

# SOLUBLE SUGARS AND STARCH ACCUMULATION ON COTTON PLANT LEAVES AFFECTED BY COTTON LEAFROLL DWARF VIRUS

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**Abstract:** The cotton blue disease, a main cotton pest in Brazil, is caused by a luteovirus, named Cotton leafroll dwarf virus (CLR DV) or Cotton vein mosaic virus (CVMV). Symptoms include shortening of internodes and stunting. Luteoviruses have been reported to cause carbohydrates accumulation in infected plants. The aim of this research was to verify if soluble sugars and starch accumulation occurs in leaf of cotton plants infected by the virus. Leaf petioles of the cotton plants with symptoms of cotton blue disease presented greater brix values than the leaves of healthy plants. Similarly, starch content in leaves of symptomatic plants were greater than those of asymptomatic plants. This is a novel virus-host system in which carbohydrates accumulation occurs, which can help to elucidate this has a hole in plant defense against infection.

**Index Terms:** virus, carbohydrate, luteovirus.

## AÇÚCARES SOLÚVEIS E AMIDO ACUMULAM-SE EM ALGODOEIROS COM DOENÇA AZUL

**Resumo:** A doença azul, uma das principais doenças do algodoeiro no Brasil, é causada por um luteovírus, designado como cotton vein mosaic virus (CVMV) ou Cotton leafroll dwarf virus (CLR DV). Os sintomas incluem encurtamento dos entrenós e nanismo. Como o acúmulo de carboidratos devido a infecção por luteovírus tem sido descrito em diferentes espécies de plantas, conduziu-se um estudo para verificar se ocorre acúmulo de açúcares solúveis e amido em folhas de diferentes espécies de algodoeiros infectados por CLR DV. Os pecíolos de algodoeiros com sintomas de doença azul apresentaram maiores valores de brix em relação a pecíolos de plantas sem sintomas. Da mesma maneira, a quantidade de amido de folhas de plantas com sintomas foram maiores que aquelas de plantas saudáveis. O conhecimento de uma nova interação entre vírus e planta hospedeira que leva ao acúmulo de carboidratos pode colaborar na elucidação do papel do acúmulo de carboidratos na capacidade de defesa da planta contra a infecção por vírus.

**Termos para Indexação:** virose, carboidratos, luteovírus.

## INTRODUCTION

Cotton blue disease is widely distributed in

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Central and Southeast regions of Brazil, the main cotton production areas, and may cause severe yield losses, for which it is considered one of the most important cotton diseases in the country. It was first reported in Brazil in 1938 (COSTA; FORSTER, 1938), and symptoms and the transmission by the aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) suggested it was caused by a virus. Symptoms characterization

and transmission by an aphid were enough to name the disease Cotton vein mosaic virus (CVMV) (COSTA; CARVALHO, 1965), otherwise it was only recently that Corrêa et al. (2005) demonstrated, by PCR amplification in which degenerate primers were used, followed by sequencing, that the presence of the symptoms is associated to the presence of a virus of the family Luteoviridae, probably of the genus *Polevirus*. The authors suggested that the virus is called Cotton leafroll dwarf virus (CLRDV), although it has been also called by the name with which it was first described, CVMV (MICHELOTTO; BUSOLI, 2006).

Symptoms include shortening of internodes and stunting. Leaves are small and curled down, with cleared veins. A reduced number of bolls is produced in infected plants, furthermore there is loss of fiber quality (COSTA; CARVALHO, 1965). Symptom intensity depends on when infection occurs. If young plants are infected, plant high can be reduced by 80%, and become completely sterile (MICHELOTO; BUSOLI, 2006). Although resistant varieties had been developed, susceptible ones are still largely planted, and virus is controlled by frequent insecticide applications with the aim to maintain the vector at levels sufficiently low to prevent virus spread.

Because of the similarity of symptoms and transmission by the same aphid, it has been suggested that the cotton blue disease observed in Brazil is the same that the one observed in Africa (CAUQUIL, 1977, CAUQUIL; FOLLIN, 1983), although there are not still molecular evidence to confirm that (CORRÊA et al., 2005).

