REACTION OF COWPEA GENOTYPES TO THE ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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Summary. Seventy cowpea genotypes were evaluated in two different screen-house pot experiments for their reaction to the root-knot nematode, *Meloidogyne incognita* host race 4. The known resistant cv. New Era (Acc 64298) and the susceptible cv. Ife Brown (Acc 73001) were included as controls. Based on reproduction, root gall and egg mass indices of the nematode, five genotypes [Vita 3, Acc 64298 (cv. New Era), 82D4532CIT'85, IT89KD-288 and TVX2724-01F], showing reproduction factors (RF) of 0.5-0.8, reproduction indices (RI) of 6.7-8.4, gall numbers (GN) of 4-8, gall indices (GI) of 2, egg mass numbers (EN) of 3-6, egg mass indices (EI) of 2, and root galling of 2-5%, were considered resistant. None of the resistant genotypes was superior to the standard control resistant cv. New Era. Two genotypes, IT8D-12228-10 and IT96D-733, with RF of 2.3 and 3.2, RI of 20.1 and 21.4, GN of 6 and 7, GI of 2, EN of 35 and 40, EI of 4 and root galling of 4 and 9%, respectively, were tolerant. Sixty-one genotypes, including the standard susceptible cv. Ife Brown, with RF of 2.3-6.4, RI of 17.6-110.4, GN of 25-98, GI of 3-4, EN of 25-90, EI 3-4 and root galling of 15-50%, were susceptible. The cv. IAR 399-1, with a RF of 0.7, RI of 9.0, GN of 35, GI of 4, EN of 4, EI of 2 and galling of 15%, was considered hyper-susceptible.

Key words: Resistance, screening, *Vigna unguiculata.*

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the world's important grain legume crops, widely grown in Africa, Eastern Europe, Australia, United States, Mediterranean area, Asia and the Caribbean (Padulosi and Ng, 1997). The production and importance of the crop have increased dramatically, reaching a world annual grain yield of 3.7 million metric tonnes from an area of 10.4 million hectares, with an average grain yield of 0.354 metric tonnes/ha (FAO, 2007). Nigeria produces 2.3 million metric tonnes annually on an area of 5.3 million ha (FAO, 2007).

Due to biotic constraints, the average grain yield (0.434 metric tonnes/ha) is poor compared with the potential yield of 2,000 kg/ha (IITA, 1982). Among the biotic constraints to yield is infection by root-knot nematodes, especially *Meloidogyne incognita* (Kofoid *et* White) Chitw. Cowpea yield losses of 59-94% in Nigeria (Ogunfowora, 1976; Olowe, 1981) and 43% worldwide (Sasser and Freckman, 1987) were estimated to be caused by this nematode.

Several workers have searched for resistance in cowpea, with some success, for the management of root knot nematodes in various countries (Fassuliots, 1979; Sasser and Kirby, 1979; Kirkpatrick and Morelock, 1987; Florini, 1997; Ehler *et al.,* 2000; Adegbite *et al.,* 2005).

Combined resistance to the four major root-knot nematodes, *M. arenaria* (Neal) Chitw., *M. hapla* Chitw., *M. incognita* and *M. javanica* (Treub) Chitw., has also been reported in several cowpea cultivars (Hare 1967; Patel *et al*., 1977; Hartman and Sasser, 1985; Onyeigwe and Ogbuji, 1991). Roberts (1982) found 30 cultivars resistant to *M. incognita*, four to *M. javanica* and four to *M. arenaria.*

However, the reaction of a cowpea cultivar also depends on the race of the nematode (Taylor and Sasser, 1978; Sasser, 1979; Witcher and Ogle, 1987). Odihirin (1981) identified the cultivar TVu 857 resistant to *M. incognita* races 1, 2 and 3 and also to *M. javanica.* Olowe (1981) rated the cv. New Era highly resistant to *M. incognita* races 1, 3 and 4 but susceptible to *M. incognita* race 2 and also to *M. javanica*.

