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INVESTIGATION ON THE BIOCHEMICAL BASIS OF RESISTANCE TO *FUSARIUM* WILT IN NEWLY SYNTHESIZED BANANA HYBRIDS

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ABSTRACT

In the present investigation developed ten hybrids were screened under pot culture condition along with their parents and reference cultivars *i.e.*Rasthali and YKM 5. The experiment was laid out in completely Randomised block design (CRD) with two replications. Among the ten hybrids the total phenol content significantly increased compared to control and was recorded highest (47.73 %) in hybrid H 926. In the case of synthetic hybrids, all of them showed increase in proline content upon inoculation and it was found maximum with hybrid H 926 (65.00 %)followed with hybrid H 915 (64.00 %). The peroxidase (PO) activity was found to be at the maximum (68.27 %) with hybrid H 926, closely followed by H-11-23 while hybrid H 902 registered the least increase in PO activity (14.16 %). Hybrid H 926 recorded the maximum increase (30.77 %) in polyphenol oxidase (PPO) activity among all hybrids. On the contrary, H 902 and H-11-23 manifested the least increase in PPO activity.The increase in Phenylalanine ammonia lyase(PAL) activity was very high (48.93 %) with hybrid H 926 while least increase in PAL activity was observed with H 902 and H 923. Hence, among them three hybrids (H 914, H 915, and H 926) were confirmed to wilt resistance while rest of seven were found to be tolerance to *Fusarium* wilt.

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INTRODUCTION

Banana and plantain (*Musa* spp.) are important tropical and subtropical fruits around the world, and also a staple food for more than 400 million people. It ranks as the fourth major crop after rice, wheat and maize and is considered as a poor man's crop in tropical and subtropical countries (Swennen *et al*., 2000; Jain and Swennen, 2004). Although it is grown in about 130 countries of the world, India has become the largest producer of bananas in the world by producing 26.50 million MT from an area of 0.77 million hectares (NHB, 2013-14).

Banana production is threatened by many biotic and abiotic factors and among the biotic factors, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) assumes greater economic importance as the loss is alarming. *Fusarium* wilt was recognised as one of the most wide spread and destructive plant diseases in the history of agriculture and still remains as a major constraint to banana production worldwide. At present, there are no economically viable biological, chemical or cultural measures of controlling *Fusarium* wilt in an infected field (Ploetz, 2006; Buddenhagen, 2009). It is widely accepted that the breeding and selection for disease tolerance or resistance is the most effective and sustainable management option (Buddenhagen, 2009).

India has two known virulent races of Foc, *viz*., race 1 affecting Rasthali, Laden, Red Banana, and Hill Banana *etc*. while race 2 is known to affect only ABB type culinary cultivars.In the present study, 'soil inoculation technique' was employed for screening resistance/susceptibility. The same technique was successfully used earlier by many workers (Das, 2007; Shanker, 2013). Fungal pathogen is capable to penetrate plant roots through invading root epidermal cells directly resulting in cell membrane damage causes the production of reactive oxygen species (ROS) and elicit the production of antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidise (POS) (Kuzniak, 2001). These enzymes would degrade fungal cell wall and inhibit growth and development of pathogen in plant cell (Wu *et al.,* 2008).The activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were found more in resistant and tolerant banana cultivars in both pot culture as well as field studies when compared with susceptible cultivars (Damodaran, 2003). The content of phenols, lignin and activity of peroxidase, polyphenol oxidase, chitinase and phenylalanine ammonia lyase was higher in resistant hybrids (Sankar, 2013). As crop improvement is a continuous programme, recently new hybrids have been identified (Karunakaran, 2010; Sankar, 2013), however, their resistance to Foc is yet to confirmed. In the present study, therefore, a total of ten economic hybrids identified were taken for assessing the biochemical basis of resistance to Foc (race 1).

MATERIALS AND METHODS

The present study was carried out in the Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2013-14. The experiment was laid out in a completely randomized block design(CRD) and replicated twice. The hybrids and their parents were screened for their resistance to *Fusarium* wilt disease by pot culture method. The inoculum was incorporated @ 50 g in each pot at 45 days after planting of suckers in the pots. These observations were recorded from the inoculated and control plants on 90thdays after inoculation of *Fusarium* culture under pot condition.

Estimation of phenols content (µg/g)

The intensity of deep blue colour developed was measured at 660nm wavelength in a Spectrophotometer (Spies, 1955). From the standard graph, amounts of phenol present were calculated and expressed as µg/g /leaf.Arnow method was followed for the estimation of Ortho-dihydroxy phenols (Malik and Singh, 1980).

