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STUDIES ON BIOCHEMICAL CHANGES IN SOYBEAN INFECTED WITH *COLLETOTRICHUM TRUNCATUM* (SCHW.)

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ABSTRACT

Present study was carried out to evaluate the changes occurring in biochemical's (*viz.*, total phenols, OD, reducing sugar, non reducing sugar, total sugar, chlorophyll a, b and total chlorophyll) in two genotypes JS-335 (susceptible) and MAUS-71 (resistant) after infection by pathogen *Colletotrichum truncatum* (Schw.) which causes tremendous losses in soybean crop. Results revealed that in diseased foliage of both the soybean cultivars, the chlorophyll content was reduced drastically over healthy cultivars, but at higher rate in susceptible JS-335 (55.8, 71.8 and 64.3 per cent) than resistant variety MAUS-71 (4.1, 31.8 and 12.9 per cent). Total sugar, reducing sugar and non reducing sugar contents were higher in healthy leaves of susceptible genotypes (7.0, 4.6 and 2.4 mg/g respectively) than resistant ones (5.3, 3.7 and 1.6 mg/g, respectively). Decrease in sugar content was at higher rates in susceptible varieties compared to resistant and moderately resistant varieties. Total phenol (0.9 mg/g) and OD phenol (0.3 mg/g) were higher in amounts in healthy leaves of resistant genotypes than susceptible (Total phenols: 0.5 mg/g and OD phenols: 0.3 mg/g). So, it is clear that the infection of *C. truncatum* causes drastic alternations in amounts of biochemicals in soybean crop.

INTRODUCTION

The plant pathogen *Colletotrichum truncatum* (Schw.) is most common species recorded on oil seed cum leguminous soybean crop (*Glycine max* L. Merrill.) causing more than 30 per cent yield losses (Khan and Sinclair, 1992 and Mittal *et al.*, 1993). The common biochemical constituents like chlorophyll, sugars and phenols are important in imparting resistance to the crop plants. But almost most all living animals and plant show biochemical changes after infected by infectious agent (in Fishes by Mahananda *et al.*, 2010 and in trees by Bora and Joshi, 2013). Sometimes, host plant is induced to synthesize these compounds upon infection. The biochemicals *viz.*, phenols, sugars, chlorophyll etc. are known to play imperative role in susceptibility and or resistance against biotrophic, hemi-biotrophic and necrotic plant pathogens (Sindhan and Jaglan, 1987, Saharan, *et al.*, 2001 and Hasabnis *et al.*, 2004). Such biochemical changes reported by many workers in different plants by different plant pathogens (Sindhan *et al.*, 1987, Bashan 1986, Amresh and Nargund 2001, Gupta *et al.*, 1990, Sindhan and Jaglan 1987, Saharan *et al.*, 2001, Hasabnis *et al.*, 2004 and Waghmare *et al.*, 2012) but there is a little reports seems to be available for biochemical changes in soybean crop infected with plant pathogen *Colletotrichum truncatum*.

Hence, present investigation was undertaken with the objective to study the different biochemical changes in soybean, infected with *Colletotrichum truncatum* (Schw.). In this manoeuvre several biochemicals from the foliage of two soybean cultivars were estimated and analysed.

MATERIALS AND METHODS

Present study was carried out at department of Plant Pathology, College of Agriculture, V.N.M.K.V., Parbhani during year 2012-13. Healthy and diseased soybean leaf samples of the resistant Cv. MAUS-71 and susceptible Cv. JS-335 were selected on the basis of their reaction to the *C. truncatum* (Schw.) from screening nursery as per scale given by Mayee and Datar (1986). Further, biochemical constituents *viz.*, chlorophyll (a, b and total chlorophyll), sugars (reducing, non reducing and total) and phenol (total and OD) were estimated from the foliage of soybean cultivars JS-335 (susceptible) and MAUS-71 (resistant) applying standard and scientific protocols as mentioned bellow

Estimation of reducing sugars is carried out by Benedict's method given by Plummer, (1988). Estimation of chlorophyll is carried out according to method given by Hitchcook, (1979). Quantitative analysis of chlorophyll was done by using DMSO (Dimethyl sulphoxide) in the test tube. The optical density of coloured extracted solution was measured at 645 nm, 652 nm and 663 nm for chlorophyll a, chlorophyll b and total chlorophyll, respectively. The chlorophyll a, chlorophyll b and total chlorophyll were estimated using the formulae as given below.

