similar to what was found for cultivar DeltaOpal, the results for cultivar NuOpal (Table 7) show that, regardless of the lot quality, there was an increase in the length of the aerial part for seeds treated in relation to the ones not treated with thiamethoxam, subjected to low temperature conditions (18 ºC) and water availability of 60%.

Seeds treated with thiamethoxam in high temperature conditions (35 ºC) and high water availability (80%), present a significant increase in the aerial part length in relation to the untreated seeds for all the lots studied from this cultivar (Table 7). Similar results were found by Thielert (2006), when significant increases were observed in the aerial part and also in the growth of barley seedlings subjected to thiamethoxam treatments under water stress conditions. This difference in the aerial part length of seeds containing thiamethoxam can be explained due to the hypothesis that thiamethoxam favors the absorption of water and the stomatal resistance, improving water balance in the plant and, therefore, better tolerating water stress (Castro et al., 2007).

Table 7. Comparison of the aerial part length (cm) of cotton seedlings, cultivar NuOpal, obtained from untreated seeds (US) and treated seeds (TS), subjected to different temperatures and water availability.

Lot	Water availability (%)	18 °C		25 °C		35 °C	
		US	TS	US	TS	US	TS
1	40	5.1 Aa	6.7 Aa	4.8 Aa	4.3 Aa	7.2 Aa	8.0 Aa
	60	5.0 Ba	8.1 Aa	5.9 Aa	5.1 Aa	6.7 Aa	7.8 Aa
	80	3.7 Aa	3.7 Ab	5.1 Aa	4.0 Aa	4.0 Bb	6.2 Aa
2	40	4.9 Ba	6.5 Aa	3.7 Aa	3.7 Ab	7.3 Aa	8.3 Aa
	60	4.9 Ba	8.0 Aa	5.0 Ba	8.4 Aa	7.1 Aa	8.2 Aa
	80	3.6 Aa	4.1 Ab	3.7 Aa	3.7 Ab	4.1 Bb	5.8 Ab
3	40	5.1 Aa	6.4 Aa	5.1 Ab	4.8 Aab	7.2 Aa	8.0 Aab
	60	4.8 Ba	7.7 Aa	8.4 Aa	6.6 Ba	7.2 Aa	8.6 Aa
	80	3.7 Aa	3.8 Ab	4.8 Ab	3.8 Ab	4.2 Bb	6.1 Ab*

*Means followed by the same uppercase letter in the rows and lowercase lettres in the columns do not differ significantly from each other by the Tukey test at 5% probability.

In the experiment carried out in the greenhouse, we can observe that the chlorophyll content increased throughout the plant cycle and adjusted more appropriately to the quadratic model, with high determination coefficients (Figure 1). The increase in chlorophyll content in initial stages of growth can be expected due to the greater investment of compounds to the formation of radiant energy capture (Taiz; Zeiger, 2004).

There was a growing tendency in this variable until 42 DAE (days after emergence), with a later reduction until 70 DAE. Regardless of the lot or cultivar, plants coming from seeds treated with thiamethoxam presented higher synthesis of this pigment, probably due to the bioactivation effect of the product on synthesis metabolic pathways. This increase may be explained by the bioactivation effect of thiamethoxam on the formation of radiant energy pigments. The bioactivators are capable of modifying or altering several specific physiologic processes, stimulating the chlorophyll synthesis and the photosynthesis (Carvalho; Ferreira; Staut, 2007).

