# SCREENING OF CERTAIN BANANA ACCESSIONS AGAINST *RADOPHOLUS SIMILIS* UNDER FIELD CONDITIONS

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**Summary.** Fifteen diploid and nine triploid banana accessions were screened under field conditions for their reaction to *Radopholus similis*. Among the diploids, Anaikomban, Pisang Lilin, Pisang Jari Buaya and Kunnan were rated resistant, while Vennettu Kunnan and Then Kunnan were rated tolerant. The remaining accessions were rated susceptible. Among the triploids, Yangambi km 5 was rated as resistant and Karpooravalli as tolerant. The triploid varieties Robusta and Red Banana were highly susceptible and the remainder were rated as susceptible.

Banana (*Musa* spp.) is one of the most important fruit crops grown in India and ranks second, next to mango, in area and production. In India, banana production is estimated at 16.91 million tonnes per annum from 0.49 million hectares (Singh, 2002). The burrowing nematode *Radopholus similis* (Cobb) Thorne, the spiral nematode *Helicotylenchus multicinctus* (Cobb) Golden, and the lesion nematode *Pratylenchus coffeae* (Zimmermann) Goodey are considered major threats to banana in several banana growing regions (Gowen, 1996). To a certain extent, *R. similis* can be controlled with chemicals, but these may cause adverse environmental effects and are too expensive for subsistence farming. For a perennial crop like banana, the best strategy for managing nematode pests is by genetic improvement.

The first systematic search for nematode resistance in banana clones was made in Honduras (Wehunt and Edwards, 1965; Wehunt *et al.*, 1978) and, subsequently, Pinochet and Rowe (1978, 1979) reported resistance to *R. similis* in an accession of Pisang Jari Buaya and a diploid hybrid (SH 3142) derived from it. This diploid hybrid has since been used as a breeding line in some of the international breeding programmes (Ortiz *et al.*, 1995). The prospect of producing disease resistant cultivars for Indian farmers has been addressed at Tamil Nadu Agricultural University (TNAU) and a programme for *Musa* improvement using conventional breeding techniques has been in progress since 1949 (Sathiamoorthy and Balamohan, 1993; Gowen *et al.*, 1998a, b). This article describes an evaluation of the responses of 24 banana accessions from the germplasm collection held at TNAU to the burrowing nematode under field conditions.

## MATERIALS AND METHODS

Seven diploid *Musa* AA accessions (including a synthetic hybrid H.59 derived at TNAU), seven diploid *Musa* AB accessions and nine triploid accessions (3 each in *Musa* AAA, AAB and ABB) were drawn from the germplasm bank maintained at the Department of Fruit Crops, TNAU and screened to assess their potential for use in breeding programmes. Screening of the accessions against *Radopholus similis* was conducted in a field naturally infested with >1 specimen of *R. similis*/cm$^3$ of soil. Other nematodes, such as *H. multicinctus* and *P. coffeae* were negligible. Pits of 0.45 m$^3$ size were dug out at 1.8 m × 1.8 m spacing after thoroughly ploughing and leveling the field. Suckers of the 24 accessions were planted in the pits with one sucker per pit. The experiment was laid out in a randomized block design with three replicate blocks. Five plants of each accession were planted next to one another in each replicated block. Standard cultural practices were followed as recommended by TNAU (Anonymous, 1999), but no nematicide was applied.

Soil and root samples were collected from around the plants after 3 months, 5 months, at flowering and at harvest. Nematodes were extracted from 200 cm$^3$ soil by Cobb's sieving and modified Baermann funnel method (Cobb, 1918; Schindler, 1961; Southey, 1986). To extract *R. similis* from the roots, a sub-sample of 5 g of fresh roots was macerated in a blender for 10 seconds. The resultant mixture was poured onto a 60-mesh sieve nested on a 350-mesh sieve. Nematodes retained on the 350-mesh sieve were collected and transferred into modified Baermann funnels and allowed to stand for a day (Flegg and Hooper, 1970). The nematode suspension was collected and the nematodes were counted using a binocular microscope.

