



Article

ROSOLEM, C.A.^{1*}
SARTO, M.V.M.²
ROCHA, K.F.¹
MARTINS, J.D.L.¹
ALVES, M.S.¹

DOES THE INTROGRESSION OF *Bt* GENE AFFECT PHYSIOLOGICAL COTTON RESPONSE TO WATER DEFICIT?

A Introgessão do Gene Bt Afeta a Resposta Fisiológica do Algodoeiro ao Déficit Hídrico?

ABSTRACT - Water deficit may affect the expression of lepidoptera-controlling proteins in cotton. However, it is unknown if there is a differential response of conventional and *Bt* cotton cultivars to water deficit, what could potentially affect the plant competition with weeds. The objective of this work was to investigate the response of *Bt* cotton cultivars to water deficit compared with their conventional near-isolines. The experiment was conducted in a greenhouse, where the cotton cultivars FMT 705, FMT 709 and IMACD 8276, with and without the *Bt* gene, were grown under two water regimens: 100% and 50% (moderate water deficit) of available soil water. Cotton phenology was severely affected by moderate water deficit, with a reduction in shoot and root dry matter production, root length and diameter, plant height and leaf area. No effect of the *Bt* gene was observed. Water deficit during cotton flowering decrease stomatal conductance, net assimilation of CO₂ and transpiration rates. The leaf water potential is lower in plants exposed to a moderate water deficit compared with non-stressed plants. However, the introgression of the *Bt* gene does not modify cotton physiological and phenotypic response to water deficit.

Keywords: available water, drought, *Gossypium hirsutum* L., Genetically Modified Organism.

RESUMO - O déficit hídrico pode afetar a expressão de proteínas que promovem o controle de lepidópteros no algodoeiro. Entretanto, não é conhecida a resposta de cultivares de algodão *Bt* e convencionais ao déficit hídrico, o que poderia afetar o potencial de competição com plantas daninhas. O objetivo deste trabalho foi avaliar a resposta de cultivares de algodão *Bt* e suas isolinhas ao déficit hídrico. Foi conduzido um experimento em casa de vegetação, onde os cultivares de algodão FMT 705, FMT 709 e IMACD 8276, com e sem o gene *Bt*, se desenvolveram sob dois regimes hídricos: 100% e 50% (déficit hídrico moderado) de água disponível no solo. A fenologia do algodoeiro foi severamente afetada pelo déficit hídrico moderado, com redução da produção de matéria seca de raiz e parte aérea, comprimento e diâmetro de raiz, massa de planta e área foliar. Não foi observado efeito do gene *Bt*. Déficit hídrico durante o florescimento do algodoeiro resulta em diminuição da condutância estomática, assimilação líquida de CO₂ e taxa de transpiração. O potencial hídrico foliar é menor em plantas expostas ao déficit hídrico moderado, em comparação a plantas não estressadas. Contudo, a introgessão do gene *Bt* não modifica a resposta fisiológica e fenotípica do algodoeiro sob déficit hídrico.

Palavras-chave: água disponível, seca, *Gossypium hirsutum* L., Organismos Geneticamente Modificados.

* Corresponding author:

<ciro.rosolem@unesp.br>

Received: November 16, 2017

Approved: March 13, 2018

Planta Daninha 2019; v37:e019187935

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.



¹ Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu-SP, Brasil. ² Soil Microbiology Agroecology Lab, Kansas State University, Manhattan, Kansas, EUA.

INTRODUCTION

Water deficit is the most limiting factor for agricultural production. Under water deficit plant transpiration is impaired and plant biomass accumulation is linearly decreased (Hay and Porter, 2006). Cotton (*Gossypium hirsutum* L.), has been described as relatively drought tolerant, because it originates from warm and arid regions (Lee, 1984). However, it is sensitive to water stress during flowering and boll development (Constable and Hearn, 1981).

Pests of the genus *Lepidoptera* severely damage agricultural production. For a long time, these pests were controlled by spraying insecticides, but currently several genetically modified cotton cultivars are grown worldwide. One example is cotton with *Bacillus thuringiensis* genes (*Bt*), which controls bollworms. The introgression of *Bt* genes in cotton has promoted the effective control of target pests with great reduction of insecticide application (Pray et al., 2001). However, it has been shown that during cotton growth, *Bt* toxin is released into the rhizosphere and the soil through plant exudates and decomposing plant material (Icoz and Stotzky, 2008). Furthermore, genetic engineering or traditional genetic changes might alter plant metabolism (Schaarschmidt et al., 2007) and root exudate components (Bais et al., 2006), which leads to inhibition of arbuscular mycorrhizal fungal development (Chen et al., 2016). There are reports of lower yields of *Bt* cotton cultivars when compared with non-*Bt* ones (Pray et al., 2001), and it was hypothesized that the insertion of genes with insecticidal expression may affect cotton genetic and phenotypic stability, what may interfere with its tolerance to periods of drought.

