Detection of Total Phenols in Relation to Red Rot Resistant and Susceptible Varieties of Sugarcane

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International Journal of Plant Protection, Vol. 2 No. 1 : 111-113 (April to September, 2009)

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SUMMARY

The total phenols in both resistant and susceptible varieties increased thirty days after inoculation with the three pathotypes of *Colletotrichum falcatum* causing red rot in sugarcane. No perceptible differences were observed between resistant and susceptible varieties of sugarcane at any stage.

Key words : Red rot, *Colletotrichum falcatum*, Pathotypes, Phenols

Accepted :* *March, 2009

Phenolic compounds have long been considered to play an important role in the biochemical aspects of disease resistance in plants (Mahadevan, 1966). Rao *et al.* (1968) studied the phenolic content of the cane juice in resistant and susceptible varieties after inoculation with red rot pathogen. They observed that it was significantly higher in varieties resistant to *Colletotricum falcatum* (*Glomerella tucumerensis*) than in susceptible varieties and that the activity of poly phenol oxidase (PPO) was greater. After inoculation, there was marked liberation of phenols in the resistant varieties but none or even a slight decrease in the susceptible ones. A higher levels of activity of PPO was also maintained in the resistant varieties. Chlorogenic acid was detected by Wilson and Srivatava (1969) in the nodal tissues of the varieties Co 285 and Co1070 moderately resistant to red rot.

These varieties have high phenolic content compared to the susceptible varieties Co331 and Co 445. Singh *et al.* (1976) reported an increase in the total phenols on fourth day after inoculation of all the varieties tested against red rot pathogen. However, they observed that there was no positive correlation between the amount of total phenols in sugarcane and degree of resistance. The phenolic content differed among cultivars, tissues and growth stages as reported by Sharma *et al.*(1982). These compounds seemed to accumulate more in the apical meristem than in roots and leaves. Healthy stem tissues contained much higher amounts of endogenous phenols in *Colletotrichum falcatum* resistant varieties than in those of susceptible genotypes at ripening. Phenolic content in the juice of immature tissues was positively correlated with resistance.

Pinon *et al.* (1984) found higher phenol content when the cane was inoculated with *Colletotrichum falcatum.* They reported that the total phenols in plant and first ratoon were higher in My 5514 resistant to *Colletotrichum falcatum* and My 5741 moderately resistant, than in susceptible Pomez-72. Average values were higher in the first ratoon than in plant cane. In a subsequent report, Pinon *et al.* (1985) observed that when resistant, moderately resistant and susceptible cultivars were inoculated with *Colletotrichum falcatum* an increase in total phenols was observed in susceptible Pomez-72, seven days later and in all the three cultivars after15 and 30 days after inoculation, although there was a general tendency of phenolic compounds to increase in all three cultivars. Based on three observations, attempts were made to compare the total phenolic content in resistant and susceptible cane varieties on infection with three pathotypes of red rot.

MATERIALS AND METHODS

Sugarcane varieties, Co 419, Co 997 and Co 671 (susceptible) and Co 7706, CoA 7602 and Co 8013 (resistant) were used for study.

All the six varieties were inoculated with three pathotypes *viz.*, Cf 419, Cf 997 and Cf 671 at eight months age by following the plug method of inoculation. After incubation period of 60 days, five g of stalk tissue was recovered with the help of cork borer from the nodes just above the inoculated internodes from all the six varieties and also healthy canes to serve as check.

The stalk tissue was cut into pieces and was immediately plugged into boiling ethyl alcohol and allowed to boil for 10 minutes. 5 ml of alcohol was used for every gram of tissue. The entire extraction was done on top of stem bath under a hood.

The extraction was led in a pan of cold water and the tissues were thoroughly crushed in a mortar with pestle for 10 minutes. The extract was passed through cheese cloth and the primary extract was collected. The ground tissues were re-examined for 3 minutes in 80% alcohol used at he rate of 2ml/g of tissue to ensure complete removal of alcohol soluble phenolic substances from the tissues. Finally both the extracts were pooled and filtered through Whatman No-41 filter paper. The final volume of the extract was made up to 25 ml in volumetric flask with 80% ethanol.

Estimation of phenols by folinciocalteu method:

Estimation of phenols was done by using folin-ciocalteu reagent where the reaction between phenols and oxidizing agent, phosphonmolybdate resulted in the formation of blue complex (Bray and Thorpe, 1954). 1 ml of the extract was pipetted into a test tube and then 1ml of folin-ciocalteu reagent followed by 2 ml of sodium carbonate was added. The tube was shaken well and placed in a boiling water bath for exactly 1 minute and cooled under running tap water. The blue solution was diluted to 25 ml with water and then absorbance was measured at 650 nm in a calorimeter. Phenols in the sample were worked out from a standard curve prepared by using alcohol. A blank containing all the reagents minus plant extract was used every time to adjust the absorbance.