Estimation of proline content (µmols/g)

500 mg of leaves were extracted with 10 ml of 3% sulpho salicylic acid. Two ml of the extract was taken in a test tube to which 2ml each of acid ninhydrin and acetic acid was added. The contents were kept in water bath for an hour. These contents were transferred to a separating funnel and 4 ml of toluene was added. After shaking for 5 min, the pink coloured solution was collected and colour intensity was measured at 520 nm (Bates *et al.,* 1973) and expressed in ìmols/g.

Enzymes assays

The banana leaf samples were taken and homogenized at the rate of 500 mg per five ml of 0.1M phosphate buffer (pH 6.5). The homogenate was centrifuged for 20 minutes at 10,000 rpm at 4^{o}C. Borate buffer 0.2M (pH 8.7) was used for the extraction of PAL. The supernatant was used as the enzyme extract and the activities were recorded as units min^{-1} g^{-1} fresh weight. Peroxidase activity was analysed spectrophoto metrically (Hartec, 1955). It was assayed using the modified method of Mayer *et al.* (1965). The PAL assay was conducted as per the method described by Ross and Sederoff (1992).

The experiment data were statically analysed as per the method suggested by Panse and Sukhatme (1984). The critical difference was worked out for 5 per cent (0.05) probability.

RESULTS AND DISCUSSION

Effect of *Fusarium* inoculation on biochemical constituents of banana hybrids under pot condition after 90th days of inoculation was recorded. Among the 10 hybrids the increased total phenol content when compared to control was more (47.73 %) with H 926 at 90th DAI. But at 90th days it was more (50.00 %) with H 923 and H-11-18 (44.80 %). In the case of parents also phenol content increased upon inoculation, the phenol content got elevated and it was maximum with cultivar Tongat followed by Ambalakadali(Table 1).Similar finding was observed by Sankar, 2013. The role of total phenol in scavenging of free radical also repoted by Pal, 2013.

Proline content estimated in the leaves depicted that the reference resistant cultivar (YKM 5) was very high 90th DAI whereas, it was trivial with Rasthali, the reference susceptible cultivar (Table 1). In the case of synthetic hybrids, all of them showed increase in proline content upon inoculation and it was maximum with hybrid H 926 and hybrid H 915. H 902

Table 1: Effect on biochemical attributes in banana hybrids and their parents inoculated with Fusarium wilt under pot culture condition

S. No.	Hybrids	(µg/g) content														
		Total phenol			Proline			PO(Peroxidase)			PPO(Polyphenol oxidase)			PAL(Phenylalanine ammonia lyase)		
		C	I	%	C	I	%	C	I	%	C	I	%	C	I	%
1.	H 902	196.0	226.0	15.31	30.12	42.00	39.44	2.19	2.50	14.16	0.19	0.21	10.53	15.93	18.01	13.06
2.	H 906	325.0	400.0	23.08	40.00	60.00	50.00	2.36	3.10	31.36	0.15	0.19	26.67	17.10	20.04	17.19
3.	H 914	245.0	295.0	20.41	20.00	29.00	45.00	2.08	2.85	37.02	0.10	0.13	30.00	18.79	25.63	36.40
4.	H 915	330.0	395.0	19.70	25.00	41.00	64.00	2.08	3.01	44.71	0.11	0.14	27.27	14.57	20.95	43.79
5.	H 916	290.0	350.0	20.69	20.00	30.00	50.00	2.08	2.71	30.29	0.12	0.15	25.00	20.50	24.90	21.46
6.	H 923	200.0	300.0	50.00	40.00	62.00	55.00	2.08	2.90	39.42	0.11	0.13	18.18	17.52	19.73	12.61
7.	H 926	300.0	425.0	41.67	20.00	33.00	65.00	2.08	3.50	68.27	0.13	0.17	30.77	23.40	34.85	48.93
8.	H-11-18	375.0	543.0	44.80	39.00	60.00	53.85	2.29	2.76	20.52	0.08	0.10	25.00	15.25	17.67	15.87
9.	H-11-23	320.0	392.0	22.50	25.00	38.00	52.00	2.08	3.16	51.92	0.12	0.13	8.33	19.10	22.61	18.38
10.	H-11-36	350.0	444.0	26.86	25.32	36.00	42.18	2.08	2.56	23.08	0.09	0.11	22.22	15.70	20.32	29.43
Parents																
1.	Rose	284.9	346.0	21.45	30.30	50.00	65.02	2.40	3.30	37.50	0.12	0.14	16.67	18.95	23.37	23.32
2.	PisangLilin	312.7	365.9	17.01	24.00	30.50	27.08	2.42	3.61	49.17	0.13	0.16	23.08	21.47	29.00	35.07
3.	Anaikomban	279.5	343.1	22.75	24.00	39.00	62.50	2.21	3.22	45.70	0.11	0.13	18.18	17.50	22.51	28.63
4.	Ambalakadali	250.7	360.2	43.68	25.00	36.00	44.00	2.04	2.91	42.65	0.09	0.11	22.22	16.31	25.15	54.20
5.	Erachivazhai	236.5	285.0	20.51	32.00	40.30	25.94	1.88	2.23	18.62	0.19	0.20	5.26	14.48	16.60	14.64
6.	H 516	291.7	355.5	21.87	43.00	49.00	13.95	2.32	3.22	38.79	0.12	0.13	8.33	18.65	23.34	25.15
7.	H 201	290.5	350.3	20.59	30.00	39.00	30.00	2.10	3.10	47.62	0.12	0.14	16.67	16.40	21.70	32.32
8.	H 911	290.5	330.3	13.70	34.00	42.00	23.53	2.90	3.50	20.69	0.13	0.14	7.69	15.40	20.70	34.42
9.	Tongat	290.5	450.3	55.01	29.00	45.00	55.17	2.50	3.90	56.00	0.12	0.16	33.33	12.40	17.70	42.74
10.	Poovan	184.6	225.4	22.10	24.00	29.00	20.83	2.28	3.48	52.63	0.09	0.10	11.11	11.62	14.07	21.08
Reference cultivars																
1.	YKM5	198.0	268.0	35.35	25.00	40.30	61.20	2.31	3.42	48.05	0.13	0.15	12.31	21.70	31.28	44.15
2.	Rasthali	220.0	250.0	13.64	19.00	22.40	17.89	1.54	1.72	11.69	0.06	0.08	34.43	13.24	13.92	5.14
SEd			-	17.54	-	1.43	2.05	-	0.108	0.150	-	-	0.007	-	0.93	1.21
CD (0.05)			-	35.35	-	2.88	4.13	-	0.218	0.303	-	-	0.014	-	1.88	2.45