The concentration of Cry1Ac and Cry2Ab proteins in *Bt* cotton plants decreases under water deficit (Parimala and Muthuchelian, 2010). Martins et al. (2008), imposed a moderate water deficit in cotton and observed differences in growth of both *Bt* and non-*Bt* plants. However, the effectiveness of *Bt* in controlling caterpillars was not affected by the lower *Bt* concentrations in leaves, flowers and bolls. Nonetheless, there are few studies on the differential response of *Bt* and non-*Bt* cotton varieties under water deficit. If cotton response to drought is differentially affected by the introgression, this may affect the plant's ability to compete with weeds for water.

Considering the hypothesis that *Bt* gene insertion can modify cotton tolerance to water deficit, the objective of this work was to evaluate the tolerance of *Bt* cotton cultivars to water deficit when compared with their non-*Bt* near-isolines.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse in Botucatu, São Paulo, Brazil, in randomized complete blocks, with four replications. The experimental units were pots containing 8 kg of soil, collected at the 0-0.2 m depth from a medium texture eutrophic Red Latosol (Embrapa, 2013). Selected chemical characteristics of the soil are shown in Table 1. Phosphorus and potassium were applied at 150 mg dm⁻³ of soil, as triple superphosphate and potassium chloride, respectively, plus 80 mg dm⁻³ of N as ammonium sulfate. Six cotton seeds were planted per pot, and plantlets were thinned to three per pot after five days. The experimental design was a 3 x 2 x 2 factorial composed by the *Bt* cotton cultivars FMT 705, FMT 709 and IMACD 8276; presence (*Bt*) or absence (*non-Bt*) *Bacillus thuringiensis* gene introgression, and two water regimens: 100 % and 50 % of available soil water (AW).

Soil water levels were calculated by subtracting the permanent wilting point from the maximum water retention capacity, and then multiplying by 100. In the 100% AW, soil water was monitored and kept between 100-70% of AW (without water deficit), and soil water was kept between 70-50% in the treatment with 50% AW (moderate water deficit). The plants were grown under 100% AW from emergence to the growth stage B1 (Marur and Ruano, 2001), which corresponds to the appearance of the first flower bud (40 days after sowing). Then the treatments were applied and kept until the end of experiment (60 days after sowing). The temperature and relative air humidity were monitored from the 15th day after sowing to the end of the experiment (Figure 1).

Gas exchange was evaluated 60 days after sowing using an infrared gas analyzer (IRGA – Infra-Red Gas Analyzer, portable, open system, model LICOR 6400 XT). Measurements were made on the third leaf completely expanded and healthy from the apex of the plant, between

Table 1 - Selected chemical characteristics of the soil

SOM	pH	P	K ⁺	Ca ⁺²	Mg ⁺²	Al ⁺³	H ⁺ + Al ⁺³	CEC	B	m
(g dm ⁻³)	(CaCl ₂)	(mg dm ⁻³)	(mmolc dm ⁻³)						(%)	
25	5.7	17	4.5	49	22	0.0	20	95	79	0.0

B = base saturation; m = aluminum saturation.

