REACTION OF SOME TETRAPLOID BANANA HYBRIDS TO *RADOPHOLUS SIMILIS*

V. Krishnamoorthy, N. Kumar and K. Poornima

Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Summary. The reactions of 18 new synthetic tetraploid banana hybrids and five parental banana cultivars to *Radopholus similis* were studied under field conditions. H-02-19, H-02-21, H-02-22, H-02-23, H-02-36, Pisang Lilin and Eraichivazhai were resistant. Among the resistant hybrids, H-02-21 recorded significantly higher bunch weights over its parents and other hybrids. H-02-30, H-02-35 and Red Banana were susceptible and H-02-32 and Robusta were highly susceptible. Karpooravalli and the remaining hybrids were moderately resistant. The resistant hybrids H-02-19, H-02-21, H-02-22, and H-02-23 and the parent cultivars Pisang Lilin and Eraichivazhai had higher contents of total and ortho dihydric phenol, and higher activities of phenylalanine ammonia lyase and polyphenol oxidase.

Bananas and plantains (*Musa* spp.) are important staple food crops in tropical countries. India is the largest producer of bananas (16.81 million tonnes per annum), which are cultivated on about 0.49 million hectares. Plant parasitic nematodes are one of the major biotic stresses affecting banana production. Among them, the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, is the most important and is widely distributed in the banana growing regions of India (Rajendran *et al.*, 1980). Chemical control is widely used to manage this nematode, although nematicides are dangerous and expensive. Screening of banana germplasm revealed that most of the diploid clones, both cultivated and wild, were less damaged by the nematode, but they yield poorly (Pinochet, 1988). Most banana cultivars are triploid and very susceptible to nematodes. In banana breeding, the inheritance of the bunch characters of the parents is difficult to achieve when developing triploid clones but it is easier with tetraploid bananas. Hence, an attempt was made to obtain tetraploid bananas at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu state, India, and, as a result, many tetraploid hybrids were developed (Krishnamoorthy, 2002), with their selection based on yield performance and fruit quality. However, the behaviour of these hybrids in the presence of *R. similis* was not known. Therefore, a study was undertaken to asses the pathogenicity of the nematode on eighteen banana hybrids and five parent cultivars and to relate the hybrid and cultivar reaction to the nematode with certain aspects of their biochemical make-up.

MATERIALS AND METHODS

The study was undertaken under field conditions. A total of 18 tetraploid banana hybrids was tested. They were obtained by pollinating the female parent Karpooravalli (Pisang Awak, a triploid of the ABB genome group) with the diploid male parent Pisang Lilin (AA) (hybrids H-02-17 and H-02-18), Eraichivazhai (AA) (hybrid H-02-19) or the triploid clone Robusta (AAA) (hybrid H-02-36). The rest of the hybrids were developed from the triploid male clone Red Banana (AAA).

The hybrids and parents were planted at 1.8 m x 1.8 m spacing in a randomised block design with three replications. The field (at Coimbatore) was infested at a rate of one specimen of *Radopholus similis* per gram of soil. No measures were adopted to control the nematodes. The population of *R. similis* in the soil at planting and three months after planting and in the roots at three months after planting was determined. Two hundred cm^3 of soil and 15 g of root were collected from a cubic sample (30 cm x 30 cm x 30 cm) of topsoil taken at a distance of 25 cm from each of the mother plant corms (Cabrales, 1995). The roots were washed free of soil particles, cut into small pieces and blended three times for 10s with 5s 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 with distilled water. Nematodes were counted in 10 ml aliquots of each sample using a binocular microscope. Nematodes were extracted from 200 cm^3 soil by Cobb's wet sieving and sedimentation technique and counted (Sosamma and Koshy, 1985). Oostenbrink's (1966) reproduction factor (RF) (final soil and root nematode population/initial soil nematode population) was calculated for each hybrid and parent cultivar and the plant reaction type was defined on the basis of this factors.

Total phenol and orthodihydric phenol contents and activities of the enzymes polyphenol oxidase and pheny-

lalanine ammonia lyase in the roots were estimated three months after planting. The procedures used are described below.

Estimation of phenols. The root samples (1 g) were chopped into small pieces and plunged into 80 per cent 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 with a pestle and mortar. The extract was squeezed through two layers of cheesecloth and collected in a beaker. The residue was again extracted with 80 per cent hot ethanol and squeezed through the cheesecloth. Both the extracts were pooled together. This extract was then centrifuged, and the supernatant was collected and allowed to evaporate completely. The residue was dissolved in 10 ml distilled water and was used for estimation of the biochemicals.

