Madras Agric. J. 88 (10-12) : 571-576 October-December 2001

https://doi.org/10.29321/MAJ.10.A00375

Accumulation of anthocyanin compounds in sugarcane tissues as a factor responsible for red rot resistance

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Abstract : Experiments were conducted to study the role of anthocyanin pigment in sugarcane red rot disease resistance in response to *C. falcatum* infection. In resistant cultivars, anthocyanin levels were always higher after pathogen inoculation between 24 and 240 h than in susceptible cultivars. In resistant cultivar anthocyanin accumulation in pathogen inoculated canes was higher over the injured canes at all intervals, however, in susceptible cultivar, such accumulation was noticed upto 48 h only. Anthocyanin extracted at 72 h after inoculation had maximum antifungal activity on the pathogen. Higher anthocyanin synthesis occurred in incompatible interactions than in comptible interactions. Partially purified pathogen toxin also induced multifold increase in anthocyanin accumulation in the resistant genotype than in the susceptible genotype. *(Key words : Sugarcane, C. falcatum, Anthocyanin, Resistance).*

The factors that contribute to biochemical resistance to red rot of sugarcane are not clearly explained. Response of sugarcane to attack by the red rot pathogen *Colletotrichum falcatum* Went (teleomorph : *Glomerella tucumanensis* (Speg) Arx & Muller) suggests that the host produces anthocyanin pigments which function as phytoalexins (Viswanathan *et al.*, 1996a,b). In infected sugarcane stalks, a red substance is released in cells and intercellular spaces near invading hyphae. The resistance of the genotype is correlated with intensity of such pigment production (Edgerton and Carvajal, 1944). Anthocyanins are reported play an important role in host-pathogen interaction in crops (Hammerschmidt and Nicholson, 1977). We have attempted to quantify anthocyanin in sugarcane in response to pathogen infection, and to study the role of pathogen metabolite's (toxin) involvement in triggering anthocyanins and their significance in disease resistance.

Materials and Methods

Extraction of anthocyanin

Live canes of 8 months age were inoculated with *C. falcatum* conidial suspension obtained from 8 days old culture grown on oat meal agar. Different sugarcane cultivars (BO 91 and CoS 767 resistant; Co 7717, CoC 671 and CoC 86062 susceptible) varying in disease resistance were used in this study A bore hole (12 mm depth and 8 mm width) was made at the centre of the 3rd internode using red rot inoculator and one ml of conidial suspension (10^6 conidia ml^{-1}) was placed in the bore hole. The bore hole was later sealed with plastic clay. In the control treatment, one ml of sterile water was placed in the place of conidial suspension. Anthocyanin concentration after pathogen inoculation was assessed by a modified method of Heim *et al* (1983). Samples were drawn from inoculated sites and from distances of 2.5 and 5.0 cm away from the inoculation site at different intervals. About 0.5 g of tissue was weighed and homogenized in 5 ml of 80% methanol acidified with 0.1% HCl. The homogenate was centrifuged at 10000 rpm for 30 min. at 4°C and anthocyanin content measured directly by measuring the absorbance of the supernatant at 525 nm.

C. falcatum toxin preparation and inoculation

The pathogen was cultured on Czapek's liquid medium in which sucrose was substituted with host extract as carbon source (30g stalk tissue/l) prepared by homogenization and filtration. After 10 days, the mycelial mats and culture filtrates were pooled and homogenized in a blender, filtered and the filtrate was used as a source of combined exo-and endo-toxins of the pathogen. The toxin metabolite(s) in the filtrate were fractionated by the modified method of Nair and Ramakrishnan (1973). The filtrate was reduced to 1/10 volume under reduced pressure, mixed with equal volume of methanol, retained overnight and filtered. The methanol was removed by vacuum evaporation at 40°C, the pH of the aqueous phase adjusted to 3.5 with diluted HCl and shaken well with equal volume of diethyl ether. The ether phase was separated, mixed and shaken with an equal volume of 5% Na_2CO_3 and the aqueous phase was rejected. The ether phase was evaporated to dryness by air drying. The puri fied tox in fraction was dissolved in sterile distilled water and 1 ml of the dissolved toxin fraction of 1000 ppm was injected into 2 cultivars, *viz.* BO 91 and CoC 671 to find out the role of toxin produced by the pathogen inducing anthocyen in production.

