RESPONSE OF DIPLOID BANANA HYBRIDS AND THEIR PARENTS TO *HELICOTYLENCHUS MULTICINCTUS*

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Summary. The reactions of nine new synthetic diploid banana hybrids and their parental clones to *Helicotylenchus multicinctus* were studied in field trials. H-201, H-02-08, Anaikomban, Ambalakadali, Pisang Lilin and Eraichivazhai were resistant, whilst the hybrids H-204, H-211, H-02-11, H-02-12, H-59, H-65 and H-110 were moderately resistant. Among the resistant hybrids, H-02-08 produced significantly higher bunch weights than their parents. H-203, H-205, H-208 and H-66 were susceptible and H-02-01 and H-89 were highly susceptible to the nematode. The resistant clone H-02-08 had an enhanced reducing sugar content and H-201 and Pisang Lilin had enhanced contents of orthodihydric phenol and activities of phenylalanine ammonia lyase.

Attack by plant parasitic nematodes is one of the major biotic stresses affecting banana production. Spiral nematodes are found to infest all varieties of banana throughout the tropics and sub-tropics. Among the 17 *Helicotylenchus* species reported to occur on banana, *H. multicinctus* (Cobb) Golden, *H. dihystera* (Cobb) Sher, *H. africanus* (Micoletzky) Andrássy and *H. erythrinae* (Zimmermann) Golden have been reported to be the most important pests of banana, causing severe economic loss. In sub-tropical regions, *H. multicinctus* can be the major nematode problem (McSorley and Parrado, 1986), such as in Israel and Taiwan. In India, it causes decline of banana and is responsible for c. 34 per cent loss of yield (Rajendran *et al*., 1980). The control of this nematode largely depends on the use of carbofuran, a carbamate. However, the continuous use of nematicides leads to soil and air pollution, thus making the development of cultivars of banana resistant to the nematode a sound alternative. In this study, new synthetic banana hybrids were screened under field conditions to test their reactions to the spiral nematode and to relate the reactions of the hybrids and parents to biochemical differences.

MATERIALS AND METHODS

Before starting the experiment, the site was planted with *Musa* AAB cv. Nendran, to ensure a high population density of *H. multicinctus*. This crop was removed before planting the experiment. Suckers of nine diploid banana hybrids and ten parental clones (Tables I, II) were planted at spacings of 1.8 m between rows and 1.8 m between plants.

The nematode infestation level of the field (at TNAU, Coimbatore) at the time of planting the experiment was one specimen of *H. multicinctus* per gram of soil. No measures were adopted to control the nematodes. All the hybrids and parent clones were grown in the field till the harvest of fruit bunches. As the maturation periods of the hybrids and parent clones varied widely, the populations of *H. multicinctus* in the soil and in roots at flowering and harvest and also in the soil at planting were determined. Two hundred cm^3 of soil and 15 g of root were collected from a cubic sample (30 cm × 30 cm × 30 cm) of top soil taken 25 cm from each mother plant corm (Cabrales, 1995). The roots were washed free of soil, cut into small pieces and blended in water three times for 10 s with 5 s intervals. The mixture was poured through a series of 250 - 106 - 40 µm pore sieves and the sieves were rinsed with tap water. Nematodes remaining on the 40 µm pore sieve were collected in a beaker and those in 10 ml aliquots of each sample were counted using a binocular microscope. Nematodes were extracted from 200 cm^3 soil by Cobb's wet sieving and sedimentation technique and counted. The reproduction factor (RF) (Final soil and root nematode population/initial soil nematode population) was calculated for each hybrid and parent clone and, based on this, the plant reaction type was defined.

The biochemicals *viz.,* orthodihydric phenol, bound phenol, total sugar, tannin, and phenylalanine ammonia lyase activity in the root were estimated at flowering. The procedures and methods used are given below.

For estimation of orthodihydric phenols, root samples of 1 g each were chopped into small pieces and plunged into 80% ethanol, which was kept in a water bath at 60 °C for 10 minutes and then cooled in running tap water. The tissues were crushed thoroughly in a pestle and mortar. The macerate was squeezed through two layers of cheesecloth and the extract was collected in a beaker. The residue was again extracted with 80% ethanol at 60 °C and squeezed through the cheesecloth. The two extracts were pooled. This extract was then

Table I. Parentage of new hybrids and parental clones.