The mean nematode population from the observations recorded in soil and root samples after three months, five months, at flowering and at harvest was also calculated for overall comparison.

The extent of nematode damage to roots and corms was assessed following the technical guidelines prescribed by INIBAP (Speijer and De Waele, 1997), which were previously developed by IITA (Speijer and Gold, 1996).

Root damage assessment was done after harvest. Roots were collected from a standard size excavation of 20 × 20 × 20 cm extending outwards from the corm and were divided into dead roots and functional roots. Five functional primary roots at least 10 cm long were selected at random from each genotype in each replication. Scoring of feeder roots assigned a score of 1 if the roots were all healthy, 2 for mostly healthy roots, 3 if roots were mostly dead, and 4 if all roots were dead.

The lengths of the five selected functional roots were all reduced to 10 cm and the roots sliced lengthwise. The percentage of root cortex showing necrosis was assessed in one half of each of the five roots. The maximum root necrosis score given per root half was 20, giving a maximum root necrosis score of 100 (per cent) for all five together.

Corm damage assessment was done after harvest and after thoroughly shaking off all soil and washing the corms with water. The outward half of the corm was assessed for damage after trimming the roots off.

The number of roots showing black-purple lesions around their bases on the selected outward half of the corm was counted. The numbers of small lesions (SL, lesions smaller in diameter than the root bases) and large lesions (LL, lesions of equal or larger diameter than the root bases) were counted and scoring was given as: no lesions, score 0; one small lesion, score 1; several small lesions, score 2; one large lesion, score 3; several large lesions, score 4.

We adopted the orientative scale of plant response to lesion-forming nematodes used earlier by Pinochet (1988) to group the accessions as tolerant, susceptible or resistant. This scale considers a plant to be immune when no lesions are present either on roots or corms. Resistant plants had less than 10% of lesions on roots and less than 1% on corms. When root lesion indices ranged from 10 to 20%, the genotypes were considered tolerant. Susceptible and highly susceptible genotypes had root lesion indices above 20% and 40%, respectively.

Statistical analyses by ANOVA were carried out to compare the soil and root populations of nematodes in the different genotypes. The data was not transformed prior to analyses.

**Table I.** Population densities of *Radopholus similis* in the soil rhizosphere of different banana accessions in the field at different sampling times.

| Genotype | Genome | Nematode specimens in 200 cm$^3$ soil | | | | |
|---|---|---|---|---|---|---|
| | | 3$^{rd}$ month | 5$^{th}$ month | at shooting | at harvest | Mean population of the four samplings |
| *Diploids* | | | | | | |
| Matti (local) | AA | 110 | 260 | 309 | 312 | 247.6 |
| Anaikomban | AA | 60 | 85 | 97 | 110 | 88.0 |
| Ambalakadali | AA | 120 | 275 | 294 | 313 | 250.5 |
| Erachivazhai | AA | 131 | 250 | 286 | 270 | 234.3 |
| Pisang Lilin | AA | 57 | 82 | 99 | 115 | 88.3 |
| Pisang Jari Buaya | AA | 95 | 110 | 142 | 140 | 121.8 |
| Adakka Kunnan | AB | 158 | 250 | 310 | 317 | 258.8 |
| Kunnan | AB | 69 | 89 | 121 | 137 | 104.0 |
| Vennettu Kunnan | AB | 102 | 300 | 318 | 327 | 261.8 |
| Kadali | AB | 160 | 290 | 329 | 345 | 281.0 |
| Poomkadali | AB | 132 | 240 | 299 | 311 | 245.5 |
| Neypoovan | AB | 140 | 267 | 329 | 338 | 268.5 |
| Then Kunnan | AB | 105 | 200 | 240 | 290 | 208.8 |
| Padalimoongil | AB | 140 | 275 | 321 | 342 | 269.5 |
| H-59 | AA | 150 | 270 | 317 | 329 | 266.5 |
| Sed | | 5.449 | 5.958 | 7.835 | 12.141 | |
| CD (0.05) | | 11.163 | 12.205 | 16.051 | 24.872 | |
| CD (0.01) | | 15.061 | 16.467 | 21.655 | 33.556 | |
| *Triploids* | | | | | | |
| Robusta | AAA | 210 | 350 | 425 | 440 | 356.3 |
| Red banana | AAA | 180 | 310 | 405 | 421 | 329.0 |
| Yangambi km5 | AAA | 53 | 80 | 112 | 121 | 91.5 |
| Rasthali | AAB | 225 | 380 | 421 | 432 | 364.5 |
| Nendran | AAB | 170 | 280 | 339 | 348 | 284.3 |
| Suganthi | AAB | 190 | 260 | 329 | 340 | 279.8 |
| Karpooravalli | ABB | 120 | 212 | 310 | 391 | 258.3 |
| Klue Teparod | ABB | 130 | 280 | 319 | 321 | 262.5 |
| Monthan | ABB | 145 | 298 | 320 | 334 | 274.3 |
| Sed | | 2.357 | 4.480 | 2.041 | 4.000 | |
| CD (0.05) | | 4.739 | 9.008 | 4.327 | 8.043 | |
| CD (0.01) | | 6.323 | 12.019 | 5.962 | 10.730 | |