In Nigeria, the main crops in the rotation scheme are essentially good hosts for root knot nematodes, thus leading to high nematode populations that can cause severe damage to the succeeding crop. There is, therefore, a need to search for more cowpea cultivars resistant to root knot nematodes as those available may possess traits that are unacceptable to local consumers. A study of the reaction of cowpea germplasm to Nigerian populations of root knot nematodes had not previously been carried out, so a screening was undertaken to evaluate cowpea genotypes for resistance to host race 4 (the most common race in the country according to Olowe, 2004) of *M. incognita* under screen-house conditions.

MATERIALS AND METHODS

Source of the cultivars. Seventy cowpea genotypes were tested (Tables I and II). They were 47 from the International Institute of Tropical Agriculture (IITA),

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Ibadan; twelve from the National Cereals Research Institute (NCRI), Ibadan; three from the Institute of Agricultural Research and Training (IAR & T), Moor Plantation, Ibadan, and eight from the Institute of Agricultural Research, Samaru (IAR), Zaria. They were evaluated for their host reaction to *M. incognita* in 2006 in two separate experiments, each testing 35 cultivars. The resistant New Era and susceptible Ife Brown cvs were included as controls in each test.

Nematode population. Stock pure monocultures of *M. incognita* were prepared from six populations from widely different geographical locations, ranging from forest to savannah vegetation, to encompass the wide variability of the nematode in Nigeria. They had been collected during earlier surveys, and identified as *M. incognita* race 4 based on observation of the perineal pattern and host race testing (Hartman and Sasser, 1985). The identification had been confirmed by experts at North Carolina State University, Raleigh (U.S.A.).

Pure monocultures of each population, derived from single egg masses, were reared on tomato (*Lycopersicon esculentum* Mill.) cv. Ibadan local. Then, ten composite cultures were prepared by taking pieces of infected roots from each of the stock pure population cultures, pooling them together and inoculating ten replicate tomato seedlings of the same cultivar. After 60 days, 25 perineal patterns of randomly selected females were observed from each of the ten composite cultures for confirmation of the species identity. The identification was repeated at the end of experiments.

Heavily galled roots from all ten composite cultures were cut into 1 cm long pieces, and eggs extracted by shaking for 3 minutes in a 0.5% solution of sodium hypochlorite (Hussey and Barker, 1973) in a glass jar. The slurry was then sieved through a 75 µm sieve nested onto a 38 µm pore sized sieve. The catch on the 38 µm sieve was collected in a 2-litre glass beaker, and the egg suspension mixed and diluted with tap water to such a volume to contain 1000 ± 22 eggs per ml of egg suspension, as per five replicate counts.

Inoculation and sowing. Plastic pots of 12.5 cm diameter were filled with steam sterilized loamy sandy soil (1118 ± 26 cm^3 soil) and a 2.5 cm deep hole was made in the surface. The egg suspension was thoroughly mixed with air pump, and a 5 ml aliquot containing 5,000 ± 49 eggs discharged into the hole. Single cowpea seeds of each cowpea genotype were sown into the pots, which were then filled to the brim with the same steam sterilized loamy sandy soil. The pots were placed on benches in screen-house, arranged in a randomized complete block design and watered as required.

Observations. Fifty days after inoculation, the plants were uprooted and gently washed to prevent disintegration of the roots. The roots were then immersed in a 0.15 g phloxine B/l of water solution for 15 minutes (Dickson and Ben Struble, 1966) to stain the egg masses, and then rated for gall index (GI) and egg mass (EI) indices on a 0 to 5 scale (Sasser *et al.*, 1984) and for percentage root galling on a 0 to 10 scale (0 = no galling and 10 = 100% galling) (Kinloch, 1990). The root systems were then cut into 1 cm long pieces and eggs extracted by shaking in a 1% water solution of sodium hypochlorite for 4 minutes (Hussey and Barker, 1973). Then, the eggs were counted, and the reproduction factor calculated (RF = final number of eggs from each cultivar divided by the inoculum density of 5,000 eggs). The reproduction index (RI = number of eggs from each cultivar expressed as a proportion of that from the known standard susceptible cv. Ife brown) was also determined (Fassuliotis, 1979). Finally, the host reaction of different cultivars of cowpea was designated according to Canto-Saenz (1983) and Sasser *et al.* (1984).