Name	Parentage	Ploidy	Genome
Hybrids			
H-02-01	Ambalakadali × Anaikomban	2x	AA
H-02-08	H-201 × Eraichivazhai	2x	AB
H-02-11	H-201 × H-110	2x	AB
H-02-12	H-201 × H-110	2x	AB
H-203	H-59 × Ambalakadali	2x	AA
H-204	H-65 × Pisang Lilin	2x	AA
H-205	H-66 × Ambalakadali	2x	AA
H-208	H-89 × Pisang Lilin	2x	AA
H-211	H-201 × Pisang Lilin	2x	AA
Parents			
H-59	Matti × Anaikomban	2x	AA
H-65	Matti × Pisang Lilin	2x	AA
H-66	Matti × Anaikomban	3x	AAA
H-89	Matti × Namarai	2x	AA
H-110	Matti × Tongat	2x	AA
H-201	Bareli Chinia × Pisang Lilin × Robusta	2x	AB

Table II. Characters of parent cultivars.

Cultivar	Ploidy	Genome	Characters
Ambalakadali	2x	AA	Resistant to nematodes and *Fusarium* wilt, susceptible to Sigatoka leaf spot, male fertile, produces small bunches.
Anaikomban	2x	AA	Resistant to nematodes and *Fusarium* wilt, susceptible to Sigatoka leaf spot, male fertile, produces small bunches.
Bareli Chinia	2x	ABB	Highly resistant to nematodes, susceptible to Sigatoka, female fertile.
Eraichivazhai	2x	AA	Resistant to nematodes and *Fusarium* wilt, susceptible to Sigatoka leaf spot, male fertile, produces small bunches.
Matti	2x	AA	Medium bunch weight, female fertile, male sterile, highly susceptible to nematodes, resistance to Sigatoka.
Namarai	2x	AA	Susceptible to nematodes and Sigatoka, Resistant to *Fusarium* wilt.
Pisang Lilin	2x	AA	Dwarf, Resistant to nematodes, Sigatoka leaf spot and *Fusarium* wilt, highly male fertile, female sterile, produces small bunches.
Robusta	3x	AAA	Resistant to Fusarium wilt, highly susceptible to Sigatoka and nematodes, female sterile, male fertile.
Tongat	2x	AA	Highly resistant to nematodes, resistant to Sigatoka and *Fusarium* wilt diseases.

Table III. Reproduction of *Helicotylenchus multicinctus* on nine diploid banana hybrids and their ten parents measured at flowering stage under field conditions.

Hybrids and Parents	Nematodes per 1 g of root		Reproduction factor		Months to harvest		Bunch weight (kg)		Host reaction*
Hybrids									
H-203	76	e	2.88	c	15.0	c	1.8	a	S
H-204	33	b	1.70	b	12.0	ab	11.9	d	MR
H-205	84	f	4.08	d	18.0	d	5.10	bc	S
H-208	43	b	2.47	c	12.5	b	6.8	bc	S
H-211	16	b	1.75	b	12.0	ab	9.6	c	MR
H-02-01	14	a	5.12	de	9.9	a	6.0	bc	HS
H-02-08	17	b	0.82	ab	12.6	b	8.8	c	R
H-02-11	14	a	1.35	b	9.0	a	5.4	bc	MR
H-02-12	17	ab	1.33	b	10.0	a	4.3	b	MR
Parents									
Ambalakadali	10	a	0.68	ab	11.4	ab	12.5	d	R
Anaikomban	10	a	0.62	ab	10.0	a	6.8	bc	R
Eraichivazhai	11	a	0.88	ab	12.6	b	5.8	bc	R
Pisang Lilin	9	a	0.57	ab	10.0	a	4.3	b	R
H-59	65	d	1.99	b	15.9	c	9.2	c	MR
H-65	46	c	1.85	b	17.4	d	3.6	b	MR
H-66	84	f	4.81	d	10.0	a	5.2	bc	S
H-89	126	g	6.92	e	13.2	bc	8.7	c	HS
H-110	18	ab	1.28	ab	11.2	ab	3.4	b	MR
H-201	8	a	0.28	a	9.6	a	1.6	a	R

* R: Resistant, MR: Moderately resistant, S: Susceptible, HS: Highly susceptible.
Original data are presented, but data of nematode numbers were transformed to $\log_{10}(x+1)$ for statistical analysis. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to the Tukey HSD test.

centrifuged at 4700 g for 20 minutes and the supernatant was collected and allowed to evaporate to dryness. The residue left was dissolved in 10 ml distilled water. Three ml of the extract was allowed to react with Arnow's reagent and the flesh colour developed was read at 515 nm (Sadasivam and Manickam, 1997). The results were expressed in mg/g of root as catechol equivalents.