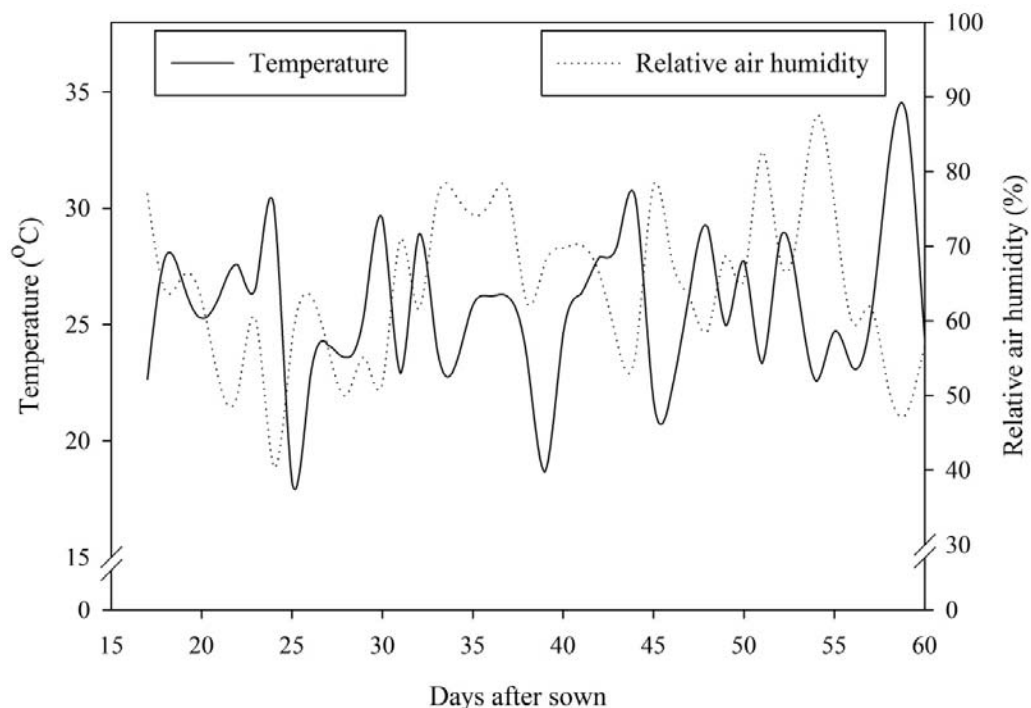


Figure 1 - Temperature and air relative humidity during cotton growth in the greenhouse.

9:00 am and 10:00 am on a sunny day. Net carbon assimilation rate (A), stomatal conductance (g_s), transpiration (E), and internal CO_2 concentration (C_i) within the substomatic chamber were determined. The water potential (Ψ_w) of the leaf was measured using a pressure chamber (Scholander et al., 1965) between 8:00 am and 9:00 am. A fully expanded, healthy third leaf from the apex of the plant was taken and analyzed immediately. Plants were cut close to the soil and the leaf area was evaluated with an electronic optical planimeter (Li-Color, model LI-3100C). Subsequently, the samples were dried to constant weight in a forced air oven air at 65 °C, and the shoot dry matter was recorded. The roots were washed in tap water over a 0.5 mm mesh screen. After washing, the roots were scanned and analyzed with the software WinRhizo version 3.8-b (Regent Instrument Inc.) to determine length, surface area and root diameter, according to Tennant (1975). The root samples were then placed in paper bags and dried to constant weight in a forced air oven at 65 °C until, and root dry matter was determined. The leaf area/root length ratio was calculated.

Data were submitted to Levene's homogeneity test and then to ANOVA. Means were compared by Tukey multi comparison test ($p < 0.05$).

RESULTS AND DISCUSSION

There was no interaction of cultivars, gene introgression and soil water content on root dry matter yield. However, it was decreased under moderate water deficit when compared with plants without deficit (Table 2). Dry matter accumulation of stressed and non-stressed plants was 4.62 g

Table 2 - Cotton root and shoot dry matter, leaf water potential (Ψ_w), root length and diameter as affected by soil available water. Averaged over three cultivars and gene introgression

Available water	Root	Shoot	Ψ_w
	(g)		(Mpa)
50 %	4.62 B*	10.52 B	-1.70 B
100 %	9.58 A	19.01 A	-1.34 A
	Root length		Root diameter
	(m)		(mm)
50 %	12.9 B		1.45 B
100 %	23.7 A		2.84 A

* Means followed by the same letters in columns are not different (F test, $p < 0.05$).

and 9.58 g, respectively, with no effects of gene introgression or cultivars. Root length and diameter were not affected by the insertion of the *Bt* gene in cotton, but root length was decreased from 23.7 m to 12.9 m under moderate water deficit (Table 2). Similarly, the root diameter was lower in plants with water deficit (1.45 mm), compared with plants without water deficit (2.84 mm). FMT 709 showed greater root length than FMT 705, and IMACD 8276 had a larger root diameter than FMT 705, which had the lowest values of root length and diameter. Cotton shoot dry matter was reduced under water deficit (Table 2), but no effect of introgression or interactions were observed. However, IMACD 8276 accumulated more dry matter in the shoot than FMT 705 (Table 3).