Increased sugar and starch content due to virus infection was reported for *Abutilon mosaic virus*, a geminivirus (LOHAUS et al., 2000) and *Sunflower chlorotic mottle virus*, a potyvirus (ARIAS et al., 2003). It has been shown that sugarcane plants infected by the luteovirus *Sugarcane yellow leaf virus* (ScYLV) have enhanced sugar concentration on leaf tissues, although in stalks it is decreased (BARROSO et al., 1995; GONÇALVES et al., 2005).

This study was conducted to verify if soluble solid contents and starch accumulation occurs in leaf tissue of cotton plants infected by cotton blue disease. Different cotton species could lead to different responses to infection, so diverse cotton genotypes present in Brazil were included. *Gossypium hirsutum* L. var. *latifolium*, also known as herbaceous or upland cotton, is the main cotton specie planted worldwide and in Brazil, and for which the virus infection causes economic losses. *G. hirsutum* race *marie galante*, known as Mocó cotton, was largely planted in the Brazilian semi-arid Northeast region, otherwise since 1970s cotton production in this area has declined. *G. barbadense* L. was cultivated by native Americans before European colonization, and is still cultivated in all Brazil regions as "dooryard" plants.

## MATERIAL AND METHODS

All the analysis were performed with 24 different Embrapa Cotton Germplasm Bank accesses from four different cotton genotypes: *G. barbadense*, *G. hirsutum* var. *latifolium*, *G. hirsutum* race *marie galante*. The accesses were chosen to include genotypes for different origins and maximize genetic diversity. They were planted in a place of history of high disease incidence, in State of Mato Grosso, at Novo São Joaquim municipalitie. Each plot consisted of three rows of ten meters in length, containing 30 plants plots of each genotype. The plants were allowed to become naturally infected.

Analysis of soluble solid contents and starch were carried out with leaf pairs composed by leafs at the same development stage, the first leaf collected from one plant with marked virus symptoms and the second leaf collected from a symptomless plant, of the same genotype, the same plant age of the same plot.

Soluble solid contents from leaf petiole of 24 different accesses, using 52 different leaf pairs, were estimated by brix degree. This was achieved by crushing the petiole and availing brix with a hand refractometer.

Starch was quantified for 38 different accesses, using 67 independent leaf pairs, by the methodology developed by McCready et al. (1950).

Starch and soluble solid contents were compared by paired Student's *t*-test.

## RESULTS

Leaf petioles of the cotton plants with symptoms of cotton blue disease presented greater brix values relative to the leaves of healthy plants, as it can be observed at Table 1. Only two leaf pairs evaluations revealed higher brix values in plants without symptoms (Figure 1). Physiological differences, instead of genotypes, caused unexpected lower brix contents for symptomless plants of these two leaf pairs, since other leaf pairs of the same genotypes presented the expected higher values for the plants with virus symptoms. One of these pairs were from a *G. barbadense* accesses, collected in Mato Grosso state, and the other from *G. barbadense* IBDAR 45 PR2, which have, respectively, five and four pairs of samples at the same experiment, showing that this values are due to variability or error, and not a characteristic of the species or variety.

The starch content was also higher in average among the foliar limbs collected from plants with blue disease symptoms than among those with no disease (Table 1).

Among 67 leaf pairs analyzed, at only eleven pairs the starch content was greater at the leaf from the plant without symptoms (Figure 1). Five

**TABLE 1.** Brix degrees and starch content (mg of glucose pr g of dry weight) in leaf petioles from cotton plants with and without blue disease symptoms.

	Symptoms	Mean	Standard deviation	t	DF	p
Brix	Present	6,52	1,4400	-	-	-
	Absent	4,34	1,0300	10,91	41	1,07E-13
Starch	Present	86,30	51,1411	-	-	-
	Absent	42,10	26,1130	6,95	66	2,03E-09

of those pairs were very similar, and differences may be considered to be due to environmental or physiological variations among plants. Two other pairs were from the accesses 1931 and 15R1, for which analysis was performed, respectively, in three and four leaf pairs of the same genotype, and the others pairs of the same genotype presented infected leaves with greater starch content than no infected ones.

The genotypes 149 F URSS and *G. barbadense* access 3 were analyzed by two leaf pairs each, and on both the leaf of the plant without symptoms presented greater starch content. The presence of the virus in the absence of symptoms can explain this results, since an evaluation of the plots from which the leaves were collected 45 days after collection showed 100% of the plants with symptoms.