For estimation of bound phenol, 1 g of banana root was ground with a pestle in a mortar containing 5 ml of 3% sodium lauryl sulphate. The contents of the mortar were transferred to a centrifuge tube and centrifuged at 2000 g for 5 minutes. The supernatant was discarded and the residue was washed successively, once with 5 ml of three per cent sodium lauryl sulphate, twice with 5 ml of water, twice with 5 ml of ethanol and twice with 10 ml of diethyl ether. After each washing the mixture was centrifuged and the supernatant discarded. The residue was then dried and suspended in 3 ml of 0.5M NaOH and incubated for 12 hours at room temperature. Afterwards, it was centrifuged and the supernatant was collected. One ml of the released phenolics was diluted to 8 ml with 0.5M NaOH. The colour intensity was measured at 290 nm (Chattopadhyay and Samadar, 1980). The concentration of bound phenols was expressed as mg/g of root as Folin-Ciocalteu reagent equivalents.

For estimation of total sugars, root samples of 2 g each were placed in a boiling tube in 5 ml of 2.5N HCl and hydrolysed by holding the tube in a boiling water bath for three hours before cooling to room temperature. They were then neutralised with solid sodium carbonate until effervescence ceased, and the volume was made to 100 ml for centrifugation. One ml of the supernatant (extract) and 4 ml of anthrone reagent were placed in a test tube and kept in a water bath at 60 °C for 10 minutes, and the colour intensity was measured at 625 nm after cooling (Sadasivam and Manickam, 1997).

For estimation of tannin, 2 g of roots were ground in 50 ml of methanol, incubated at 30 °C for 24 hours with occasional swirling, centrifuged for 20 minutes at 900 g and the supernatant was collected. One ml of the ex-

tract was reacted with vanillin hydrochloride reagent (8% HCl and 4% vanillin in 1:1 proportion) for 20 minutes. The tannin content was measured in terms of phloroglucinol produced at 500 nm (Robert, 1971)

For phenylalanine ammonia lyase activity, 1 g of banana roots were homogenized with 5 ml of 0.2M borate buffer (pH 8.7) and centrifuged at 4700 g for 20 minutes at 4 °C. The enzyme extract was removed and a reaction mixture of 2 ml was prepared with 0.5 ml borate buffer, 0.2 ml enzyme extract and 1.3 ml distilled water. The reaction was allowed to proceed for one hour at 32 °C and was stopped by the addition of 0.5 ml of 1M trichloroacetic acid. The amount of transcinnamic acid produced was measured at 290 nm and expressed as units min^{-1} g^{-1} fresh weight (Ross and Sederoff, 1992).

The experiment was laid out in a fully randomized design with three replicates of 5 plants for each hybrid and parental clone. Prior to statistical analysis, the numbers of nematodes were converted to Log_{10} (x+1). All the data were subjected to analysis of variance (ANOVA) and means of the parameters were compared using Waller Duncan's K-ratio test at $P < 0.05$. Correlation and regression analyses (Sokal and Rohlf, 1981) were performed to determine linear relationships between hybrids and parents and biochemical traits. A multiple regression predictive model, $Y = a+\Sigma biXi$ was developed to assess the reliability of biochemical traits (Xi) in predicting the numbers of nematodes in roots (non-log transformed) (y). The Durbin-Watson test was used to analyse the residuals of the regression model (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

The reactions of the banana hybrids and parental clones to *H. multicinctus* are given in Tables III and IV. In general, the nematode populations collected from roots of the parents showed significant differences. Pisang Lilin, Eraichivazhai, Anaikomban, Ambalakadali, H-201, H-65 and H-59, which have been reported earlier as resistant types (Sathiamoorthy and Balamohan, 1993), supported significantly lower numbers of nematodes. H-110, H-66 and H-89 supported significantly

Table IV. Biochemical content (µg/g of fresh roots) and phenylalanine ammonia lyase activity (units/min/g of fresh root) in the roots of nine banana hybrids and their parents infested with *H. multicinctus* measured at flowering stage under field conditions.