Table 3 - Cotton shoot dry matter, root length and diameter as affected by cultivars. Averaged over two soil water availabilities and gene introgression

Cultivar	Shoot	Root length	Root diameter
	(g)	(m)	(mm)
IMACD 8276	15.6 A*	195.8 AB	2.44 A
FMT 709	15.1 AB	202.8 A	2.12 AB
FMT 705	13.6 B	150.1 B	1.87 B

* Means followed by the same letters in columns are not different (F test, $p < 0.05$).

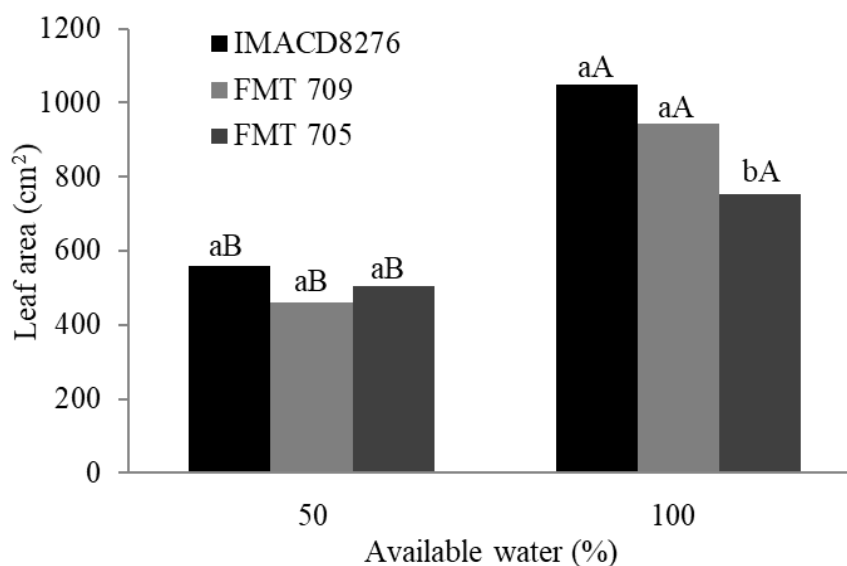
When grown without water deficit, the transgenic cultivars were taller than their non-transgenic counter parts (Table 4), but under water deficit there was no difference between them. Water deficit reduced cotton plant height by 28 % on average (Table 4). An interaction of cultivars and soil water content was observed on cotton leaf area (Figure 2), with no difference under moderate water deficit. However, when soil water was not limiting, a lower leaf area was observed for FMT 705 when compared with FMT 709 and IMACD 8276. In addition, when subjected to moderate water deficit, cotton leaf area was, on average, smaller than without water deficit, and gene introgression had no effect on leaf area. The relationship leaf area/root length was not affected by treatments and there were no interactions, with a general average of $0.52 \text{ cm}^2 \text{ cm}^{-1}$. Despite the differences found in root and shoot dry matter accumulation, leaf area and root length, plants seem to have an internal mechanism that regulates root and shoot growth, keeping the relationship constant.

Leaf water potential was lower in plants with water deficit compared with non-stressed plants, but there was no significant difference due to introgression and cultivars or interactions (Table 2). A decrease in CO_2 assimilation was observed in plants submitted to moderate water deficit compared with those without water deficit (Table 4), but it was not affected by the introgression of the *Bt* gene in cotton or by its interaction with the water deficit. Available soil water interacted with *Bt* gene introgression, affecting cotton stomatal conductance and transpiration (Table 4). Plants under water deficit showed lower stomatal conductance and transpiration when compared with non-stressed plants, with no difference between transgenic and non-transgenic cultivars. The internal CO_2 concentration was $-3451.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ on average, and it was not affected by treatments.

Table 4 - Net CO₂ assimilation by cotton plants, plant height and stomatic conductance as affected by soil water availability and *Bt* introgression. Average of three cultivars

Soil water availability	Non- <i>Bt</i>	<i>Bt</i>
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		
50 %	-10.5 B*	-5.1 B
100 %	16.6 A	16.1 A
Plant height (cm)		
50 %	44.86 aB	44.31 aB
100 %	55.44 bA	60.13 aA
Stomatic conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$)		
50 %	0.0072Ab	0.0399 Ab
100 %	0.1499 Aa	0.1010 Aa
Transpiration ($\text{mmol m}^{-2} \text{ s}^{-1}$)		
50 %	0.242 Ab	1.403 Ab
100 %	4.782 Aa	3.608 Aa

* Means followed by the same lower-case letter within water availability and capitals within *Bt* and non-*Bt*, are not different (Tukey test, $p < 0.05$).



