Some physiological characteristics in resistant and susceptible cotton cultivars infected with cotton leaf curl virus

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Abstract

Eight cultivars/lines of *Gossypium hirsutum* (CIM-443, CIM-448, CIM-1100, FH-634, S-111, S-113, Cedix, and LRA 5166) resistant to cotton leaf curl virus (CLCuV), one moderately-resistant (cv. NIAB-Krishma), and one susceptible (cv. S-12) were used. All the resistant lines remained free of all disease symptoms, whereas in moderately-resistant and susceptible cvs. leaf curling and vein thickening occurred. Cultivars with varying degree of CLCuV-resistance had different pattern of accumulation of macronutrients. Leaf N content was lowest in S-12, but increased considerably due to disease. Leaf K and Ca contents of S-12 were lower in the diseased leaves than in healthy ones. Chlorophyll *a* and *b* contents were highest in lines S-111, S-113 and S-12. A marked reduction in chlorophyll *b* content was observed in the diseased leaves of S-12. Leaf water potential in S-12 and NIAB-Krishma was also decreased due to disease. The most distinctive characteristic to differentiate between lines was epicuticular wax content, since all the resistant lines had considerably higher wax content on their leaf surfaces than the moderately-resistant or susceptible cultivars.

Additional key words: chlorophyll content, epicuticular wax content, *Gossypium hirsutum*, macronutrients, soluble proteins.

Introduction

Considerable progress has been made over the past few years in understanding the mechanism of disease resistance or susceptibility, and it has been pointed out that resistance to any virus depends on plant metabolism (Dawson and Hilf 1992). For instance, Khalifa and Gameel (1983) found large differences in phenolic compounds,

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amino acids, proteins, and RNA between lines of cotton differing in CLCuV resistance. Similarly, Rehmat (1995) showed that amount of total sugars and non-reducing sugars decreased as a result of infection of cotton leaves by CLCuV in cv. S-12 (CLCuV-susceptible) and CIM-240 (CLCuV-tolerant). However, the amount of reducing sugars decreased in the infected leaves of cv. S-12, but increased in those of CIM-240. In another study it was found that total minerals decreased more in the diseased leaves of both cvs. S-12 and CIM-240 compared with the resistant cv. CIM-434 (Hussain 1995). Long ago, Massey and Andrew (1932) discovered that diseased plants had higher nitrogen content than the healthy plants. They found that the diseased leaves had much more chlorophyll than that in the normal leaves, but the sugar and starch contents were not significantly affected. Similarly, Ahmad (1995) reported that the leaves of diseased plants of S-12 and CIM-240 had higher concentration of N than the healthy leaves.

These studies led us to hypothesize that the resistant and susceptible lines possess different levels of certain biochemicals and essential nutrients in their leaves. Comparison between diseased and healthy leaves of individual lines can uncover the extent of biochemical damage due to viral infection.

Materials and methods

Seeds of *Gossypium hirsutum* L. cvs. S-12 (susceptible to cotton leaf curl virus, CLCuV), NIAB-Krishma (moderately-resistant), and CIM-443, CIM-448, and CIM-1100 (resistant) were obtained from the Central Cotton Research Institute Multan, Pakistan, whereas those of the five CLCuV-resistant cultivars/lines, S-111, S-113, Cedix, LRA 5166, and FH-634 were obtained from the Cotton Research Station Multan, Pakistan. All seed samples were surface sterilized in 5 % sodium hypochlorite solution for 10 min before planting. The experiment was conducted in a glasshouse (30°11'N and 71°28'E) with natural sunlight during the summer 1997 with mean day temperature of 38.6 ± 9.4 °C and night temperature of 22.3 ± 7.6 °C, and photoperiod from 12 to 14 h.

The experiment was laid out in a completely randomized design, with 10 replicates, in a naturally-lit glasshouse in the Botanic Gardens of the Bahauddin Zakariya University, Multan, Pakistan. The first disease symptoms, *i.e.*, thickening of veins and leaf curling, appeared when S-12 plants were 10 weeks old. Disease symptoms also appeared on NIAB-Krishma but with relatively low severity and leaves of only 7 % of the plants were disease affected. In contrast, all the eight CLCuV-resistant cultivars/lines were free of disease symptoms. Infected and normal leaves of S-12 and NIAB-Krishma, and normal leaves of all the eight resistant cultivars/lines were sampled in the last week of August 1997 (16 d after the appearance of disease symptoms) for analysis of macronutrients, epicuticular wax content, chlorophyll content, soluble proteins and total free amino acids. One day prior to measurement of leaf water potential all plants were watered to field capacity. Next morning at 09:00, a fully expanded leaf from healthy and diseased plants was excised and its water potential measured with a Scholander type pressure chamber (*Cook and Sons*, Birmingham, UK).

Epicuticular wax content was determined following Silva Fernandez *et al.* (1964). Fresh leaves (2.0 g) of the same age and size were excised from plants. Before treating the material with the solvent, the area of each leaf was measured with a leaf area meter (*Delta T Devices*, Burwell, Cambridge, England) as the wax content was expressed per leaf area unit.