**RESULTS**

Significant differences were observed in the numbers of nematodes in soil from the various banana accessions (Table I). In the diploids, the lowest figures for the mean of the four samples were observed in Anaikomban (88.0) and Pisang Lilin (88.2). The soil populations were also low in Kunnan and Pisang Jari Buaya. The highest mean population was recorded in Kadali (281.0). Among the triploids, the greatest mean nematode population was in Rasthali (364.5) followed by Robusta (356.2), and the lowest was in Yangambi km5 (91.5).

The root population of nematodes differed significantly among the genotypes (Table II). Among the diploids, the mean nematode population was lowest (99.7) in Anaikomban, followed by Pisang Lilin (104.2), and was highest (292.0) in Kadali (Table II). Kunnan (AB) and Pisang Jari Buaya (AA) had comparatively low root populations (117.5 and 128.2, respectively). Among the triploids, all except Yangambi km5 (98.0) had quite large root populations.

Based on the intensity of lesions, roots and corms were graded and levels of resistance were assessed (Table III). Numbers of dead roots in the 20 x 20 x 20 cm excavations ranged between 2 and 12 and functional roots varied from 14 to 27. Among the diploids, there were more dead roots in Kadali and hybrid H-59 (7) and fewer in Pisang Lilin and Then Kunnan (2). Among the triploids, there were more dead roots in Red banana (12) and less in Karpooravalli (3).

The maximum numbers of functional roots were recorded in the diploid cultivar Kunnan (27) followed by Then Kunnan (25), and the least were found in Pisang Jari Buaya. Among the triploids, there were more functional roots in Karpooravalli (26) and less in Suganthi (14). The score for feeder roots varied from 1 to 3 in diploids and triploids.

Root necrosis varied from 4% in Pisang Lilin to 16% in Robusta. The lesion index in roots varied from 10% to 45%. The corm grade ranged between 1 and 4 among the various accessions.