* Means followed by the same lower-case letter within water availability and capitals within cultivars, are not different (Tukey test, $p < 0.05$).

Figure 2 - Cotton leaf area as affected by cultivar and soil water availability. Averaged over *Bt* and non-*Bt*.

In general, soil water availability affected cotton growth, since plant shoot and root growth were decreased under moderate water deficit. Water deficit impairs cell elongation and cell wall synthesis, which results in reduced growth due to a decrease in cell turgor. The decreased cell volume results in lower turgor pressure and higher solute concentration in plant cells, thus affecting cell expansion and root elongation (Taiz and Zeiger, 2006). Root growth is important for plant growth and yield, especially in soils where water and nutrient resources are scarce. Root elongation is slower in dry soil due to a combination of water stress and mechanical strength (Bengough et al., 2011). Ayalew et al. (2014), also observed a reduction in root length in wheat cultivars submitted to water deficit compared with the control. Root diameter decrease is an important mechanism for plants to adapt to water deficit environments because it increases the specific surface area, thus facilitating water absorption (van der Weele et al., 2000).

Martins et al. (2008), observed that leaf dry matter, leaf area and total dry matter did not differ between *Bt* and non-*Bt* cotton plants, as observed in the present experiment. However, leaf and shoot growth were reduced by soil water deficit. Leaf area is an important factor in determining yields, because plant water use depends on the leaf area, and the potential of leaf production is severely inhibited when they are exposed to water deficit (Fernández et al., 1996). Water deficit also resulted in lower leaf water potential. When it is lower than -1.5 MPa, CO₂ assimilation, transport of inorganic sap in the xylem, organic sap flow in the phloem and respiration decrease, while the activity of hydrolytic enzymes increases (Smith and Cothren, 1999). Water deficit during anthesis results in significant reductions in water potential in cotton (Loka and Oosterhuis, 2014). The stomatal conductance was also impaired by water deficit, but was not affected by *Bt* introgression. Under stress, lower soil water availability may have resulted in partial closure of plant stomata, leading to a decrease in stomatal conductance. Under stress, low values of stomatal conductance become extremely important (Sinclair and Ludlow, 1986). In pot experiments it has been observed that cotton plants under water stress have lower rates of stomatal conductance (Costa and Cothren, 2011; Loka and Oosterhuis, 2014). Similarly, CO₂ assimilation was impaired by water deficit but it was not affected by genetic modification. Previous studies with cotton have reported that water deficit imposed at any stage of development, and at different intensities, results in a large reduction in the net assimilation of foliar CO₂ (Costa and Cothren, 2011), through a combination of stomatal and non-stomatal limitations (Loka et al., 2011). The stomata begin to close as a reaction to the decline in leaf water potential, thus decreasing the rate of water loss. However, gas exchange and photosynthesis also decrease. Non-stomatal limitations are due to metabolic decline and are thought to occur under severe drought. The high leaf temperature produces thermal inhibition of RuBisCO and other enzymes, which is more likely to occur in hot and dry climates (Carmo-Silva et al., 2012). However, Catuchi et al. (2011) observed that CO₂ assimilation was decreased by 81% in a conventional soybean cultivar, while the transgenic cultivar showed a 52% decrease due to water deficit. The decrease in soil water availability resulted in partial closure of the stomata, reduction in stomatal conductance, transpiration and CO₂ assimilation, since both are diffusive processes, and, eventually, the impairment of photoassimilate synthesis (Loka and Oosterhuis, 2014). In this study, no evidence was found that *Bt* gene insertion in cotton modifies the response to water deficit.

Cotton phenology was severely affected by water deficit, with decreased shoot and root growth. Water deficit during cotton bloom compromises cotton physiology by impairing stomatal conductance, photosynthesis, and transpiration rates. However, there is no evidence of modifications in cotton response to water deficit as affected by the insertion of *Bt* gene, and so, a differential competition with weeds under a moderate drought is not expected.