Leaf chlorophyll content was determined according to Witham *et al.* (1971) using a spectrophotometer *Hitachi U-2000* (Tokyo, Japan). Total soluble proteins were determined according to Lowry *et al.* (1951) and total free amino acids according to Hamilton and Van Slyke (1943).

For the analysis of macronutrients (K, Ca, Mg, N, and P) a fully expanded youngest leaf from each plant was sampled. The K content was determined with a flame photometer (*Jenway PFP7, Gransmore Green*, Dunmow, UK), Ca and Mg contents with an atomic absorption spectrometer *Analyst 100* (*Perkin Elmer*, Beaconsfield, Germany), P was determined using a spectrophotometer *Hitachi U-2000*, and N by titration method following Allen *et al.* (1986).

The results for each variable (based on $n = 6$) were subjected to a two-way analysis of variance using a *COSTAT* package (*Cohort Software*, Berkeley, USA) and the least significant differences (LSD) were calculated following Snedecor and Cochran (1980).

Results and discussion

The first symptoms of the disease, *i.e.*, vein thickening and leaf curling, appeared on S-12 and NIAB-Krishma when the plants were 10 weeks old. The incidence of disease was 100 % in S-12 and 7.0 % in NIAB-Krishma, whereas all the other cultivars were free of disease symptoms. On S-12, leaf curling and vein thickening were of severe type (Ali *et al.* 1995), whereas on NIAB-Krishma it varied from medium to severe type. It is now widely reported that leaf curling is one of the most distinctive characteristics of cotton plant as a result of virus infection (Mansoor *et al.* 1993, Ali *et al.* 1995). The average population of the disease vector, whitefly (*Bemisia tabaci*), on all the cultivars under study ranged from 2.8 to 4.6 per leaf throughout the growth season.

Ten cotton cultivars/lines differed significantly in accumulation of different nutrients in their leaves (Table 1*A*). Of the CLCuV-resistant cultivars N was maximum in FH-634. NIAB-Krishma was as good as most of the CLCuV resistant lines in N accumulation. In contrast, cv S-12 (CLCuV-susceptible) had a significantly lower concentration of N in its leaves compared with the other lines. The results for N content in S-12 can be easily related to the earlier findings of Massey and Andrew (1932), Rashid *et al.*(1995), and Ahmad (1995). However, N content in the diseased leaves of S-12 was considerably higher than in healthy leaves, but this trend was not observed in the moderately-resistant, NIAB-Krishma. In addition, it is interesting to note that N content in all cultivars/lines of varying degree of CLCuV-resistance was

Table 1*A*. Nitrogen, phosphorus, potassium, calcium, and magnesium contents [mg g^{-1}(d.m.)] and leaf water potential, ψ_w [-MPa] in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines. Means in columns assigned by the same letters are not significantly different at $P = 0.05$, $n = 6$.

Cultivar/line	N	P	K	Ca	Mg	ψ_w
CIM-443	23.30ac	2.37ac	25.35ad	38.9ad	4.42ac	1.81ab
CIM-448	24.09ac	1.54bc	11.82bc	44.5ac	3.98ac	1.63a
CIM-1100	29.75a	2.57a	27.46ad	59.8b	8.82bc	1.73ab
S-111	27.71ac	2.70a	11.49b	46.1c	10.70b	1.84abc
S-113	23.65ac	2.23ac	8.87b	35.0de	5.93c	1.89bc
FH-643	44.51b	1.92a	11.80b	39.2ad	6.26ac	1.78ab
Cedix	22.11ac	1.59bc	16.92abd	53.0b	8.40bc	1.85abc
LRA 5166	29.27a	1.75bc	20.41acd	46.6c	4.02ac	1.81ab
NIAB-Krishma (H)	26.88a	2.08ac	13.99bcd	45.7c	4.68ac	1.69ab
NIAB Krishma (D)	27.80a	1.54bc	12.83bcd	41.0c	4.17ac	2.07c
S-12 (H)	19.73c	2.43ac	21.21ad	43.4ac	7.30bc	1.84abc
S-12 (D)	38.50b	2.43ac	10.78b	32.0e	5.07ac	2.62d

Table 1*B*. Wax content [μg cm^{-2}] and contents of soluble proteins [mg g^{-1}(f.m.)], free amino acids [μg g^{-1}(f.m.)], and Chl *a* and *b* [mg g^{-1}(f.m.)], and Chl *a*/*b* ratio in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines. Means in columns signed by the same letters are not significantly different at $P = 0.05$, $n = 6$.