**Table II.** Population densities of *Radopholus similis* in root samples of banana accessions grown in the field.

| Genotype | Genome | Nematode specimens in 5 g of roots | | | | |
|---|---|---|---|---|---|---|
| | | 3rd month | 5th month | at shooting | at harvest | Mean population of the four samplings |
| *Diploids* | | | | | | |
| Matti (local) | AA | 125 | 268 | 309 | 320 | 255.5 |
| Anaikomban | AA | 68 | 97 | 105 | 129 | 99.8 |
| Ambalakadali | AA | 135 | 280 | 305 | 319 | 259.8 |
| Erachivazhai | AA | 136 | 262 | 295 | 309 | 250.5 |
| Pisang Lilin | AA | 90 | 88 | 110 | 129 | 104.3 |
| Pisang Jari Buaya | AA | 105 | 124 | 130 | 154 | 128.3 |
| Adakka Kunnan | AB | 170 | 265 | 320 | 349 | 276.0 |
| Kunnan | AB | 80 | 94 | 135 | 161 | 117.5 |
| Vennettu Kunnan | AB | 110 | 280 | 290 | 299 | 244.8 |
| Kadali | AB | 172 | 300 | 345 | 351 | 292.0 |
| Poomkadali | AB | 135 | 250 | 311 | 320 | 254.0 |
| Neypoovan | AB | 162 | 274 | 330 | 344 | 277.5 |
| Then Kunnan | AB | 102 | 171 | 194 | 215 | 170.5 |
| Padalimoongil | AB | 155 | 286 | 320 | 337 | 274.5 |
| H-59 | AA | 174 | 289 | 325 | 341 | 282.3 |
| Sed | | 4.229 | 2.062 | 3.737 | 3.380 | |
| CD (0.05) | | 8.664 | 4.225 | 7.655 | 6.925 | |
| CD (0.01) | | 11.690 | 5.700 | 10.329 | 9.342 | |
| *Triploids* | | | | | | |
| Robusta | AAA | 259 | 374 | 437 | 456 | 381.5 |
| Red banana | AAA | 209 | 332 | 424 | 435 | 350.0 |
| Yangambi km5 | AAA | 58 | 99 | 110 | 125 | 98.0 |
| Rasthali | AAB | 241 | 350 | 415 | 429 | 358.8 |
| Nendran | AAB | 182 | 294 | 342 | 354 | 293.0 |
| Suganthi | AAB | 180 | 270 | 335 | 344 | 282.3 |
| Karpooravalli | ABB | 101 | 181 | 310 | 342 | 233.5 |
| Klue Teparod | ABB | 148 | 292 | 395 | 317 | 288.0 |
| Monthan | ABB | 157 | 270 | 310 | 322 | 264.8 |
| Sed | | 2.301 | 1.062 | 4.016 | 5.338 | |
| CD (0.05) | | 4.625 | 2.253 | 8.515 | 11.317 | |
| CD (0.01) | | 6.173 | 3.104 | 11.733 | 15.593 | |

**Table III.** Assessment of damage caused by *R. similis* to roots and corms of banana accessions in the field.

| Genotype | Roots | | | Root necrosis (%) | | | | | Total RN% | Corm grade | Root lesion index (%) | Level of resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DR | OK | Grade of FR | 1 | 2 | 3 | 4 | 5 | | | | |
| *Diploids* | | | | | | | | | | | | |
| Matti (local) | 5 | 16 | 2 | 5 | 1 | 2 | 2 | 1 | 11 | 2 | 24 | S |
| Anaikomban | 3 | 19 | 1 | 1 | 1 | 2 | - | 1 | 5 | 1 | 10 | R |
| Ambalakadali | 4 | 18 | 2 | 2 | 2 | 5 | 1 | 1 | 11 | 2 | 35 | S |
| Erachivazhai | 4 | 18 | 2 | - | 2 | 1 | 5 | 1 | 9 | 2 | 24 | S |
| Pisang Lilin | 2 | 16 | 1 | 1 | - | 2 | - | 1 | 4 | 1 | 11 | R |
| Pisang Jari Buaya | 3 | 15 | 2 | 2 | - | 1 | 2 | 2 | 7 | 2 | 19 | R |
| Adakka Kunnan | 5 | 17 | 2 | 2 | 1 | 2 | 2 | 1 | 8 | 2 | 23 | S |
| Kunnan | 4 | 27 | 1 | 1 | - | 1 | 2 | 1 | 5 | 2 | 12 | R |
| Vennettu Kunnan | 6 | 19 | 2 | 2 | - | 2 | 2 | 1 | 7 | 2 | 18 | T |
| Kadali | 7 | 16 | 3 | 5 | 2 | 2 | 2 | 2 | 13 | 3 | 30 | S |
| Poomkadali | 6 | 21 | 2 | 2 | 5 | 2 | 2 | 2 | 13 | 2 | 22 | S |
| Neypoovan | 5 | 18 | 3 | 2 | 1 | 5 | 2 | 1 | 11 | 2 | 25 | S |
| Then Kunnan | 2 | 25 | 1 | 1 | - | 2 | 1 | 1 | 5 | 2 | 10 | T |
| Padalimoongil | 5 | 20 | 2 | 2 | 2 | 2 | 1 | 2 | 9 | 1 | 20 | S |
| H-59 | 7 | 17 | 2 | 2 | 1 | 2 | 1 | 2 | 8 | 2 | 27 | S |
| *Triploids* | | | | | | | | | | | | |
| Robusta | 9 | 21 | 3 | 2 | 5 | 2 | 2 | 5 | 16 | 4 | 45 | HS |
| Red banana | 12 | 23 | 3 | 5 | 1 | 5 | 1 | 2 | 14 | 4 | 42 | HS |
| Yangambi km5 | 4 | 24 | 1 | 1 | 2 | - | 2 | - | 5 | 1 | 12 | R |
| Rasthali | 11 | 18 | 2 | 2 | 2 | 5 | 1 | 5 | 15 | 4 | 34 | S |
| Nendran | 6 | 17 | 2 | 1 | 2 | 2 | 5 | 2 | 12 | 3 | 21 | S |
| Suganthi | 5 | 14 | 2 | 2 | 1 | 5 | 2 | 1 | 11 | 3 | 36 | S |
| Karpooravalli | 3 | 26 | 1 | 1 | 2 | 1 | 1 | - | 5 | 2 | 14 | T |
| Klue Teparod | 4 | 24 | 2 | 5 | - | 2 | 2 | 1 | 10 | 2 | 23 | S |
| Monthan | 6 | 19 | 2 | 1 | 2 | 2 | 2 | 1 | 8 | 2 | 30 | S |