To estimate total phenols from the ethanol extract, one ml was placed in each of two test tubes and the volumes were made up to 3 ml with distilled water. One ml of Folin Ciocalteau reagent was added to each test tube, followed by the addition of 2 ml of 20% sodium carbonate solution. The tubes were held in a boiling water bath for one minute, cooled and diluted to 10 ml with distilled water. The intensity of the deep blue colour developed was measured at 660 nm wavelength in a spectrophotometer (Sadasivam and Manickam, 1997). From the standard graph, amounts of phenol present were calculated and expressed as mg/g root.

For orthodihydroxy phenol determination, 3 ml of ethanol 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.

Enzyme activity. Activities of polyphenol oxidase and phenylalanine ammonia lyase in the roots were estimated at three months after planting.

For polyphenol oxidase activity, 1 g of banana root was homogenized in 5 ml of 0.1 M phosphate buffer (pH 6.5). The homogenate was centrifuged for 20 minutes at 10,000 rpm (4700 g) at 4 °C. The supernatant was used as the enzyme extract and the activities were measured spectrophotometrically and recorded as units min^{-1} g^{-1} fresh weight (Mayer *et al.*, 1965). Standard reaction mixture contained 1.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.5 ml of the enzyme extract and 0.5 ml of 0.01 N catechol. The changes in the absorbance were recorded at 495 nm at 30 sec interval for 3 minutes.

For phenylalanine ammonia lyase activity, 1 g of the banana root was homogenized with 5 ml of 0.2 M borate buffer (pH 8.7). The homogenate was centrifuged for 20 minutes at 10,000 rpm (4700 g) at 4 °C. The supernatant was used as the enzyme extract and the activities were recorded as units min^{-1} g^{-1} fresh weight. The activity was determined spectrophotometrically by the formation of trans cinnamic acid. The reaction mixture consisted of 0.5 ml borate buffer, 0.2 ml enzyme extract and 1.3 ml of distilled water. The reaction was incubated for 60 minutes at 32 °C and then was stopped by addition of 0.5 ml of 1 M trichloroacetic acid. The amount of trans cinnamic acid produced was determined at 290 nm (Ross and Sederoff, 1992).

RESULTS AND DISCUSSION

The reactions of the banana hybrids and cultivars to *Radopholus similis* are given in Table I. Banana hybrids H-02-19, H-02-21, H-02-22, H-02-23, H-02-36 and cvs Pisang Lilin and Eraichivazhai were considered resistant to the nematode as the reproduction rate of the parasite was less than one. Hybrids H-02-30, H-02-35 and cv. Red Banana were susceptible (reproduction rate 4.27 - 4.68), H-02-32 and cv. Robusta were highly susceptible (reproduction rate 6.02 - 7.89), and the remaining hybrids were moderately resistant. Thus, the tetraploid banana hybrids tested were either resistant or moderately resistant to *Radopholus similis,* except for H-02-30 and H-02-35, which were susceptible, and H-02-32, which was highly susceptible. It is therefore evident that *R. similis* was less pathogenic to most of the tetraploid banana hybrids. Moreover, the reproduction rate of the nematode was less in many of the tetraploid hybrids than in the triploid cultivars (Karpooravalli, Red Banana and Robusta). Among the tetraploid banana hybrids, H-02-21 gave a bunch weight of 18 kg, which was greater than that of the parent cultivar Karpooravalli. Ortiz *et al.* (1995) reported that tetraploid hybrid (FHIA-1) was resistant to *Radopholus similis* and recorded bunch weights greater than the triploid parent, and Afrehnumanah *et al.* (1996) found similar results when they compared the tetraploid hybrid PITA-8 with the female parent Bluggoe (ABB).

The resistant hybrids exhibited higher contents of total phenol and orthodihydric phenol and their respective enzymes polyphenol oxidase and phenylalanine ammonia lyase (Table II), 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).

It can be concluded that the tetraploid banana hybrids H-02-19, H-02-21, H-02-22, H-02-23, and H-02-36 and cvs Pisang Lilin and Eraichivazhai are resistant to *R. similis* populations of Coimbatore. They may or may not be resistant to *R. similis* populations from other locations in the world. These hybrids can be utilized in future breeding programmes for synthesis of secondary triploids. Indeed, the highest yielding hybrids could also be released for cultivation.

