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Screening of cultivars/genotypes for anthracnose resistance and estimation of biochemical factors of resistance in bitter gourd (*Momordica charantia*)

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Abstract
Bitter gourd (*Momordica charantia*) also known as Balsam pear or bitter melon is one of the most important vegetable crop grown in India including Konkan region of Maharashtra. Among various diseases of bitter gourd anthracnose caused by *Colletotrichum lagenarium* is the most destructive disease of bitter gourd. Screening of fifteen promising genotypes against anthracnose disease under field condition revealed that, Co-Long, PBIG-1, RHRBG-5, DVBTG-1, Hirakani, PBIG-3 and ARBTH-1 showed moderate resistance, PBIG-2, Konkan Tara, Preeti, BG-2, BG-1 and Co-1 were susceptible while only one variety RHRBG-4-1 showed high susceptibility. The biochemical studies revealed that chlorophyll 'a', 'b' and total chlorophyll were present in higher amount in moderately resistant cultivars than susceptible and highly susceptible ones. The rate of decrease was found higher in highly susceptible than susceptible varieties. Total sugars, reducing sugar and non-reducing sugar contents were found higher in moderately resistant varieties than susceptible and highly susceptible cultivars whereas, in diseased leaves their amount decreased in all the varieties. In case of phenol content, higher amount of total phenol and ortho-dihydroxy phenol was found in healthy leaves of moderately resistant genotypes than susceptible varieties. In diseased leaves, their amount increased in both the genotypes.

Keywords: Bitter gourd, ortho-dihydroxy phenol, *Colletotrichum*, chlorophyll

Introduction
Bitter gourd (*Momordica charantia*) also known as Balsam pear or bitter melon is one of the most important vegetable crop grown in India including Konkan region of Maharashtra. Bitter gourd is susceptible to many fungal and viral diseases like downy mildew, powdery mildew, anthracnose, watermelon mosaic and other cucurbit viruses. Among such diseases anthracnose caused by *Colletotrichum lagenarium* is the most destructive disease of bitter gourd and other cucurbits grown in warm season with frequent rains. Early infection of the disease may lead to premature fruit dropping and malformed fruits. Cankered fruits are also vulnerable to the attack of many other soft rotting fungi. Such diseased fruits loose market value. Yield losses of marketable fruits up to 99.5 per cent have been reported Ullasa and Amin, 1986) [14]. In Konkan region of Maharashtra, bitter gourd also known to suffer regularly due to the anthracnose disease causing huge loss of produce. Considering the destructive nature of the pathogen and importance of the disease, the present investigation was conducted.

Materials and Methods
Screening of bitter gourd cultivars/genotypes against anthracnose
A field experiment was conducted at Central Experiment Station, Wakawali to know the resistance levels in the promising cultivars during *kharif*, 2008-09 and 2009-10. Fifteen promising cultivars/genotypes of bitter gourd were evaluated. The experiment was conducted with randomized block design with three replications. The anthracnose severity was recorded by following 0-9 scale of Phytopathometry (Mayee and Datar, 1986) [10]. Further, the per cent disease index was calculated at 30 days interval with the above scale using the formula of Wheeler (1969) [15].

Estimation of biochemical factors of resistance

Effect of anthracnose of bitter gourd on biochemical constituents *viz.,* chlorophyll, sugars and phenols was determined both in healthy and diseased leaves. For this, two moderately resistant (Co-long and RHRBG-5), two susceptible (Preeti and Konkan Tara) and one highly susceptible (RHRBG-4-1) cultivars were selected. The healthy as well as diseased leaves of plants were tested for biochemical constituents under study.

a) Estimation of chlorophyll content

Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' contents were determined (Arnon, 1949) [1]. Leaf tissue from the middle portion of the plant was cut into small pieces. One hundred mg of sample was homogenized with 80 per cent acetone. The final volume of the extract was made up to 25 ml. The absorbance of the extract was measured at 645, 652 and 663 nm in spectrophotometer. The total chlorophyll, chlorophyll 'a' and chlorophyll 'b' contents were calculated using the following formula.

$$\text{Total chlorophyll} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\text{fr.wt.})$$

$$\text{Chlorophyll (a)} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\text{fr.wt.})$$

$$\text{Chlorophyll (b)} = 22.9\ (A_{645}) - 14.65\ (A_{652}) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\text{fr.wt.})$$

Where**,**

V = Volume of the extract (25 ml)

W = fresh weight of the sample (100 mg)

a = Path length of light (1 cm)

b) Estimation of sugars and phenols

Five grams of leaf material was extracted in ethanol as per the procedure followed by Jaypal and Mahadevan (1968) and clarified with saturated solution of lead acetate. The excess lead acetate was precipitated by the addition of sufficient quantity of standard solution of di-sodium orthophosphate.