Table 1. Anthocyanin accumulation (absorbance at 525 nm/g fresh tissue) in sugarcane internodal tissues after *C. falcatum* (pathotype Cf671) inoculation

Cultivar	Sampling distance (cm)	Hours after inoculation						
		24	48	72	96	168	240	Mean
BO 91	0.0	0.108	0.203	0.261	0.292	0.723	0.366	0.326
	2.5	0.127	0.116	0.170	0.057	0.178	0.165	0.235
	5.0	0.123	0.161	0.112	0.079	0.179	0.145	0.133
CoS 767	0.0	0.268	0.133	0.181	0.255	0.236	0.396	0.244
	2.5	0.367	0.413	0.157	0.236	0.223	0.210	0.268
	5.0	0.248	0.139	0.182	0.130	0.087	0.166	0.159
Co 7717	0.0	0.144	0.137	0.099	0.09	0.155	0.165	0.133
	2.5	0.234	0.103	0.087	0.083	0.202	0.392	0.184
	5.0	0.144	0.084	0.083	0.055	0.464	0.304	0.189
CoC 671	0.0	0.170	0.123	0.141	0.078	0.164	0.131	0.134
	2.5	0.133	0.129	0.146	0.074	0.164	0.159	0.134
	5.0	0.163	0.201	0.157	0.106	0.189	0.208	0.171
CoC 86062	0.0	0.180	0.323	0.097	0.096	0.105	0.258	0.161
	2.5	0.169	0.219	0.068	0.074	0.165	0.114	0.135
	5.0	0.198	0.147	0.151	0.117	0.220	0.249	0.180

Values are mean of 6 replications
CD (P=0.05%) 0.058 (Cultivar) 0.100 (Interaction)

Table 2. Anthocyanin accumulation (absorbance at 525 nm/g fresh tissue) in sugarcane internodal tissues after *C. falcatum* pathotype cf671 inoculation

Treatment	Sampling distance (cm)	Hours after inoculation						
		24	48	72	96	168	240	Mean
BO 91 control	0.0	0.176	0.143	0.124	0.239	0.325	0.357	0.241
	2.5	0.359	0.296	0.263	0.194	0.204	0.371	0.290
	5.0	0.351	0.220	0.217	0.117	0.122	0.119	0.206
BO 91 treated	0.0	0.723	0.273	0.379	0.533	0.435	0.582	0.496
	2.5	0.821	0.463	0.431	0.303	0.537	0.466	0.496
	5.0	0.293	0.379	0.280	0.274	0.140	0.167	0.260
CoC 86062 control	0.0	0.263	0.240	0.346	0.244	0.420	0.448	0.323
	2.5	.0.538	0.329	0.121	0.469	0.194	0.204	0.285
	5.0	0.261	0.475	0.188	0.177	0.208	0.259	0.250
CoC 86062 treated	0.0	0.211	0.259	0.125	0.310	0.202	0.263	0.220
	2.5	0.293	0.232	0.156	0.191	0.274	0.233	0.214
	5.0	0.227	0.386	0.154	0.282	0.353	0.344	0.292

Values are mean of 6 replications
CD (P=0.05%) 0.062 (Cultivar) 0.108 (Interaction)

