

STRUCTURAL AND NON-STRUCTURAL CARBOHYDRATES IN RELATION TO BACTERIAL BLIGHT RESISTANCE IN FORAGE GUAR (*CYAMOPSIS SP.*)

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SUMMARY

The present investigation was conducted to study the changes in structural and non-structural carbohydrate contents and their role in bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*) resistance in forage guar. Guar crop (cvs. HG 563, FS 277, PNB and *C. serrata*) was raised in pots filled with 5 kg of sieved sandy soil in a naturally lit net house. Two sets of 15 pots of each genotype were maintained. One set was kept as control and another set was inoculated with *X. axonopodis* bacterial suspension at 30-35 days after sowing (DAS) and humidity was maintained before and after inoculation. Lesion size was observed to be in direct correlation with the disease susceptibility. Non-structural carbohydrate content decreased in inoculated leaves as compared to uninoculated one, while structural carbohydrates (NDF, ADF, hemicellulose, cellulose, lignin and silica) increased in inoculated leaves. This study enables to determine the relative disease resistance in relation to changes in carbohydrate content along with the role of carbohydrates in bacterial blight resistance in forage guar.

Key words : Bacterial blight, carbohydrate, guar, *Xanthomonas*

Guar is an important forage legume crop of arid and semi-arid region of India. It is mainly grown in north-western zone of India viz., Haryana, Rajasthan, Punjab and parts of U. P. and M. P. (Pahuja *et al.*, 2010), but during growth period, guar plants are attacked by several pathogens which cause bacterial blight, powdery mildew, fusarium blight, root-rot and wilt diseases. Among them, bacterial blight caused by *X. axonopodis* pv. *cyamopsidis* is a serious disease, which appears almost every year and seriously threatens the guar production in certain areas. The disease was first recorded in India as bacterial leaf spot in 1953. Bacterial blight is a vascular disease; that develops in the form of necrotic areas mostly from the margin of the leaves. These necrotic areas enlarge and sometimes cover the entire leaf lamina. From the leaf, infection advances systematically through petiole into the stem, which results in blackening and cracking of stem. Despite using good quality seeds and fertilizers, the farmers are unable to harvest good crop due to bacterial blight disease as it is the major constraint in the productivity. Many workers use different antibiotics and bactericides as seed treatment and foliar spray for

controlling bacterial diseases in crops but chemicals pose several problems, such as threats to ecosystem and human health, increase in production cost and induction of pathogen resistance to chemicals following their long-term use. Khatri and Nanda (2010) showed that chemical control was not always effective in controlling bacterial blight disease. Plant also contains a variety of substances, which are involved in resistance or susceptibility to infection by pathogens. Many plant species employ a diverse array of defense that minimizes losses during pathogen attack, among which, variation in structural carbohydrate, total soluble sugars, reducing sugars and non-reducing sugars after inoculation has been reported (Rao and Nayudu, 1979). Soluble sugar content is related to pathogen invasion, as it is the major source of energy for the development of plant pathogen. On the other hand, the increase in reducing sugar content is associated with higher resistance (Yadav, 1994), while structural carbohydrates play first line of defense against pathogen invasion. The wild species *C. serrata* of guar is resistant and Pusa Nav Bahar (PNB) is susceptible to bacterial blight but information regarding the effect of bacterial

blight on such species is not available. In view of this, present investigation was planned to ascertain the effect of disease on carbohydrate contents and their role in disease resistance in forage guar.

MATERIALS AND METHODS

Seeds of guar species *Cyamopsis serrata* (Highly resistant) and *Cyamopsis tetragonoloba* [HG 563 and FS 277 (Moderately resistant) and Pusa Nav Bahar (Highly susceptible)] were procured from Forage Section, CCS Haryana Agricultural University, Hisar. The homogenized, sieved sandy soil (5 kg soil/pot) was filled in large pots, lined with polythene bags.

Pathogen Inoculation and Raising of Crop

The bacterial suspension from crude leaf extract of infected leaves was prepared (10 cfu/ ml) and was sprayed on guar leaves and humidity was maintained before and after inoculation. Two sets of 15 pots of each genotype (five seeds per pot) were maintained. One set was kept as control and another set was inoculated with *X. axonopodis* bacterial suspension at 30-35 days after sowing (DAS). In order to prevent insect attack, the plants were sprayed with malathion as per recommendation.

Sampling

Guar leaves were collected at 2, 10 and 25 days after inoculation and brought to the laboratory in polythene bags. The lesion size (cm) was recorded at the time of sampling and for carbohydrate content estimation, plant parts were dried in a hot air oven maintained at 70°C and stored in paper bags with proper labelling.