The precipitate was removed by filtering the alcohol extract through Whatman No. 1 filter paper and the filtrate was made up to 25 ml volume with 80 per cent alcohol. Reducing sugar, non-reducing sugars, phenols and ortho-dihydroxy phenols were estimated in alcohol extract of fresh leaves.

Sugars

Reducing sugars in leaf samples were estimated by Lane and Eynon method (Lane and Eynon, 1923) [9]. Ten ml of mixed Fehling's solution was poured into each of two 250 ml conical flasks. Fifty ml burette was filled with the titrated solution. The contents of the flask were mixed and heated to boil moderately for 2 min. Then nine drops of methelene blue solution were added without touching it to the side of the flask. The titration was completed within 1 minute by adding 2 to 3 drops of sugar solution at 5 to 10 sec intervals, until the indicator was completely decolorized. At the end point, the boiling liquid assumed the brick-red colour of precipitated cuprous oxide, which it had before the indicator was added. The volume of the solution required was recorded.

Non- reducing sugars were hydrolyzed by using concentrated 5 ml HCL and then estimated as in case of reducing sugars to get total sugars. Non-reducing sugars were calculated by subtracting the reducing sugar from total sugars.

$$\text{a. \% Reducing sugars} = \frac{\text{Mg of Invert sugar X Dilution X 100}}{\text{Titre X Wt. or volume of the sample X 100}}$$

b. % Total sugars as = Calculated as in (a) making the titre value obtained in the invert sugar determination of total sugars after inversion

c. % Sucrose = (% Total invert sugars- %Reducing sugars originally present) X 0.95

d. Total sugars = (% Reducing sugars + % Sucrose)

Total Phenols

Estimation of total phenols present in plant samples was carried out following Colourimetric method. One ml of alcohol extract was taken into 100 ml volumetric flask to which 5 ml Folin-Denis reagent was added followed by 10 ml of Na_2CO_3 solution. When the solution became blue in colour, the distilled water was added to the flask to make up the volume up to 100 ml. The flask was kept in stand for 30 min after shaking and then the intensity of the colour was measured at 760 nm against experimental blank adjusted to 0 absorbency. The amount of phenols present in the sample was estimated from a standard curve prepared from 0 to 10 series of tannic acid.

Ortho-dihydroxy phenols

Arnow's reagent specially reacts with ortho-dihydroxy phenols by producing a pink coloured complex, the intensity of which is measured in a colourimeter. One ml of the alcohol extract was pipetted into a test tube to which 1.0 ml of 0.05 N HCL, 1.0 ml of Arnow's reagent, 10.0 g sodium nitrate ($NaNO_2$) and 10 g of sodium molybdate (Na_2MoO_4) were dissolved in 100 ml distilled water, 10.0 ml of distilled water and 2.0 ml of 1N NaOH were added.

Soon after the addition of NaOH, the content of test tube turned to pink colour. The intensity was read at 515 nm in spectrophotometer. The ortho-dihydroxy phenol content in the unknown samples was determined from the standard curve of catechol.

Results and Discussion

Screening of bitter gourd cultivars/genotypes against anthracnose

Data depicted in table 1 revealed that the terminal (105 DAS) per cent disease intensity of anthracnose in different genotypes/cultivars of bitter gourd screened was ranged from 41.40% (Co-Long and PBIG-1) to 63.00% (RHRBG-4-1). Among 15 cultivars tested for their reactions against anthracnose none of them was either immune or resistant to anthracnose. Eight cultivars *viz.,* Co-Long and PBIG-1 (41.40% each), RHRBG-5 (43.20%), Hirkani (45.00%), DVBTG-1 (45.00), Priya (46.80%), PBIG- 3 (47.70%) and ARBTH-1 (48.60%) found moderately resistant to anthracnose.

Six of the bitter gourd varieties *viz.,* PBIG-2, Konkan Tara, BG-1, Preeti, BG-2, and Co-1 were susceptible with 54.00, 55.80, 55.80, 57.60, 57.60, and 59.40 per cent disease intensity while only one variety RHRBG-4-1 was highly susceptible to anthracnose with 63.00 PDI. These findings are almost similar to those of Jadhav (2007) [6] who screened different varieties of bitter gourd against anthracnose disease under Konkan conditions.

The present studies indicate the possibility of testing moderately resistant varieties for their yield potential so that

the best among them can either be recommended for cultivating in Konkan or may be utilized in resistance breeding programme at university level.