$$\text{VChlorophyll a} = \frac{(12.7 \times \text{OD at 663 nm}) - (2.69 \times \text{OD at 645 nm}) \times V}{1000 \times W}$$

$$\text{Chlorophyll b} = \frac{(22.9 \times \text{OD at 645 nm}) - (4.68 \times \text{OD at 663 nm}) \times V}{1000 \times W}$$

$$\text{Total Chlorophyll} = \frac{(\text{OD at 652 nm}) \times V}{1000 \times W}$$

Where, V = Volume, W = Weight

Phenol was estimated by following Folin –Ciocalteau Reagent. The orthodihydroxy (O.D.) phenols were estimated using Arnow's reagent (Mahadevan, 1966) method. The O. D. phenols were calculated in terms of catechol in μg / g of tissue from standard curve of Chlorogenic acid.

RESULTS AND DISCUSSION

Experimental results obtained in present investigation revealed the significant variations in resistant and susceptible cultivars of soybean (Table No.1 & Fig. No.1) for different biochemical constituents as discussed below.

Chlorophyll (chlorophyll a, b and total)

Results revealed that chlorophyll (a, b and total) content of both the soybean cultivars, *viz.*, JS-335 (susceptible) and MAUS-71 (resistant) were significantly varied in the healthy and diseased foliage. MAUS-71, healthy foliage contained higher i.e. 0.8, 0.7 and 1.7 mg/g of chlorophyll a, chlorophyll b and total chlorophyll, respectively. Whereas, JS-335, healthy foliage contained comparatively minimum amount of chlorophyll a (0.7 mg/g), b (0.4 mg/g) and total (1.3 mg/g). In the healthy foliage of both the cultivars chlorophyll content was found to be more than its diseased counterpart.

Further, in diseased foliage of both the soybean cultivars, the chlorophyll (a, b and total) content was reduced drastically, but at higher rate in susceptible JS-335 than resistant variety MAUS-71. This reduction in chlorophyll content of susceptible cultivar may be due to death of leaf tissues caused by infection of *Colletotrichum.*

However, the diseased foliage of JS-335 contained comparatively reduced amount of chlorophyll a (0.3 mg/g), b (0.1 mg/g) and total (0.5 mg/g). On the contrary, diseased foliage of MAUS-71 contained comparatively maximum amount of chlorophyll a (0.8 mg/g), b (0.5 mg/g) and total (1.5 mg/g). It may be cause of either necrosis of leaf vein, leaf rolling, petiole canker or defoliation occurred in susceptible variety. The rate of reduction of all three chlorophyll in the diseased foliage of JS-335 was higher to the tune of 55.78, 71.80 and 64.30 per cent, respectively of chlorophyll a, b and total. While, in resistant MAUS-71 the rate of chlorophyll reduction was comparatively minimum to the tune of 4.05, 31.78 and 12.86 per cent, of chlorophyll a, b and total, respectively. This is due to production of biochemicals in ample amount in resistant varieties that imparts the resistance against particular disease. These results of the present study obtained on the foliage contents of chlorophylls (a, b and total) and their reduction thereafter *C. truncatum* infection in both the soybean cultivars were in consonance with the findings of those reported earlier by several workers. Comparatively higher amounts of the chlorophylls (a, b and total) in the *Alternaria* blight resistant cultivars of cluster bean (Saharan and Saharan, 2001) and mustard (Atwal *et al.*, 2004), rust resistant cultivars of wheat (Hasabnis *et al.*, 2004) and anthracnose resistant French bean cultivars (Gupta *et al.*, 2010).

Further post infection disease reductions in the chlorophylls (a, b and total) at higher rates in the foliage of susceptible host plants and comparatively at lower rates in the foliage of resistant host plants were reported earlier by several workers. Drastic reductions in the chlorophylls (a, b and total) due to infection with *A. macrospora* in cotton, *Cercospora* leaf spot in groundnut, *Alternaria* blight in cluster bean, leaf rust in wheat, early blight in tomata and *Alternaria* leaf spot in wheat were reported earlier by Padmanaban and Narayanswamy (1978), Bala and Dhillan (1987), Saharan and Saharan (2001), Hasabnis *et al.* (2004), Mate *et al.* (2007) and Rathod *et al.* (2007), respectively.