## DISCUSSION

It was shown that cotton plants with blue disease symptoms have a marked tendency to accumulate soluble solids at the petiole and starch at foliar limbs. The association between virus infection and increased sugar or starch contents has been reported previously for the genus *Comovirus* (TECSI et al., 1992); *Tobamovirus* (OLESINSKI et al., 1996); *Geminivirus* (LOHAUS et al., 2000); *Potyvirus* (ARIAS et al., 2003) and the genus *Luteovirus* (BARROSO et al., 1995; HERBERS et al., 1997),

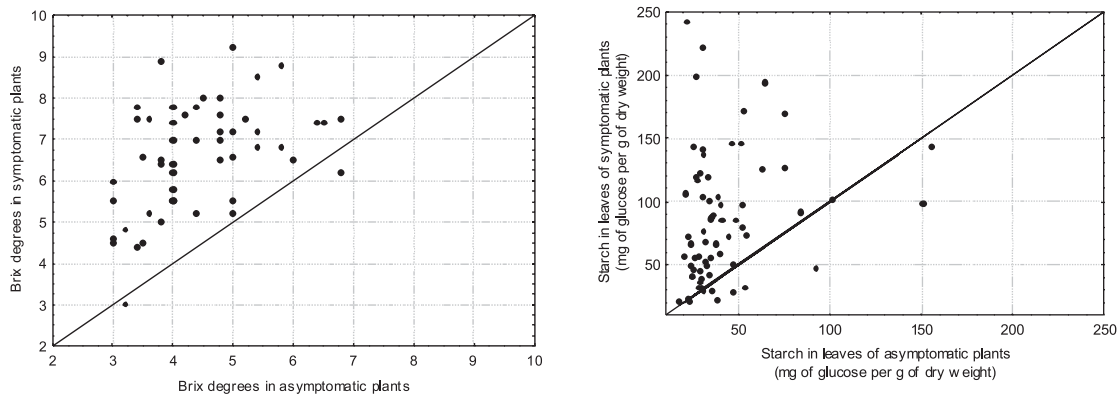


FIGURE 1. A) Brix degrees in cotton leaf petioles. B) Starch in foliar limb. Each point represents a pair of leaf petioles collected from plants from the same variety and age, but one from a plant with blue disease symptoms and the other from a symptomless plant. The line represents the expected values if disease and healthy plants didn't differ on brix values, and points above the line represents the plants in which the petiole from the symptom plant had the greater brix value.

although, to our knowledge, there has been no previous report relating carbohydrate accumulation and cotton leaf roll dwarf virus.

Interestingly, carbohydrate accumulation has been seen both as symptom caused by virus infection as well as a defense response. The pattern of starch accumulation in pea plants infected with red clover mottle comovirus can be mimicked by removal of the apex in uninfected plants, suggesting starch accumulation is an indirect consequence of sealing the physiological sink for photosynthate (TECSI et al., 1992). Otherwise, a series of studies with transgenic plants presented evidence that carbohydrate accumulation is mediated by the movement protein of the virus. Inhibition of sucrose export leading to soluble sugars and starch accumulation was observed on transgenic potato plants expressing tobacco mosaic tobamovirus movement protein (OLESINSKI et al., 1996) and tobacco plants transgenic to the movement protein of potato leafroll luteovirus (PLRV) presented stunted phenotype associated with a drastic accumulation of carbohydrates and altered plasmodesmal structures (HERBERS et al.,

1997). Tobacco plants constitutively expressing PLRV movement protein are partially resistant to *Potato virus Y*, which is mediated by movement proteins through plasmodesmas, although efficiency determined by carbohydrate status rather than by protein amount (HOFIUS et al., 2001). Sealing of plasmodesmas was also observed in transgenic tobacco plants constitutively expressing tomato spotted wilt tospovirus, in which symptoms expression seems to be due to various degrees of inhibition of sucrose export from the leaves (RINNE et al., 2005).

Both CLRDV and PLRV are luteovirus, which isometric particles remain restricted to phloem during infection; so similarities between movement proteins are expected. Possibly, accumulation of starch and sugars is mediated by CLRDV movement proteins in cotton plants infected by the virus.

## CONCLUSION

Carbohydrate accumulation occurs in cotton plants infected with CLRDV.

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