Hybrids and Parents	OD[@] phenol	Bound phenol	Total sugar	Tannin	Phenylalanine ammonia lyase
Hybrids					
H-203	20 b	0.31 a	25 a	93.0 h	428 b
H-204	25 b	0.35 a	30 ab	35.7 c	690 cd
H-205	98 e	0.71 d	24 a	97.0 h	417 b
H-208	78 d	0.57 bc	67 d	38.0 cd	574 bc
H-211	90 e	0.64 c	80 de	33.6 c	681 cd
H-02-01	3 a	0.22 a	32 ab	53.0 f	351 a
H-02-08	28 b	0.53 bc	68 d	24.6 b	456 c
H-02-11	5 a	0.29 a	40 c	50.5 f	400 b
H-02-12	6 a	0.28 a	47 c	43.6 cd	340 a
Parents					
Ambalakadali	68 d	0.40 b	70 d	38.0 cd	617 c
Anaikomban	76 d	0.42 b	87 de	32.7 c	684 cd
Eraichivazhai	70 d	0.41 b	150 f	26.2 b	802 e
Pisang Lilin	134 f	0.46 b	97 e	16.7 a	1088 f
H-59	54 c	0.57 bc	72 d	44.0 e	615 c
H-65	76 d	0.58 bc	76 d	46.4 e	648 c
H-66	24 b	0.27 a	30 ab	80.8 g	411 b
H-89	30 bc	0.28 a	25 a	88.4 g	421 b
H-110	4 a	0.40 b	33 ab	45.0 e	302 a
H-201	70 d	0.46 b	27 a	14.5 a	712 d

[@] OD = orthodihydric phenol.
Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to the Tukey HSD test.

Table V. Multiple regression analysis between biochemical traits and *H. multicinctus* populations in the roots of banana hybrids and their parents.

Biochemical	Partial regression coefficients (bi)	Standard error	't' test	Correlation (r^2)	Regression coefficient (β)
Total sugar	-653.20	1.378	0.219	-0.445*	0.029
Orthodihydric phenol	0.301	1.098	1.128	-0.301*	0.152
Bound phenol	1.239	0.233	2.270*	-0.284*	0.300*
Tannin	0.529	1.839	7.658*	-0.840*	1.124*

* Significant at P = 0.05

higher numbers of nematodes in their roots. These were reported earlier as susceptible in field conditions (Gowen *et al.*, 1999), as judged from necrosis percentage and nematode populations. Among the hybrids, H-02-01, H-02-08, H-02-11 and H-210 supported significantly fewer nematodes than H-110, H-66 and H-89 (which were reported earlier as susceptible types) and the nematode counts in these hybrids were on a par with those of the resistant parents. The hybrids H-205, H-203, H-208 and H-204 had higher nematode populations than the resistant hybrids and parents and lower populations than the susceptible parents, indicating their susceptibility. Nematode reproduction on H-02-08 was significantly lower than that on H-205, H-203, H-208 and H-204.

Banana hybrids and parental clones H-02-08, H-201, Pisang Lilin, Eraichivazhai, Anaikomban and Ambalakadali were considered resistant to nematodes as the reproduction rate of the parasite was less than one. H-204, H-02-11, H-02-12, H-211, H-110, H-59 and H-65 were moderately resistant, hybrids H-203, H-205, H-208 and H-66 were susceptible (reproduction rate 2.47 - 4.81), and H-02-01 and H-89 were highly susceptible (reproduction rate 5.12-6.92). The resistant hybrids exhibited higher contents of orthodihydric phenol, bound phenol, reducing sugar and phenylalanine ammonia lyase (Table IV), thus confirming that high contents of these biochemicals is an indication of plant resistance to nematodes. Similar results were also reported in banana by Giebel (1982) and Fogain and Gowen (1996). Collingborn *et al.* (2000) and Sundararaju *et al.* (2002) also have recorded higher contents of these biochemicals in the roots of certain banana cultivars.

Bunch weight and crop duration varied significantly among the hybrids and parent clones. The duration ranged from 9 to 18 months in hybrids and 9.6 to 17.4 months in parent clones. Generally, the diploids produce low bunch weights compared to commercial triploid cultivars. Bunch weights were 4.3 kg to 11.9 kg in hybrids and 1.6 to 12.5 kg in parent clones. Among the resistant hybrids, H-02-08 recorded the higher bunch weight of 8.8 kg.

Multiple regression analysis equations (Table V) clearly indicated that all the biochemical traits together accounted for 89.50% (R^2 = 0.800**) of the total variation in the nematode population. Total sugar, orthodihydric phenol, bound phenol and tannin showed positive associations with nematode population build-up but the correlations were only significant for bound phenol and tannin. The partial regression coefficients showed positive associations in ortho dihydric phenol, bound phenol and tannin. The variation in nematode population in the roots was mainly explained by tannin (91.73%) and bound phenol (6.50%).

It can be concluded that the banana hybrid H-02-08 and cvs Pisang Lilin, Anaikomban, Ambalakadali and Eraichivazhai are resistant to *H. multicinctus* and could be utilized in a breeding programme for synthesis of new hybrids.

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