Sugar content

The results (Table No. 1) indicated that total sugar, reducing sugar and non reducing sugar contents were higher in healthy leaves of susceptible genotypes than resistant ones. In diseased leaves, their amount was decreased in both resistant and susceptible genotype. But, this decrease in sugar content was at higher rates in susceptible varieties compared to resistant and moderately resistant varieties.

The healthy foliage of JS-335 (susceptible) contained comparatively maximum reducing sugar (4.6 mg/g), non reducing sugar (2.4 mg/g) and total (7mg/g) sugars. Whereas, the healthy foliage of MAUS-71 (resistant) contained comparatively minimum amount of reducing sugar (3.70 mg/ g), non reducing sugar (1.6 mg/g) and total (5.3 mg/g) sugars than susceptible variety JS-335. It may be due to some aspects

Table 1: Biochemical variations in soybean variety susceptible and resistant to *C. Truncatum*

Biochemicals	JS-335		Per cent increase ordecrease over healthy	MAUS-71		Per cent increase ordecrease over healthy
	H	D		H	D	
Chlorophyll a (mg/g)	0.7	0.3	55.8 (-)	0.8	0.8	4.1 (-)
Chlorophyll b (mg/g)	0.4	0.1	71.8 (-)	0.7	0.5	31.8 (-)
Total Chlorophyll (mg/g)	1.3	0.5	64.3 (-)	1.7	1.5	12.9 (-)
Reducing sugar (mg/g)	4.6	4.2	8.9 (-)	3.7	3.4	8.1 (-)
Non reducing sugar (mg/g)	2.4	2.2	5.9 (-)	1.6	1.5	5.7 (-)
Total sugar (mg/g)	7.0	6.4	7.9 (-)	5.3	4.9	7.2 (-)
Total phenols (mg/g)	0.5	0.6	19.2 (+)	0.9	1.1	25.5 (+)
OD = phenols (mg/g)	0.3	0.4	21.9 (+)	0.7	0.9	25.3 (+)

Note:- H = Healthy leaf sample, D = Diseased leaf sample

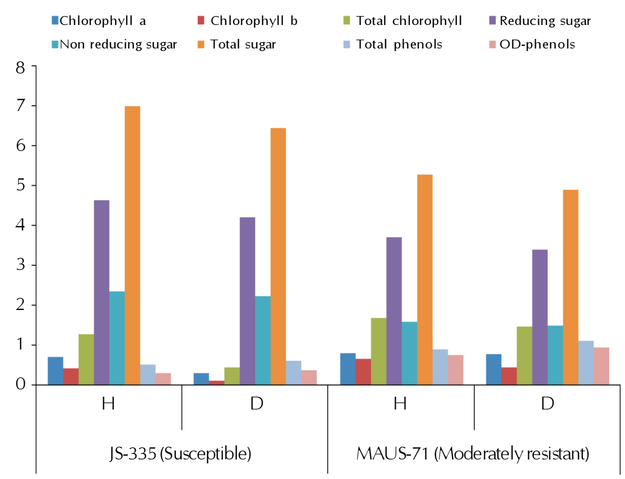

Figure 1: Biothemical variations in soybean variety susceptible and resistant to C. truncutm

of biochemical defence mechanisms, operating in the resistant host.

The disease infection causes the decrease in contents of all of three sugars significantly in the diseased foliage of both soybean cultivars tested. The diseased foliage of JS-335 (susceptible) contained reducing sugar (4.2 mg/g), non reducing sugar (2.2 mg/g) and total (6.4 mg/g), whereas, the diseased foliage of MAUS-71 contained reducing sugar (3.40 mg/g), non reducing sugar (1.5mg/g) and total (4.9 mg/g) sugar. The rate of decrease in these sugars (reducing, non reducing and total) was higher in susceptible JS-335 than resistant MAUS-71. The diseased foliage of susceptible JS-335 contained about 8.9, 5.9 and 7.9 per cent lower reducing, non reducing and total sugars, respectively over its healthy counterpart. While resistant MAUS-71 diseased foliage contained about 8.10, 5.7 and 7.2 per cent lower reducing, non reducing and total sugars, respectively over its healthy counterpart. It may be the cause of biochemical chemical change occurred due to infection by pathogen. So, form this study it can be clear that low amount of carbohydrates in resistant genotypes may be responsible for resistance of genotypes against C. truncatum.