Table 1: Reaction of bitter gourd genotypes/cultivars against anthracnose under field condition

S. No	Variety	Percent disease Intensity						Reaction
		30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	
1	Konkan Tara	13.50	20.70	31.50	37.80	46.80	55.80	S
2	Preeti	14.40	24.30	32.40	41.40	48.60	57.60	S
3	Hirkani	10.80	20.70	32.40	39.60	45.00	45.00	MR
4	Priya	11.70	20.70	30.60	36.00	42.30	46.80	MR
5	CO- 1	15.30	21.60	30.60	37.80	48.60	59.40	S
6	CO-Long	9.90	15.30	18.90	26.10	36.00	41.40	MR
7	BG- 1	18.90	25.20	35.10	41.40	50.40	55.80	S
8	BG-2	16.20	24.30	33.30	39.60	46.80	57.60	S
9	DVBTG - 1	11.70	24.30	32.40	37.80	41.40	45.00	MR
10	PBIG - 2	16.20	23.40	35.10	41.40	48.60	54.00	S
11	RHRBG-4-1	25.20	39.60	45.00	50.40	57.60	63.00	HS
12	RHRBG -5	9.00	18.00	28.80	36.00	39.60	43.20	MR
13	PBIG- 3	10.80	17.10	28.80	37.80	43.20	47.70	MR
14	ARBTH - 1	9.90	18.00	30.60	36.00	42.30	48.60	MR
15	PBIG-1	9.00	18.00	26.10	31.50	35.10	41.40	MR

Estimation of biochemical factors of resistance

Estimation of different biochemical factors *viz.,* chlorophyll, sugars and phenol content was carried out in moderately resistant, susceptible and highly susceptible bitter gourd varieties to compare their variation in healthy and diseased plants of respective varieties.

Chlorophyll Content

Destruction of chloroplast is a common feature of diseased tissues. Disease may reduce photosynthetic rate, phosphorelation and CO_2 assimilation. These changes may be partially or completely accounted by reduction in chlorophyll content. Hence, the rate of chlorophyll loss in bitter gourd crop as influenced by the development of anthracnose was studied. The data pertaining to chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of different genotypes is presented in Table 2.

The data revealed that T3 (RHRBG-5 H) was significantly superior over all treatments. Moderately resistant varieties recorded higher amount of chlorophyll 'a', 'b' and total chlorophyll content than susceptible and highly susceptible varieties. Their concentrations were also observed to be decreased as a result of infection, but the rate of decrease was higher in highly susceptible than in susceptible varieties. In all varieties there was decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content in diseased leaves. The lowest decrease in chlorophyll 'a'content (8.78 per cent) was observed in Co-Long followed by RHRBG- 5 (10.52 per cent). Both these varieties were found moderately resistant. The highest decrease of 18.31 per cent was observed in highly susceptible variety RHRBG 4-1 followed by susceptible varieties, Konkan Tara (14.81 per cent) and Preeti (12.05 per cent). Chlorophyll 'b' content in susceptible and highly susceptible bitter gourd cultivars showed maximum decrease of 40.15, 34.26 and 39.21 per cent chlorophyll content in Konkan tara, Preeti and RHRBG-4-1, respectively.

As the total chlorophyll is the cumulative effect of chlorophyll 'a' and chlorophyll 'b', it was also reduced in the same trend. Its reduction was found to be more in highly susceptible variety, RHRBG-4-1 (25.48%) than in moderately resistant varieties, Co-Long and RHRBG- 5 (9.56 and 15.58%, respectively). These results are in conformity with those of Benagi (1995) [2] who studied leaf spot of groundnut and Kulkarni (2009) [8] who worked on anthracnose disease of green gram.

Table 2: Effect of anthracnose on chlorophyll content (mg/g of fresh weight) in varieties of bitter gourd with different levels of resistance

Treatments	Chlorophyll 'a' (mg/g)	% decrease over healthy	Chlorophyll 'b' (mg/g)	% decrease over healthy	Total Chlorophyll (mg/g)	% decrease over healthy
T1-CO-Long (H)	0.911		0.700		1.611	
T2-CO-Long (In)	0.831	8.78	0.626	10.57	1.457	9.56
T3-RHRBG-5 (H)	1.049		0.864		1.913	
T4-RHRBG-5 (In)	0.939	10.52	0.676	21.73	1.615	15.58
T5-Konkan tara (H)	0.875		0.520		1.395	
T6-Konkan tara (In)	0.746	14.81	0.311	40.15	1.057	24.25
T7-Preeti (H)	0.844		0.517		1.360	
T8-Preeti (In)	0.742	12.05	0.340	34.26	1.082	20.49
T9-HRBG-4-1 (H)	0.861		0.450		1.311	
T10-RHRBG -4-1 (In)	0.704	18.31	0.273	39.21	0.977	25.48
S. Em $\pm$	0.003		0.004		0.002	
CD at 5%	0.012		0.017		0.009	

H-Healthy leaves In- Infected leaves

Sugar Content

The present study revealed the variation in sugar content between healthy and infected bitter gourd leaves.