Biochemical Analysis

Non-structural carbohydrate contents were estimated in the sugar extract, prepared by mixing 100 mg of powdered leaves tissue in a 100-ml flat-bottomed volumetric flask with 10 ml of 80 per cent ethanol. The flask was maintained at 70°C for 1 h in a water bath and the filtrate was collected in a 25-ml volumetric flask. The extraction procedure was repeated five times. The final volume was made to 25 ml with 80 per cent ethanol. Total soluble sugar content was estimated by the method

of Dubois *et al.* (1956). Reducing sugar content was determined by Nelson (1944) and Somogyi's method (1945). The concentration of non-reducing sugars was calculated by subtracting the reducing sugars content from the total soluble sugars content. Structural components viz., acid detergent fibre (ADF), neutral detergent fibre (NDF), hemicellulose, cellulose, lignin and silica were analyzed by the method of Goering *et al.* (1956).

Statistical Analysis

The experimental data were analyzed as a complete randomized design using OPSTAT software available on CCSHAU home page (www.hau.ernet.in).

RESULTS AND DISCUSSION

Lesion Size

Lesion size measurement is a direct indication of disease resistance and susceptibility. In the present investigation, bacterial blight disease was recorded in all the genotypes after inoculation with *X. axonopodis*. Yellowish brown lesion appeared at margin of guar leaf tip after two days of inoculation with bacterial blight inoculum.

The data (Table 1) showed that there was a significant increase in lesion size among four genotypes at all the sampling stages after inoculation. Mean value of lesion size was minimum (0.68 cm) in case of *C. serrata*, while maximum lesion size occurred in PNB (1.50 cm) followed by FS 277 (1.03 cm) and HG 563 (0.80 cm). This view is supported by earlier study of the

TABLE 1
Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on lesion size on guar leaves

Genotype	Lesion size (cm)			
	2 DAI	10 DAI	25 DAI	Mean
<i>C. serrata</i>	0.25	0.55	1.25	0.68
<i>C. tetragonoloba</i> , HG 563	0.25	0.75	1.40	0.80
FS 277	0.45	0.95	1.70	1.03
PNB	0.55	1.70	2.25	1.50
Mean	0.38	0.99	1.65	1.00
C. D. (P=0.05)	V=0.19, D=0.16, VXD=0.33			

DAI (D)–Days after inoculation and V–Genotype.

development of bacterial blight and changes in the resistant and susceptible genotypes of clusterbean (Lodha *et al.*, 1993), in which, maximum blight intensity (76%) and apparent infection rate (0.052) were recorded in Pusa Nav Bahar. Similar observations were also recorded in tomato, in which, resistant cv. Sun hybrid and cv. Iandam had minimum disease impact, whereas cv. Quality (highly susceptible) had recorded maximum disease impact during the study of induction of bacterial spot pathogenesis (Chandrashekar and Umesha, 2012).

Non-structural Carbohydrates

Total soluble sugar content increased with advancement in growth in both uninoculated and inoculated leaves, but a significant decrease in the total soluble sugar content, with advancement in growth of pathogen, was observed after infection of the guar leaves with *X. axonopodis* pv. *cyamopsidis* (Tables 2, 3 and 4). Post-infectious reduction in the non-structural carbohydrate contents was recorded in many host-pathogen systems (Sharma *et al.*, 2012; Wadhwa *et al.*,

2013). The amount of total soluble sugar content was also determined in the stem bark of healthy and quick lime disease infected mango tree and reduction of 12.5 per cent in total sugars was found in infected stem bark as compared to healthy parts (Saeed *et al.*, 2016). The variation in the total soluble sugar in uninfected and infected banana fruits with *L. theobromae* was also observed at different ripening stages (Nath *et al.*, 2015) and the total soluble sugar content was found lower in infected than uninfected fruits. Maximum reduction in total soluble sugar content occurred in case of PNB (48.07 %) followed by *C. serrata* (25.70%) and HG 563 (25.70%), while minimum occurred in FS 277 (11.11%) over control at 25 DAI (Table 2).

A significant increase was found in reducing sugar content in both uninoculated as well as inoculated guar leaves with time period, but after inoculation, non-reducing sugar content declined in guar leaves. Maximum reduction occurred in PNB (55.50%) and minimum reduction was observed in *C. serrata* (21.70%) after 25 days of inoculation as compared to control (Table 3). Similar trend was observed for non-reducing sugar

TABLE 2

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on total soluble sugar content (mg/g dry weight) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	80.76	109.45	114.03	101.41	61.60	68.61	84.72	71.64	88.20
<i>C. tetragonoloba</i> HG 563	110.07	121.25	125.97	119.10	71.04	93.19	96.32	86.85	105.61
FS 277	79.24	122.43	141.88	114.51	54.58	112.71	125.90	97.73	113.31
PNB	55.83	104.51	172.64	111.00	40.56	78.82	89.65	69.68	95.19
Mean	81.48	114.41	138.63	111.50	56.94	88.33	99.15	81.48	100.58

C. D. (P=0.05) : T=2.07, D=2.54, V=2.93, T x D=3.59, T x V=4.15, V x C=5.08 and T x DXV=7.18
DAI (D)—Days after inoculation, V—Genotype and T—Treatment.