DAI - Days after inoculation; C - Control; I - Inoculated; % - Per cent difference over control

registered the least increase in proline content.Damodaran (2003) also reported similar higher proline content in resistant cultivars of banana.

The enzyme activity also recorded of banana hybrids under pot inoculation after 90th days. The Peroxidase (PO) activity assessed in the leaves revealed that the reference resistant cultivar (YKM 5) showed the highest increase than the susceptible reference cultivar (Rasthali) (Table 1). Among the 10 hybrids screened the PO activity was found to be at the maximum (68.27 %) with hybrid H 926 90th DAI, closely followed by H-11-23 while hybrid H 902 registered the least increase in PO activity (14.16 %) at 90thDAI. Among the 10 parents evaluated, Tongat recorded the maximum (56.00 %) increase in PO activity closely followed by Poovan at 90th DAI. Erachivazhai recorded the least increase in PO activity at 90th DAI. Association of the peroxidase activity with hybrids resistant to *Fusarium* has been also earlier reported by many workers (Gunavathi, 2000; Krishnamoorthy, 2002; Damodaran, 2003; Kavitha, 2005; Das, 2007). The poly phenol oxides (PPO) activity estimated in the reference cultivar YKM 5 showed that it had maximum increase both at 90th DAI (Table 1), while the minimum increase was observed with susceptible Rasthali.Among the 10 hybrids screened the maximum increase (30.77 %) in PPO activity was observed with hybrid H 926 at 90th DAI. On the contrary, H 902 and H-11-23 manifested the least increase in PPO activity. In case of parents, Tongat expressed higher increase (33.33 %) PPO activity at 90th DAI while Erachivazhai had the least increase in PPO activity at both the stages. In the present study, increase in polyphenol oxidase activity was observed more with resistant genotypes compared to the susceptible genotypes. However, the level of activity was always higher in resistant cultivars indicating their role in the production of phenolic substances. A similar result of increased polyphenol oxidase activity was observed by Gunavathi, 2000; Damodaran, 2003; Kavitha, 2005 and Karunakarn (2010) in banana.

The Phenylalanine ammonia lyase (PAL) activity assayed in the reference cultivar did reveal that resistant cultivar (YKM 5) had higher PAL activity at both the stages while the susceptible cultivar had the least activity (Table 1). Among the 10 hybrids screened, the increase in PAL activity was very high (48.93 %) with hybrid H 926 at 90th DAI while least increase in PAL activity was observed with H 902 and H 923 at 90th DAI. The estimation of this enzyme in the present study revealed that increased activity of PAL was observed in the resistant genotypes compared to susceptible genotypes. Similar increase in phenylalanine ammonia lyase activity was also recorded while screening banana hybrids by Krishnamoorthy and Kumar (2004) and Kumar *et al*. (2009).

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