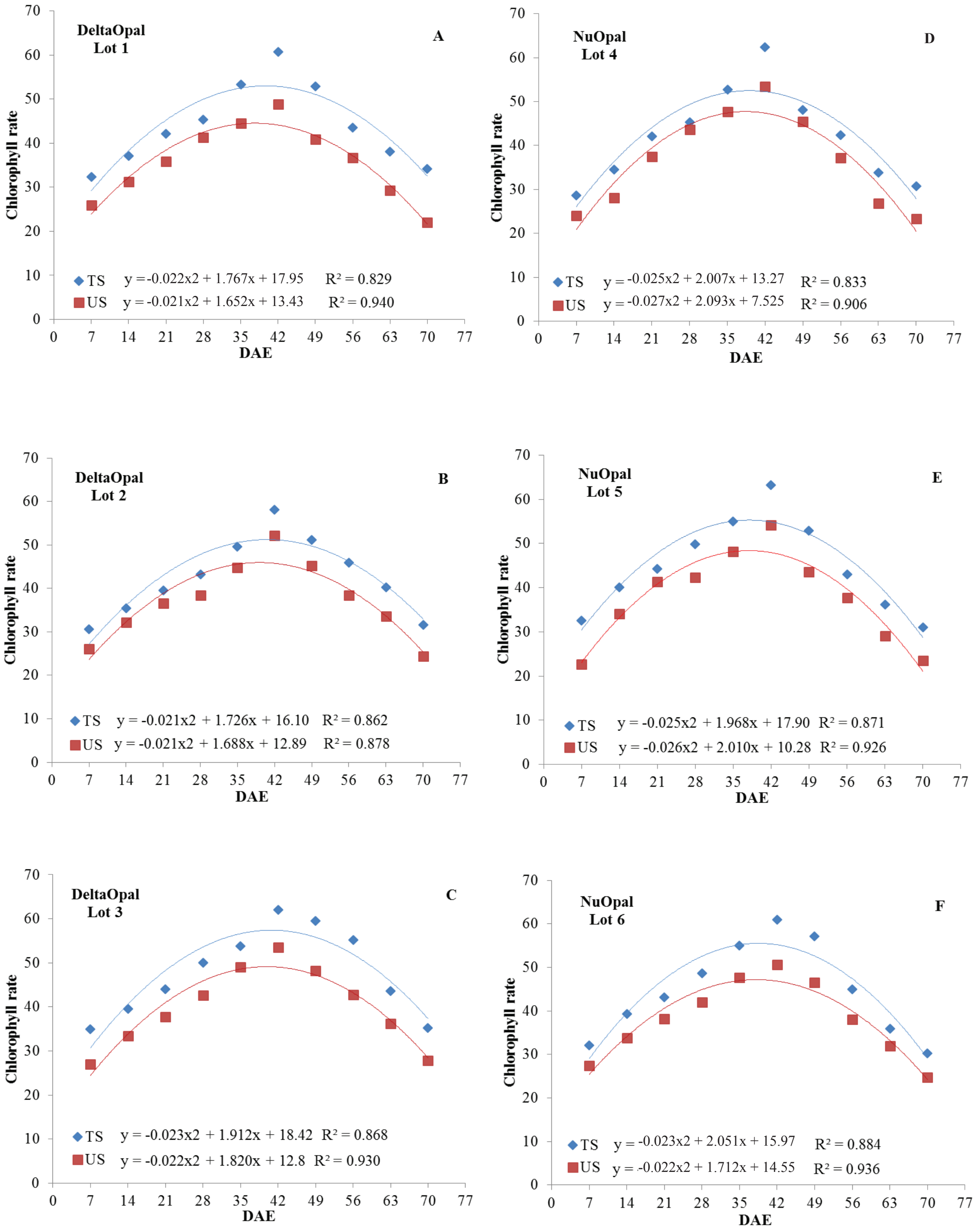

Figure 1. Chlorophyll rate in cotton plants from cultivars DeltaOpal and NuOpal, originated from different lots of seeds subjected or not to the treatment with Thiamethoxam. Days after emergence (DAE), Treated seeds (TS), Untreated seeds (US).

Conclusions

The growth of cotton seedlings from cultivars DeltaOpal and NuOpal, subjected to low temperature conditions and water availability of 40 and 60% is favored by the treatment of seeds with thiamethoxam.

The treatment of seeds with thiamethoxam increases the chlorophyll content during the development of cotton plants.

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