RESULTS AND DISCUSSION

The total phenols in red rot resistant and susceptible varieties were estimated from stalk tissues at twenty four hours, fifteen days and thirty days after inoculation. The samples from healthy stalks were estimated at the time of inoculation and thirty days after inoculation. The results on the total phenols in both resistant and susceptible varieties at different periods of inoculation as well as from healthy un inoculated varieties are presented in Table1. The data presented in the table clearly indicate that the native total phenols were higher in the uninoculated susceptible varieties initially as well as thirty days later, compared to the total phenols in uninoculated resistant varieties at both the stages of the sampling. However, a reasonable increase in the total phenols was observed in uninoculated healthy tissues of both resistant and susceptible varieties with age. A significant difference in the total phenols was noticed in all the uninoculated varieties irrespective of susceptibility and resistance.

The variety, Co997 exhibited significantly higher (40.75 mg/g) quantity of total phenols and the resistant variety C07706 recorded significantly lower quantity of phenols (32.58mg/g) in initial stages of sampling (Table 1).

But that trend did not continue thirty days later. The healthy tissues of susceptible variety C0671 recorded significantly higher total phenols (53.14 mg/g) followed by Co 419, Co8013, CoA 7602 and Co 9967 and significantly lower quantity of total phenols in the resistant healthy CoA7602 at thirty days of sampling. The healthy tissues of susceptible variety Co997 recorded significantly lower quantities of total phenols (42.79 mg/g) compared

Table 1 : Total phenols in stalk tissues of resistant and susceptible varieties at different intervals after inoculation with three pathotypes of inoculation with three pathotypes of *Colletotrichum falcatum* (mg/g fresh tissue weight)

Varieties	Inoculated*									Un inoculated*	
	Cf 419			Cf 997			Cf 671				
	24 hrs	15 days	30 days	24 hrs	15 days	30 days	24 hrs	15 days	30 days	15 days	30 days
Co 419	42.93	33.68	77.97	40.28	39.46	68.52	36.60	69.53	59.87	38.23	52.25
Co 997	33.20	35.10	65.66	38.51	34.36	52.80	49.05	39.67	78.65	40.75	42.79
Co 671	44.56	63.00	76.48	42.39	71.10	83.40	47.28	66.13	47.56	38.85	53.14
Co 7602	41.84	44.22	67.02	48.10	46.67	59.19	37.01	53.48	59.19	36.06	43.68
Co 7706	44.09	29.80	53.41	47.97	35.72	64.10	40.89	49.39	56.27	32.38	41.43
Co 8013	41.30	40.89	54.57	35.79	58.10	61.23	40.62	556.40	55.93	34.91	47.28
S.E.±	0.58	0.49	0.57	0.60	0.43	0.60	0.35	0.17	0.17	0.27	0.21
C.D. (P=0.05)	1.75	1.49	1.73	1.80	1.28	1.81	1.06	0.50	0.51	0.81	0.64
CV %	2.80	2.40	1.70	2.80	1.80	1.90	1.70	1.90	1.70	1.50	2.90

* Mean of three replications

to the total phenols of resistant CoA 7602 (43.69 mg/g) and Co 8013 (47.28 mg/g).

The data presented in the table1 clearly indicated as increase in the total phenols in the uninoculated susceptible and resistant hosts at thirty days of sampling over the initial content of total phenols. It is also very evident that on inoculation with *Colletotrichum falcatuum* irrespective of pathotype a reasonable increase in the quantity of total phenols was noticed in both resistant and susceptible varieties at thirty days of inoculation, comparative to the content of total phenols at thirty days sampling in the healthy uninoculated resistant and susceptible varieties. The synthesis and accumulation of total phenols in resistant and susceptible varieties on inoculation with three pathotypes has not exhibited an uniform trend of either increase or decrease when compared to the initial phenolic quantities and at fifteen days after inoculation. However, a steep increase I the content of total phenols was observed in both resistant and susceptible varieties on inoculation with three pathotypes *viz.*, Co 419, Co671 and Co997 at thirty days of inoculation compared to the content of total phenols present in susceptible and resistant varieties at twenty four hours after inoculation. The data also clearly indicate that there was a higher accumulation of total phenols in all the three susceptible varieties *viz*., Co 419, Co 671 and Co 997 compared to the accumulation of total phenols in the resistant varieties at 30 days after inoculation with the three pathotypes.

The total phenols of healthy tissues of susceptible varieties were higher initially than the resistant varieties. On inoculation the total phenols at thirty days after inoculation showed an increase in both resistant and susceptible varieties. The trend remains almost the same at different stages of sampling with minor deviations. No specific trend or any remarkable differences among the pathotypes was observed with respect to total phenols in resistant and susceptible varieties.

These findings are more or less disagreement with the observations made by earlier workers (Singh *et al.*, 1976 and Pinon *et al.,* 1985).

However, Rao *et al*. (1968) and Wilson and Srivsthava (1969) observed that the phenolic content of resistant varieties was significantly higher than susceptible varieties.

CONCLUSION

In view of the varied observations of different workers, it seems appropriate to continue critical investigations in this regard.

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