Conidial germination and mycelial growth assay

The effect of anthocyanin extract on conidial germination and radial mycelial growth of *C.falcatum* was determined. To assess conidial germination, the anthocyanin extract was made up to 5 ml in methanol in all treatments and 50 ml of the extract was allowed to dry on cavity slides and then 50 ml of *C. falcatum* conidial suspension from a 8 days old culture (10^6 conidia/ml) was added and allowed to germinate in a moist chamber at room temperature (28 ± 2°C). The spore germination was recorded after 18 h. To determine mycelial growth, one ml of dissolved extract was mixed with 50 ml of hot sterilized oat agar (45°C) and poured into the Petri plates. The plates were kept in the laminar flow chamber for 24 h for complete evaporation of methanol. Later, the plates were inoculated with 8 mm diameter discs of pathogen grown on oat agar. In the control plates, methanol alone was used in place of anthocyanin extracts.

Results and Discussion

In resistant cultivars accumulation of anthocyanin was significantly higher than in susceptible cultivars throughout the study period at the point of inoculation and also 2.5 cm away from inoculation point. However, at 5.0 cm away from the site of inoculation, no difference in anthocyanin concentrations was noticed. The resistant cultivar BO 91 recorded the lowest anthocyanin concentration at that point (Table 1). These results indicate that anthocyanins accumulate faster and at higher quantities near the pathogen inoculation site in resistant cultivars whereas, in susceptible cultivars the anthocyanin is diffused throughout the internode of canes. In the susceptible host the pathogen ramifies adjacent internodes completely within 10 days after pathogen inoculation but in resistant hosts no such symtpoms were noticed suggesting a positive relationship between pathogen colonisation and anthocyacnin accumulation.

Anthocyanin accumulation in pathogen inoculated and injured canes of resistant and susceptible cultivars was compared, anthocyanin content was much higher in inoculated canes than in injured canes of resistant cultivars. The increase was more than 100% and 20% at the point of inoculation and 5 cm away respectively. However, in the susceptible cultivar anthocyanin accumulation was more to injury than in response to pathogen inoculation except at a distance of 5.0 cm from the point of inoculation (Table 2). These results show that the pathogen had a specific role in triggering anthocyanin accumulation in the resistant host and in the susceptible host, the role of the pathogen in induction of anthocyanin is less or the pathogen may outrightly kill the host tissues in the internodal areas, which results in lesser accumulation of anthocyanins. Antifungal activity of anthocyanin extracts from 5 cultivars showed that all extracts recorded maximum inhibition of spore germination at 72 hr and not at any other stage of host pathogen interaction. Extracts from BO 91 (84.58%) were highly inhibitory followed by those from CoS 767 (81.66%). Extracts from Co 7717 were not inhibitory except at 72 h after inoculation (Table 3). This information suggests that all cultivars accumulate the highest quantity of antifungal pigments by 72 h after inoculation while in resistant cultivars it is maintained throughout. In maize and *Helminthosporium maydis* interaction anthocyanin accumulation was correlated with disease resistance (Heim *et al.*, 1983).

Results of the studies with 10 host differentials against two pathotypes (Cf1148 and Cf7717) of *C. falcatum* showed that variation in anthocyanin accumulation among cultivars in relation to the reaction of the clone to the different pathotypes. All the cultivars had exhibited resistance to one isolate and susceptibility to the other isolate in artificial testing. Anthocyanin accumulation was higher in all incompatible interactions than in compatible interactions. In Co 7717, in compatible interaction recorded 3 fold higher anthocyanin accumulation after *C. falcatum* inoculation as compared to the compatible interaction (Table 4). In soyabean following infection with an incompatible race of *Phytophthora megasperma* f. sp. *glycinea* the levels of phytoalexins (glyceollin and glycinol) increased from undetectable amounts to greater than 10% of the dry weight of the infected tissue within 24 to 48 h after inoculation (Keen and Horsch, 1972).