These results concerned with higher amount of sugars (reducing, non reducing and total) in the foliage of susceptible Cv. JS-335 than that of resistant MAUS-71 are in agreement with the earlier findings of many workers. Sindhan and Jaglan (1987) and Sindhan *et al*. (1987) reported lower amounts of sugars (reducing and total) in *Cercospora* resistant groundnut cultivars as compared to the susceptible one. Suryawanshi *et al*. (1993), also reported higher amounts of sugars (reducing and total) in *P. personata* susceptible groundnut Cv. JL-24. Similarly, Waghmare, *et al*. (2012) reported that the plant infected with leaf spot of rose caused by *Alternaria alternate* showed a decrease in the quantity of total sugar and reducing sugar compared to the healthy plant. So, present study revealed that the low amount of carbohydrates in resistant genotypes imparts resistance against *C. truncatum*.

Phenols

Phenols also play an important role in imparting disease resistance. In the present investigations, both total phenol and ortho-dihydroxy phenols were estimated. The present study indicated that healthy leaves of resistant genotypes contained higher amount of total phenol and OD phenol than susceptible. In diseased leaves their amount increased in both the genotypes.

The healthy foliage of resistant soybean Cv. MAUS-71 contained higher amounts of total (0.90 mg/g) and OD (0.7 mg/g) phenols than susceptible Cv. JS-335 (Total phenols: 0.5 mg/g and OD phenols: 0.3 mg/g). The infection of *C. truncatum* accumulated/ increased total as well as OD phenols in the diseased foliage of both the soybean cultivars. However, there was higher accumulation of total (1.1 mg/g) and OD (0.9 mg/g) phenol, in the diseased foliage of resistant soybean Cv. MAUS-71, whereas, there was comparatively lower accumulation of total (0.6 mg/g) and OD (0.4 mg/g) phenols in the diseased foliage of susceptible variety JS-335. It indicates that presence of maximum amount of phenol and OD phenol in plant part at the time of infection may give resistance against infection. The rate of increase in total (25.47%) and OD (25.33%) phenols were higher in resistant Cv. MAUS-71, the susceptible Cv. JS-335 (Total phenols: 19.23% and OD phenols. 21.87%).

These findings are in conformity with those reported earlier by many workers. Waghmare, *et al*. (2012) reported that the rose plants infected with leaf spot of rose showed significant increase in phenol content as compared to the healthy plant. Similarly, higher amounts of phenols were recorded for leaf spot resistant in groundnut cultivars by Gupta *et al*., (1985), Sindhan and Jaglan (1987) and Sindhan *et al*. (1987); in cotton Cv. Akala for *Alternaria* resistance by Bashan (1986); in mustard cultivars for *Alternaria* blight tolerance by Gupta *et al*. (1990), Singh *et al*. (1999), Saxena and Prasad (2003) and Atwal *et al*. (2004); for *A. helianthi* resistance in sunflower hybrids MSFH-17 and KBSH-1 by Amresh and Nargund, (2001); for rust resistance in groundnut cultivars by Sunkad and Kulkarni, (2006), for *A. solani* resistance in tomato cultivars by Mate *et al*. (2007) and for anthracnose resistance in French bean cultivars by Gupta *et al*. (2010) were reported earlier, which supports the findings.

So, from the present findings it can be concluded that there were significant reductions in chlorophylls (a, b and total) and sugars (reducing, non reducing and total) at higher rate in susceptible soybean Cv. JS-335 than that of resistant Cv. MAUS-71. While, phenols (total and OD) were increased at higher rate, respectively in susceptible Cv. JS-335 and resistant MAUS-71. It signifies that the infection of *C. truncatum* causes drastic alternations in amounts of biochemicals present in crop like soybean. So, there is need of development of such cultivars which will exhibit least changes in biochemical components after disease incidence.

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