Table I. Reaction of the banana hybrids and their parent cultivars to *Radopholus similis*.

Hybrids and parents	Parentage*	No. of *R. similis*/g of root	Reproduction factor	Reaction#	Bunch weight (kg)
Hybrids					
H-02-17	KV X PL	70.0	1.91	MR	14.1
H-02-18	KV X PL	58.2	1.56	MR	12.2
H-02-19	KV X EV	44.2	0.66	R	9.4
H-02-21	KV X RB	9.4	0.34	R	18.0
H-02-22	KV X RB	28.0	0.26	R	9.0
H-02-23	KV X RB	32.4	0.52	R	12.2
H-02-25	KV X RB	54.2	2.00	MR	10.1
H-02-26	KV X RB	58.4	1.42	MR	13.0
H-02-27	KV X RB	68.4	1.84	MR	12.1
H-02-28	KV X RB	74.2	1.99	MR	10.9
H-02-29	KV X RB	74.2	1.67	MR	13.1
H-02-30	KV X RB	78.0	4.68	S	14.2
H-02-31	KV X RB	56.4	2.00	MR	14.0
H-02-32	KV X RB	102.2	6.02	HS	8.4
H-02-33	KV X RB	73.2	1.98	MR	10.0
H-02-34	KV X RB	69.2	1.99	MR	15.0
H-02-35	KV X RB	76.4	4.27	S	12.0
H-02-36	KV X RA	44.2	0.76	R	10.0
Parents					
Pisang Lilin		19.2	0.30	R	3.9
Eraichivazhai		44.5	0.56	R	4.8
Karpooravalli		58.0	2.78	MR	13.3
Red Banana		83.4	4.63	S	8.5
Robusta		102.2	7.89	HS	25.0
[a]S.Ed. ±		3.62	-	-	3.40
[b]C.D. (P=0.05)		7.54	-	-	7.21

[a]Standard error deviation

[b]Critical difference at five degrees of freedom between hybrids and parents.

*KV : Karpooravalli, PL : Pisang Lilin, EV : Eraichivazhai, RB : Red Banana, RA : Robusta

#R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

Table II. Phenol content and enzyme activities (units/min/g fresh weight) in the roots of banana hybrids and parents, in soil infested with *R. similis*.

Hybrids and parents	Parentage*	Total phenol ($\mu g\ g^{-1}$)	OD phenol ($\mu g\ g^{-1}$)	Phenylalanine ammonia lyase	Polyphenol oxidase
Hybrids					
H-02-17	KV X PL	457.3	15.5	343.5	54.7
H-02-18	KV X PL	463.9	16.7	396.0	55.9
H-02-19	KV X EV	489.7	19.3	435.5	58.1
H-02-21	KV X RB	513.9	24.0	470.0	63.8
H-02-22	KV X RB	608.1	26.5	488.0	65.7
H-02-23	KV X RB	505.1	21.2	460.0	58.5
H-02-25	KV X RB	411.3	14.0	317.0	50.7
H-02-26	KV X RB	477.0	18.1	409.0	56.0
H-02-27	KV X RB	461.2	16.0	345.0	55.1
H-02-28	KV X RB	442.2	14.1	324.0	52.7
H-02-29	KV X RB	463.5	16.0	391.0	55.7
H-02-30	KV X RB	361.5	13.0	291.0	46.1
H-02-31	KV X RB	431.5	14.0	321.5	52.1
H-02-32	KV X RB	230.2	8.2	235.0	34.3
H-02-33	KV X RB	456.0	15.0	342.5	54.1
H-02-34	KV X RB	453.4	14.1	340.5	53.6
H-02-35	KV X RB	372.3	13.9	297.0	48.0
H-02-36	KV X RA	489.4	18.5	430.5	57.2
Parents					
Pisang Lilin		811.1	66.8	544.0	81.2
Eraichivazhai		488.7	37.0	430.5	56.2
Karpooravalli		241.9	6.4	216.0	35.3
Red Banana		135.0	1.3	177.0	19.4
Robusta		16.9	1.2	155.5	13.2
[a]S. Ed. ±		39.5	4.1	7.6	2.7
[b]C.D. (P=0.05)		230.2	8.2	235.0	34.3

[a] Standard error deviation

[b] Critical difference at five degrees of freedom between hybrids and parents.

*KV : Karpooravalli, PL : Pisang Lilin, EV : Eraichivazhai, RB : Red Banana, RA : Robusta

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