REFERENCES

- Ayalew H, Ma X, Yan G. Screening wheat (*Triticum* spp.) genotypes for root length under contrasting water regimes: potential sources of variability for drought resistance breeding. *J Agron Crop Sci.* 2014;201:189-94.
- Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann Rev Plant Biol.* 2006;57:233-66.
- Bengough AG, McKenzie BM, Hallett PD, Valentine TA. Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *J Exp Bot.* 2011;62:59-68.
- Catuchi TA, Vitolo HF, Bertolli SC, Souza GM. Tolerance to water deficiency between two soybean cultivars: transgenic versus conventional. *Cienc Rural.* 2011;41(3):373-8.
- Carmo-Silva AE, Michael AG, Sanchez PA, French AN, Hunsaker DJ, Salvucci ME. Decreased CO₂ availability and inactivation of RuBisCO limit photosynthesis in cotton plants under heat and drought stress in the field. *Environ Exp Bot.* 2012;83:1-11.
- Chen X-H, Wang F-L, Zhang R, Ji L-L, Yang Z-L, Lin H, et al. Evidences of inhibited arbuscular mycorrhizal fungal development and colonization in multiple lines of *Bt* cotton. *Agric Ecosy Environ.* 2016;230:169-76.
- Constable GA, Hearn AB. Irrigation of crops in a subhumid climate, 6: effects of irrigation and nitrogen fertilizer on growth, yield and quality of cotton. *Irrig Sci.* 1981;2:17-28.

- Costa VA, Cothren JT. Drought effects on gas exchange, chlorophyll, and plant growth of 1-methylcyclopropene treated cotton. *Agron J*. 2011;103:1230-41.
- Empresa Brasileira de Pesquisa Agropecuária – Embrapa. Sistema Brasileiro de Classificação de Solos. 3ª.ed. Brasília, DF: 2013.
- Fernández CJ, McInnes KJ, Cothren JT. Water status and leaf area production in water-and nitrogen-stressed cotton. *Crop Sci*. 1996;36(5):1224-33.
- Hay R, Porter J. The physiology of crop yield. Ames: Blackwell Publishing; 2006.
- Icoz I, Stotzky G. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. *Soil Biol Biochem*. 2008;40(3):559-86.
- Lee JA. Cotton as a world crop. In: Kohel RJ, Lewis CF, editors. *Cotton Agronomy Monograph*. Madison: American Society of Agronomy; 1984. p.1-25.
- Loka DA, Oosterhuis DM. Water-deficit stress effects on pistil biochemistry and leaf physiology in cotton (*Gossypium hirsutum*, L.). *South Afr J Bot*. 2014;93:131-6.
- Loka DA, Oosterhuis DM, Ritchie GL. Water-deficit stress in cotton. In: Oosterhuis DM, editor. *Stress physiology in cotton*. Memphis: The Cotton Foundation; 2011. p.37-72.
- Martins CM, Beyene G, Hofs JL, Krüger K, van der Vyver C, Schlüter U, et al. Effect of water-deficit stress on cotton plants expressing the *Bacillus thuringiensis* toxin. *Ann Appl Biol*. 2008;152(2):255-62.
- Marur CJ, Ruano O. A reference system for determination of developmental stages of upland cotton. *Rev Oleag Fibr*. 2001;5:313-7.
- Parimala P, Muthuchelian K. Physiological response of non-*Bt* and *Bt* cotton to short-term drought stress. *Photosynthetica*. 2010;48:630-4.
- Pray CE, Ma D, Huang J, Qiao F. Impact of *Bt* cotton in China. *World Dev*. 2001;29(5):813-25.
- Schaarschmidt S, Kopka J, Ludwig-Müller J, Hause B. Regulation of arbuscular mycorrhization by apoplastic invertases: enhanced invertase activity in the leaf apoplast affects the symbiotic interaction. *Plant J*. 2007;51(3):390-405.
- Smith CW, Cothren JT. *Cotton: Origin, history and production*. New York: John Wiley & Sons; 1999.
- Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. Sap pressure in vascular plants. *Science*. 1965;148(3668):339-46.
- Sinclair TR, Ludlow MM. Influence of soil water supply on the plant water balance of four tropical grain legumes. *Aust J Plant Physiol*. 1986;13:329-41.
- Taiz L, Zeiger E. *Plant physiology*. Sunderland: Sinauer Associates Press; 2006.
- Tennant DA. Test of a modified line intersect method of estimating root length. *J Ecol*. 1975;63:995-1001.
- van der Weele CM, Spollen WG, Sharp RE, Baskin TI. Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *J Exp Bot*. 2000;51(350):1555-1562.