DR = Dead roots; OK = Functional roots; FR = Feeder roots; RN = Root necrosis; HS = Highly susceptible; S = Susceptible; T = Tolerant.

## DISCUSSION

Resistance can be considered as the ability of the plant to suppress development of pests or pathogens, whereas tolerance is the ability of the plant to grow well despite infection by a pathogen (Bos and Parlevliet, 1995). In the present trial, although the diploid cultivars Vennettu Kunnan and Then Kunnan and the triploid Karpooravalli registered lower lesion indices, they were considered tolerant and not resistant because their population levels in the roots were higher. The diploid accessions Anaikomban, Pisang Lilin, Pisang Jari Buaya and Kunnan were rated resistant as they suppressed nematode populations both in the soil and in the roots and had relatively low root lesion indices.

The resistance of Pisang Jari Buaya confirms the resistance reported to a population of *R. similis* in Honduras (Pinochet and Rowe, 1978) and suggests that this clone might have resistance to different populations of this nematode. Kunnan has also been recorded as resistant to *R. similis* and *P. coffeae* (Collingborn and Gowen, 1997; Johnson and Sathiamoorthy, 1999). Roots of this cultivar have relatively high levels of condensed tannins that could account for its resistance to *R. similis* (Collingborn *et al.*, 2000).

Among the triploids, Yangambi km5 was resistant and Karpooravalli was tolerant. Yangambi km5 is a cultivar that was found in Africa and shows good resistance to diseases; its low susceptibility to *R. similis* was reported by Price (1994) and its resistance to populations of *R. similis* and *Pratylenchus goodeyi* Sher *et* Allen in Cameroon was confirmed by Fogain and Gowen (1998). Hitherto, plant breeders have been unable to use this cultivar in improvement programmes.

As *R. similis* is an endoparasite that causes root destruction and corm damage, the data on the root population densities of nematodes were more meaningful than those on soil population denisities. The diploids Anaikomban, Pisang Lilin, Pisang Jari Buaya, Kunnan, Vennettu Kunnan and Then Kunnan, and the triploids Karpooravalli and Yangambi km5, have been selected for the TNAU hybridization programme to be used either as male or female parents either directly or by ploidy manipulations. A form of Pisang Jari Buaya has been used successfully by the FHIA breeding programme. This cultivar also has resistance to the fungus pathogens *Mycosphaerella* spp. and *Fusarium oxysporum* f.sp. *cubense* (Ortiz *et al.*, 1995).

Finally, it would be worthwhile to screen other accessions of Indian collections of bananas to identify more potential sources of resistance that could be used for banana improvement in India and elsewhere.

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