The pathogen toxin triggered more amount of anthocyanin accumulation than it was induced by pathogen inoculation in both cultivars viz. BO 91 and CoC 671. Anthocyanin accumulation was about 3 times greater in BO 91 than in CoC 671, after toxin treatment. Likewise, in the cultivar BO 91 anthocyanin accumulation was about 4 times higher than CoC 671 following pathogen inoculation (Table 5). Earlier studies have proved that the toxin produced by the pathogen has a role in *C. falcatum* pathogenesis in sugarcane (Mohanraj *et al.*, 1997). The results suggest that the pathogen toxin also triggers anthocyanin accumulation in both susceptible and resistant host cultivars.

Table 3. Anthocyanin extracts from sugarcane cultivars on *C. falcatum* (pathotype Cf671) conidial germination.

Cultivar used for anthocyanin extraction	Anthocyanin extraction from sugarcane internodes – h after *C. falcatum* inoculation.					
	24	48	72	168	240	Mean
BO 91	23.72	16.26	1.59	21.32	14.22	15.42
CoS 767	15.02	9.74	4.10	30.68	37.67	19.44
Co 7717	93.79	46.68	25.10	94.65	94.40	70.92
CoC 671	63.04	20.67	13.57	43.57	85.47	45.28
CoC 86062	40.19	77.37	8.37	73.29	78.83	55.61

CD (P=0.05%) 9.62
The values are transformed (arc sine) before performing statistical analysis.
Per cent germination of conidia in the control was 92.56.

Table 4 Anthocyanin accumulation in sugarcane differentials in response to *C. falcatum* pathotypes.

Cultivar	*C. falcatum* pathotype used	Red rot reaction	Anthocyanin A_{525} nm/gfw
Co 62175	1	MR	0.192 ±.012
	2	HS	0.168 ±.014
Co 7717	1	MR	0.858 ±.052
	2	HS	0.242 ±.030
Co 7514	1	HS	0.116 ±.021
	2	MR	0.170 ±.018
Co 8014	1	MR	0.432±.022
	2	HS	0.264±.024
Co 1148	1	HS	0.206±.019
	2	MR	0.500±.047
Co 997	1	HS	0.150±.008
	2	MR	0.242±.028
Co 6806	1	S	0.114±.006
	2	MR	0.122±.011
Co 419	1	MR	0.296±.024
	2	HS	0.102±.017
Co 6914	1	MR	0.330±.034
	2	HS	0.216±.025
Co 6907	1	MS	0.404±.037
	2	S	0.180±.014

Pathotypes 1 and 2 refer to pathotypes of Cf 1148 and Cf 7717 respectively.
MR = Moderately Resistant; MS = Moderately Susceptible; S= Susceptible;
HS = Highly Susceptible
gfw = Gram fresh weight of sugarcane internode tissues.

Table 5. Induction of anthocyanin in sugarcane by *C. falcatum* toxin

Variety	Treatment	Red rot reaction	Anthocyanin A525 nm/g fresh weight
BO 91	Pathogen	MR	0.864b
BO 91	Toxin	-	1.404a
CoC 671	Pathogen	HS	0.184d
CoC 671	Toxin	-	0.456c

Values followed by same letters are not significantly different at 5% by DMRT.

Phenolics were initially thought to play a role in red rot resistance (Rao *et al.*, 1968). Although quantitative differences existed among cultivars in their phenolic content, that could not be correlated with the degree of resistance to *C. falcatum* and phenolic content increased as a result of infection, but not as a function of resistance (Singh *et al.*, 1976; Godshall and Lonergan, 1987). From the earlier findings it is clear that red rot resistance is governed by the factors other than the concentration of phenolics. The anthocyanins are the products of phenylpropanoid pathway and they are implicated in disease resistance in different crops. Although complete resistance to red rot in sugarcane does not exist (Abbott and Hughes, 1961) some antifungal substances produced by the host upon pathogen invasion might inhibit pathogen establishment and restrict further spread in the host. Anthocyanins are potential antifungal compounds and their role in *C. falcatum* resistance in sugarcane is established. Further studies showed that the pathogen completes its life cycle in highly susceptible genotypes within 72 h and pigmentation was diffused, whereas in resistant genotypes only dark and intense pigmentation with much restricted distribution was observed (Mohanraj *et al.* 1997). Previous studies of Viswanathan *et al.* (1996a, b) showed the presence of 3-deoxyanthocyanidin phytoalexins *viz.* luteolinidin, apigeninidin and caffeic acid ester of 5-0-apigeninidin in the anthocyanin compounds. Present studies prove the role of anthocyanin compounds in *C. falcatum* resistance in sugarcane. Studies are also in progress to utilize the phytoalexin compounds as molecular marker in screening sugarcane clones to red rot resistance.