TABLE 3

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on reducing sugar content (mg/g dry weight) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	8.48	16.71	20.25	15.15	8.63	11.92	11.33	10.63	12.89
<i>C. tetragonoloba</i> HG 563	8.13	12.38	16.79	12.43	9.43	11.95	15.50	12.29	12.36
FS277	12.85	21.83	35.08	23.25	11.62	17.73	26.58	18.64	20.95
PNB	10.20	19.71	22.52	17.48	9.08	12.54	11.88	11.17	14.32
Mean	9.92	17.66	23.66	17.08	9.69	13.54	16.32	13.18	15.13

C. D. (P=0.05) T=0.52, D=0.64, V=0.74, T x D=0.90, T x V=1.04, V x C=1.28 and T x D x V=1.81.
DAI (D)—Days after inoculation, V—Genotype and T—Treatment.

TABLE 4

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on non-reducing sugar content (mg g⁻¹ dry weight) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	72.28	92.74	93.78	86.27	52.96	57.38	73.39	61.25	73.76
<i>C. tetragonoloba</i> HG 563	100.64	108.64	109.18	106.15	62.92	81.24	80.82	74.99	90.57
FS277	66.39	100.60	106.79	91.26	42.97	94.98	99.32	79.09	85.17
PNB	45.63	84.81	150.12	93.52	31.47	66.28	69.78	55.84	74.68
Mean	71.23	96.70	114.97	94.30	47.58	74.97	80.83	67.79	81.05

C. D. (P=0.05) T=1.94, D=2.38, V=2.75, T x D=3.37, T x V=3.89, V x C=4.76 and T x D x V=6.73.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

content (Table 4).

In the present case, decrease in the quantity of total soluble sugars might have occurred due to decrease of photosynthesis assimilative surface because of formation of water soaked and brown spot on leaves, disruption of chloroplast, wasteful host respiration due to pathogenesis and utilization of soluble sugar for host defense reactions such as synthesis of polyphenols and phytoalexins (Asahi *et al.*, 1980). The present findings showed high level of total soluble sugars, reducing and non-reducing sugars in resistant and moderately resistant genotypes than in susceptible genotypes after inoculation.

Structural Components

Cell wall modifications, by increasing related gene expression, are among the induced defense mechanisms by Systemic Acquired Resistance (Ebrahim *et al.*, 2011).

In present study, gross effect on forage quality was attributed to increase in cell wall structural carbohydrate content i. e. NDF, ADF, cellulose and lignin contents in resistant and moderately resistant genotype, while decrease in these structural carbohydrates was observed in susceptible genotypes, except silica content, which was high in susceptible genotypes.

The structural carbohydrate contents (NDF, ADF, cellulose, hemicellulose, lignin and silica) increased in the leaves of the inoculated guar leaves as compared to uninoculated ones (Tables 5, 6, 7, 8, 9 and 10) at all the developmental stages. Similar increase in acid detergent fibre, cellulose, lignin and silica content was also reported in the leaves of inoculated seedlings compared with the uninoculated control in guar infected with *Rhizoctonia* sp. (Wadhwa *et al.*, 2013).

The increasing concentration of the structural carbohydrates such as lignin and silica deposition in cell wall of the host was mainly done by cross-linking of the phenylpropanoid monomers, which contribute in

TABLE 5

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on neutral detergent fibre content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	15.40	16.00	16.35	15.92	16.00	16.55	16.85	16.47	16.19
<i>C. tetragonoloba</i> HG 563	16.30	16.85	16.95	16.70	16.65	17.65	17.85	17.38	17.04
FS277	12.55	14.30	15.55	14.13	13.60	15.65	16.55	15.27	14.70
PNB	14.00	14.05	14.85	14.30	13.85	13.90	14.15	13.97	14.13
Mean	14.56	15.30	15.93	15.26	15.03	15.94	16.35	15.77	15.52

C. D. (P=0.05) T=0.10, D=0.13, V=0.14, T x D=NS, T x V=0.20, V x C=0.25 and T x D x V=NS.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