The data presented in Table 3 indicated that total sugars, reducing sugar and non-reducing sugar contents were higher in moderately resistant varieties than susceptible and highly susceptible ones. In diseased leaves, their amount decreased in all moderately resistant, susceptible and highly susceptible

varieties The lowest rate of decrease in reducing sugar content was observed in diseased leaves of moderately resistant varieties Co-Long (3.13 per cent) followed by RHRBG-5 (4.16 per cent), while highest decrease (12.81 per cent) was observed in highly susceptible variety RHRBG-4-1. Similarly, lowest per cent decrease in non-reducing sugar content (14.05 per cent and 20.90 per cent) was observed in moderately resistant varieties *viz.*, Co-Long and RHRBH-5, respectively as compared to highly susceptible and susceptible varieties. Overall, the decrease in total sugar content was 4.20 and 5.52% in moderately resistant varieties, while it was 15.48, 17.59 and 17.03 per cent in susceptible and highly susceptible varieties, respectively. These results are in conformity with those of Sindhan and Parashar (1996) [12], Sindhan *et al.* (1999) [13] and Kulkarni (2009) [8] who studied leaf spot of groundnut, leaf spot of mungbean and anthracnose of greengram, respectively. The reduction in carbohydrate content after infection may be due to rapid hydrolysis of sugar during pathogenesis through enzymes secreted by pathogens and subsequent utilization by pathogen for their development. The present study revealed that the higher amount of carbohydrates in moderately resistant genotypes may be responsible for resistance in bitter gourd genotypes against anthracnose disease.

Table 3: Effect of anthracnose on sugar content (mg/g of fresh weight) in varieties of bitter gourd with different levels of resistance

Treatments	Reducing sugar (mg/g)	% Decrease over control	Non Reducing sugar	% Decrease over control (mg/g)	Total Sugar (mg/g)	% Decrease over control
T1- CO-Long (H)	0.164	3.13	0.018	14.05	0.182	4.20
T2- CO-Long (In)	0.159		0.015		0.174	
T3- RHRBG-5 (H)	0.143	4.16	0.013	20.90	0.155	5.52
T4-RHRBG-5 (In)	0.137		0.010		0.147	
T5- Konkan tara (H)	0.145	7.90	0.076	29.86	0.222	15.48
T6- Konkan tara (In)	0.134		0.054		0.187	
T7- Preeti(H)	0.161	12.72	0.046	34.53	0.207	17.59
T8- Preeti (In)	0.141		0.030		0.171	
T9- RHRBG-4-1 (H)	0.155	12.81	0.040	33.333	0.195	17.03
T10- RHRBG-4-1 (In)	0.135		0.027		0.162	
S.Em ±	0.001		0.001		0.002	
CD at 5 %	0.01		0.002		0.01	

H-Healthy leaves In-Infected leaves

Total phenols and ortho-dihydroxy phenols

In present studies, both total and ortho-dihydroxy phenols (O.D.) were estimated. The results are presented in Table 4. The study indicated that healthy leaves of moderately resistant genotypes contained higher amount of total and O.D. phenol than susceptible varieties. In diseased leaves their amount increased in both the genotypes. The data revealed that the increase in total phenols was higher in moderately resistant varieties *viz.*, RHRBG-5 (7.37 per cent) and Co-Long-1 (6.88 per cent), but it was lower in highly susceptible variety RHRBG-4-1 (3.57 per cent). Similarly, highest per cent increase in ortho-dihydroxy phenols (4.77 and 4.57) was found in both moderately resistant varieties while, it was only 2.47 per cent in highly susceptible variety.

The results of present investigation on phenols are in agreement with the findings of Gupta *et al.* (1985), Benagi (1995) [2], Sindhan and Parashar (1996) [12] and Sindhan *et al.* (1999) [13], who reported that total phenols increased due to infection by *Cercospora personatum* in resistant varieties as compared to susceptible varieties of groundnut. The higher amount of phenolic compounds in diseased leaves may be due to either enhancement of synthesis or translocation of phenolics to the site of infection or hydrolysis of phenolic glycosides by fungal glycosides to yield free phenols, which helped in arresting the spread of the pathogen (Sharma *et al.* 1983) [11].