Acknowledgements

The author is thankful to the Director of the institute for providing facilities and encouragement.

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(Received : April 2000; Revised : November 2001)

Madras Agric. J. 88 (10-12) : 576-582 October-December 2001

Influence of intercropping system on insect pests and viral diseases of tomato, *Lycopersicon esculentum* Mill.

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Abstract: Studies to assess the effects of intercropping in tomato under irrigated and rainfed conditions for the management of key pests at Regional Research Station, Paiyur, TamilNadu Agricultural University, TamilNadu during 1998-2000. Experiments were conducted in a factorial randomized block design using intercrops *viz*, clusterbean, green gram and mustard and two rounds of spraying with NSKE 5% and monocrotophos 0.07%, once on 30th day after transplanting and second at the time of maximum flowering and fruit set. The results revealed that tomato intercropped with mustard at 2:1 ratio had recorded reduced incidence of whitefly and thrips population and fruit borer damage as well in two different field studies. Natural incidence of tomato spotted wilt virus (TSWV) and leaf curl virus (LCV) diseases were noticed on the rainfed tomato crop raised during August-September, 1999 planting season. The incidence of TSWV and LCV diseases were less under intercropped tomatoes than sole crop tomato and this was significantly low on the mustard intercropped plots. Nearly 30% of plants were lost due to these diseases in the tomato sole crop plots and this loss in plant population was nearly 45-60% more than the mustard intercropped plots. The combined yield of undamaged and damaged fruits was 16.319 t ha^{-1} and 16.028 t ha^{-1} for the sole tomato and mustard intercropped tomato under irrigated condition. However, under rainfed condition due to the severe incidence of viral diseases, an yield of 5.832 t $ha^{-1}$was recorded in sole crop while it was 8.527 t ha^{-1} in mustard intercropped plots. The undamaged fruit yield recorded was 15.00 and 7.810 t ha^{-1} for the mustard intercropped plots, whereas it was 13.386 and 4.833 t ha^{-1} for sole crop tomato under irrigated and rainfed conditions respectively. The mustard intercropping had given an additional net return of Rs.7080 and Rs.12400 because of reduced pest infestation under irrigated and rainfed conditions respectively.

Vegetables are indispensible for healthy nutrition and thus gains importance. Tomato, *Lycopersicon esculentum* Mill. is one such important vegetable crop commercially grown worldwide. It is raised both as an irrigated and rainfed crop in India and particularly in TamilNadu. The average productivity of tomatoes in India is still 9.78 t ha^{-1} as against world average of 23.51 t ha^{-1} (Bose *et al*, 1993). Vulnerable to pests, the crop is known to be infested by an array of insect pests, of which sucking pests *viz.* thrips, *Thrips tabaci* (Genn.), whitefly, *Bemisia tabaci* (Genn.) and fruit borer, *Helicoverpa armigera* (Hub.) are considered to be important. The use of highly toxic insecticides, though a cheap source of pest control, is considered not only injurious to living organisms but leads to serious ecological disturbances in the agroecosystem on long term approach. Further, pesticide free produces are gaining importance both in the inland and foreign markets. Intercropping is believed to be a vital field tool that minimises pest population in any