TABLE 6

Effect of inoculation with *Xanthomonas axonopodis* pv. *cymopsidis* on acid detergent fibre content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	8.70	10.25	14.05	11.00	9.20	10.80	14.90	11.63	11.32
<i>C. tetragonoloba</i> HG 563	10.75	11.25	14.75	12.25	10.90	11.90	13.25	12.02	12.13
FS277	11.75	12.25	13.25	12.42	12.35	12.70	13.75	12.93	12.68
PNB	8.88	10.45	11.00	10.11	8.65	10.40	10.75	9.93	10.02
Mean	10.02	11.05	13.26	11.44	10.28	11.45	13.16	11.63	11.54

C. D. (P=0.05) T=10, D=0.13, V=0.15, T x D=0.18, T x V=0.21, V x C=0.26 and T x D x V=0.36.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

TABLE 7

Effect of inoculation with *Xanthomonas axonopodis* pv. *cymopsidis* on cellulose content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	5.65	6.30	9.25	7.07	5.75	6.45	9.85	7.35	7.21
<i>C. tetragonoloba</i> HG 563	8.80	9.05	12.30	10.05	8.85	9.45	10.40	9.57	9.81
FS277	8.75	8.90	8.95	8.87	8.85	8.95	9.35	9.05	8.96
PNB	6.10	6.65	7.45	6.73	6.35	7.95	8.05	7.45	7.09
Mean	7.33	7.73	9.49	8.18	7.45	8.20	9.41	8.35	8.27

C. D. (P=0.05) T=0.06, D=0.07, V=0.12, T x D=0.10, T x V=0.12, V x C=0.14 and T x D x V=0.20.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

TABLE 8

Effect of inoculation with *Xanthomonas axonopodis* pv. *cymopsidis* on hemicellulose content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	6.70	5.75	2.30	4.92	6.80	5.75	1.95	4.83	4.88
<i>C. tetragonoloba</i> HG 563	5.55	5.60	2.20	4.45	5.75	5.75	4.60	5.37	4.91
FS 277	0.80	2.05	2.30	1.72	1.25	2.95	2.80	2.33	2.03
PNB	5.13	3.60	3.85	4.19	5.20	3.50	3.40	4.03	4.11
Mean	4.54	4.25	2.66	3.82	4.75	4.49	3.19	4.14	3.98

C. D. (P=0.05) T=0.16, D=0.20, V=0.23, T x D=NS, T x V=0.33, V x C=0.40 and T x D x V=0.57.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

TABLE 9

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on lignin content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	2.90	3.10	3.60	3.20	3.10	3.40	3.70	3.40	3.30
<i>C. tetragonoloba</i> HG 563	1.20	1.40	1.20	1.27	1.20	1.30	1.30	1.27	1.27
FS 277	2.10	2.30	2.20	2.20	1.80	2.10	2.30	2.07	2.13
PNB	0.90	1.30	1.40	1.20	0.90	1.00	1.10	1.00	1.10
Mean	1.78	2.03	2.10	1.97	1.75	1.95	2.10	1.93	1.95

C. D. (P=0.05) T=NS, D=0.07, V=0.77, T x D=NS, T x V=0.11, V x C=0.14 and TXDXV=0.19.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

TABLE 10

Effect of inoculation with *Xanthomonas axonopodis* pv. *cyamopsidis* on silica content (% dry weight basis) in guar leaves

Genotype	Uninoculated				Inoculated				Grand mean
	2 DAI	10 DAI	25 DAI	Mean	2 DAI	10 DAI	25 DAI	Mean	
<i>C. serrata</i>	0.20	0.70	1.25	0.72	0.35	0.95	1.35	0.88	0.89
<i>C. tetragonoloba</i> HG 563	0.75	0.95	1.25	0.98	0.85	1.20	1.55	1.20	1.15
FS277	1.30	1.45	2.10	1.62	1.35	1.40	2.10	1.62	1.66
PNB	1.85	2.15	2.15	2.05	1.25	1.45	1.65	1.45	1.78
Mean	1.03	1.31	1.69	1.34	0.95	1.25	1.66	1.29	1.37

C. D. (P=0.05) T=NS, D=0.10, V=0.11, T x D=NS, T x V=0.16, V x C=0.19 and T x D x V=NS.
DAI (D)–Days after inoculation, V–Genotype and T–Treatment.

formation of defense barriers by changing the cell structure defense system against pathogens (Thilagavathi *et al.*, 2007).

CONCLUSION

In the present study, the structural and non-structural carbohydrate contents were found higher in resistant genotypes as compared to the susceptible genotypes. This study, therefore, concludes the negative relation between relative disease resistance and carbohydrate contents along with the role of carbohydrates in bacterial blight resistance in forage guar.

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