A definite correlation exists between the resistance of plants to pathogens and the state of their phenolic complex (Hare, 1966 and Kosuge, 1969) [5, 7]. For realization of their protective action, phenolic compounds must be liberated from inactive forms, since they are precisely in the Free State (Friend, 1979) [4]. Among all the biochemical components in imparting resistance to several plant diseases, high concentration causes an instant lethal action by general tannin effect while, low concentration causes gradual effect on the cellular constituents of the parasite. If the concentration does not occur in toxic level, the inhibition will be obviously slow. Besides, the pathogens readily detoxify low concentrations of the toxicant rather than high concentrations (Dasgupta, 1988) [3]. The present study revealed that high amount of phenolic compounds in moderately resistant genotypes may be responsible for moderate resistance of genotypes against anthracnose of bitter gourd.

Table 4: Effect of anthracnose on phenols content (mg/g of fresh weight) in varieties of bitter gourd with different levels of resistance

Treatments	Total Phenols	% Increase over healthy	Ortho-dihydroxy Phenols	% Increase over healthy
T1- CO-Long (H)	0.950		0.692	
T2- CO-Long (In)	1.015	6.88	0.725	4.77
T3- RHRBG-5 (H)	0.950		0.671	
T4-RHRBG-5 (In)	1.020	7.37	0.701	4.57
T5- Konkan tara (H)	0.940		0.649	
T6- Konkan tara (In)	0.991	5.46	0.669	3.13
T7- Preeti(H)	0.940		0.660	
T8- Preeti (In)	0.990	5.32	0.678	2.78
T9- RHRBG-4-1 (H)	0.860		0.581	
T10- RHRBG-4-1 (In)	0.890	3.57	0.595	2.47
S.Em ±	0.001		0.001	
CD at 5%	0.003		0.003	

H-Healthy leaves In-Infected leaves

Reference

1. Arnon DJ. Copper enzymes in isolated chloroplasts polyphenoloxidase in Beta vulgaris. Pl. Physiol. 1949; 24:1-15.
2. Benagi VI. Epidemiology and management of late leaf spot of groundnut caused by *Phaeoisarioipsis personata* (Berk and Curt.) V. Arx. Ph. D. Thes. Univ. Agric. Sci., Dharwad, Karnataka, India, 1995.
3. Dasgupta MK. Principles of Plant Pathology, Allied Publishers Pvt. Ltd. India, 1988, 470-500.
4. Friend J. Phenolic substances and plant disease. In: Biochemistry of Plant Phenolics (Swin T, Harbone BJ. and Sumere FV. Eds.), Plenum Press, New York, 1979, 557-558.
5. Hare RG. Physiology of resistance to fungal diseases in plants. Botanical Rev. 1966; 32:95.
6. Jadhav DK. Management of anthracnose and root-knot nematode in bittergourd (*Momordica charentia* L.) M.Sc. (Agri.) *Thes.* Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India, 2007.
7. Kosuge T. The role of phenolics in host response to infection. Ann. Rev. Phytopathol. 1969; 9:195.
8. Kulkarni SA. Epidemiology and integrated management of anthracnose of green gram. Ph.D. Thes. Univ. Agric. Sci. Dharwad, Karnataka, India, 2009.
9. Lane JH, Eynon L. J Soc. Chem. Ind, 1923, 42:32.
10. Mayee CD, Datar VV. *Phytopathometry*. Technical Bulletin-I, Marathawada Agricultural University, Parbhani, India, 1986, 146.
11. Sharma SG, Ram Narayan, Sangam Lal, Chaturvedi C. Role of phenolic compounds in resistance of maize to leaf blight caused by Drechslera state of Cochliobolus heterostrophus. Indian Phytopath. 1983; 36(1):43-46.
12. Sindhan GS, Parashar RD. Biochemical changes in groundnut leaves due to infection by early and late leaf spot pathogens. Indian J Mycol. Pl. Path. 1996; 26(2):210-212.
13. Sindhan GS, Indra Hooda, Parashar RD. Sources of resistance to *Cercospora* leaf spot in mungbean and biochemical parameters for resistance. Indian J Mycol. Pl. Path. 1999; 29(1):130-132.
14. Ullasa BA, Amin KS. Trop. Pest Mgt. 1986; 32:277-282.
15. Wheeler BEJ. An introduction to Plant Disease and fungi, John Wiley. Phytopathology. 1969; 22:837-845.