Cultivar/line	Wax	Proteins	Amino acids	Chl *a*	Chl *b*	Chl *a*/*b*
CIM-443	76.6ac	10.06ab	657.5ae	1.01ad	0.46ac	2.21ac
CIM-448	65.1ab	10.91ab	742.5ace	1.11ad	0.35a	3.14a
CIM-1100	59.9be	11.15ab	672.5ae	1.13ad	0.53acd	2.13ac
S-111	62.5abe	11.23ab	1030.2bc	1.84bc	0.81bc	2.27ac
S-113	89.5c	8.11a	1010.1bc	1.52c	0.97b	1.57bc
FH-643	85.8c	8.05a	797.5abce	1.08a	0.69bc	1.57bc
Cedix	52.2be	8.91a	1075.2bc	1.35ac	0.47ac	2.87ac
LRA 5166	70.3c	22.08c	810.3abc	0.81ad	0.34ac	2.35ac
NIAB-Krishma (H)	26.6d	13.24b	1655.2d	0.99a	0.51ac	1.94c
NIAB-Krishma (D)	47.9e	9.24a	1065.3bc	1.07ad	0.41ac	2.61ac
S-12 (H)	23.8d	10.71ab	587.4e	1.41ac	0.78bd	1.79abc
S-12 (D)	36.9de	10.02ab	635.1ae	1.16ad	0.41ac	2.91ac

within the critical limit (2.0 %; average of all commercial cultivars) as reported by Rashid *et al.* (1995).

Maximum accumulation of P was observed in a newly-developed line S-111 (Table 1*A*). The healthy and diseased leaves did not differ significantly in this variable. Cvs. CIM-1100 and Cedix had significantly higher Ca content in their leaves than the other cultivars. Mg content was the highest in S-111. It was worth-noting that a marked decrease in K and Ca concentrations and a slight decrease in Mg concentration in the diseased leaves in comparison with healthy ones was only in

S-12. The considerably lower concentration of K in the diseased leaves of S-12 can be explained by the fact that K is mobile nutrient (Mengel and Kirkby 1987, Marschner 1995) and under normal conditions it readily moves from older to younger leaves through phloem. Since the diseased leaves of S-12 were severely affected and curled, and occurrence of substantial/partial blockage of vascular system of these leaves has already been reported in scanning electron microscope studies (Ashraf *et al.* unpublished data), low accumulation of K in young diseased leaves can be expected. In contrast, the diseased leaves of NIAB-Krishma were not as much curled as those of S-12. Very little effect of disease has been reported on the blockage of vascular system of these leaves in our earlier studies (Ashraf *et al.* unpublished data), thus almost uniform maintenance of K in both healthy and diseased leaves is also expected. Since Ca is known to play a crucial role in maintaining the integrity of cell wall, it is possible that leaf curling is associated with a marked reduction in Ca content of the diseased leaves of S-12. However, it is not possible from these results to assess whether the reduction in Ca has occurred in the cytosol or apoplast, although it is known that a major proportion of the Ca absorbed by plant is transported to the apoplast (Hanson 1984, Marschner 1995).

Maximum chlorophyll (Chl) *a* and *b* contents (Table 1*B*) were found in S-111 and S-113 and the minimum in LRA 5166. There was a slight decrease in Chl *a* and a marked decrease in Chl *b* contents in the diseased leaves of S-12 which can be related to lower accumulation of Mg in these leaves compared with that in the healthy leaves. The reduction in Chl *b* content in CIM-448 is surprising in view of its considerable resistance to CLCuV since not a single infected leaf was found in this cultivar. However, the low Chl content in CIM-448 can be related to its low leaf Mg content. The maximum Chl *a*/*b* ratio was observed in CIM-448 and minimum in S-113 and FH-634. It is important to note that Chl *a*/*b* ratio in the diseased leaves of both NIAB-Krishma and S-12 was considerably higher than in the healthy ones.

Maximum content of soluble proteins was observed in the healthy leaves of NIAB-Krishma and minimum in those of S-12. It is interesting to note that this variable decreased significantly in the diseased leaves of NIAB-Krishma, but such decrease was not observed in the diseased leaves of S-12. The results for soluble proteins and free amino acids show non-consistent pattern among the resistant cultivars.

Water potential was considerably lower in the diseased leaves of NIAB-Krishma and S-12 compared with that of their healthy leaves. These cultivars had significantly lower epicuticular wax content per unit leaf area than all resistant lines. In the diseased leaves of these two cultivars epicuticular wax content was higher compared with that in their healthy leaves. The significant increase in wax content on the diseased leaves of both moderately-resistant and susceptible lines may have been due to the curled and shrivelled surface of these leaves with many slight grooves. Although it was tried to make the leaf surface plan and smooth by slightly pressing the leaf, it was not possible to make it fully smooth. Thus in view of this technical reason some inflated values of epicuticular wax content of diseased leaves are expected.

In summary, this study provides important information linking various plant morphological and physiological/biochemical characteristics, and CLCuV-resistance in cotton. The most prominent characteristic is the epicuticular wax content which might be used to identify CLCuV-resistant/susceptible lines. It has been found that some macronutrients such as leaf K and Ca contents decreased, whereas N content increased considerably due to the disease. Chl *b* content was reduced more considerably than Chl *a* due to the disease attack. An examination of the relationship between disease and plant growth regulators may provide additional information to help assess what constitutes a mechanism for CLCuV-resistance of plants.

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