Statistical Analysis. The data were subjected to analysis of variance, and the means separated with Fisher's (LSD) test at $P \leq 0.05$. The data on percentage of galling were arcsine transformed before analysis and retransformed for presentation.

RESULTS

The minimum and maximum temperature were 23 ± 2 °C and 35 ± 4 °C during the first experiment and 21 ± 1 °C and 36 ± 5 °C during the second experiment, respectively.

Considering the nematode RF, EI and GI indices, the cowpea cultivars were ranked as resistant (GI ≤2, RF ≤1), tolerant (GI ≤2, RF >1), hyper susceptible (GI >2, RF <1) and susceptible (GI >2, RF >1) to *M. incognita* (Canto-Saenz, 1983; Sasser *et al.*, 1984).

Five genotypes [Vita 3, Acc 64298 (cv. New Era), 82D4532CIT85, IT89KD-288 and TVX2724-01F] were resistant to the host race 4 of *M. incongita* (Tables I and II). For these genotypes, the RF (0.5-0.8), RI (6.7-8.4), egg mass number per root (EN) (3-6), EI (2), gall number per root (GN) (4-8), GI (2), and percentage root galling (2-5) were significantly lower than in the susceptible control, and there were no significant differences among them, including the resistant control Acc 64298 (cv. New Era) (Tables I and II). The genotypes IT8D-1228-10 and IT96D-733 were considered tolerant to the nematode as the RF ranged from 2.3 to 3.2, the RI from 20.1 to 21.4, EN from 35 to 40, and EI was 4; all these values were larger than in resistant and hyper susceptible genotypes (Tables I and II). In the tolerant genotypes GN (7-9) and GI (2) were lower than but did not differ significantly from those of resistant and hyper susceptible genotypes. The cultivar IAR 399-1 was rated hyper susceptible as it showed low RF (0.7), low RI (9.0), low EN (4) and low EI (2) but large GN (35) and GI (4) Moreover, there were no significant differences in RF and RI, but larger and significant differences oc-

Table I. Per cent root galling, root galls per plant (GN), root gall (GI) and egg mass (EI) indices, egg masses per plant (EN), reproduction index (RI) and reproduction factor (RF) of *Meloidogyne incognita* host race 4 on cowpea cultivars. (First experiment).

Genotype	Source	% galling	Root galls (GN)	GI	Egg masses (EN)	EI	RI	RF	Host reaction[1]
Vitas 3	IITA[2]	2	8	2	6	2	8.2	0.5	Resistant
TVX2724-01F	IITA	5	6	2	5	2	7.0	0.7	"
IAR399-1	IAR	15	35	4	9	2	9.0	0.86	Hypersusceptible
IT96D-733	IITA	9	7	2	40	4	20.2	3.2	Tolerant
ACC10	NCRI	30	40	4	38	4	18.9	3.5	Susceptible
ACC 68018	NCRI	25	36	4	29	3	18.0	3.5	"
TVXHR-026-IF	IITA	35	46	4	40	4	18.0	3.6	"
TI821C-60	IITA	22	45	4	38	4	18.9	3.6	"
TVX1948-01F	IITA	20	39	3	32	4	19.3	3.7	"
835-844	IITA	25	35	4	28	3	20.1	3.7	"
830850CIT3'83	IITA	20	30	3	25	3	23.7	3.7	"
IT 820289	IITA	25	45	4	40	4	22.2	3.7	"
TVX2394	IITA	28	50	4	45	4	28.2	3.8	"
8181007C1T1'85	IITA	20	48	4	42	4	30.4	3.8	"
83D871CITI'85	IITA	35	75	4	70	4	27.2	3.8	"
Hope	IAR	30	60	4	55	4	31.3	3.8	"
Popose	IAR	45	81	4	75	4	30.3	3.8	"
88D889	IITA	23	54	4	50	4	34.0	3.9	"
82D875CIT	IITA	25	46	4	40	4	39.0	3.9	"
82D544-4	IITA	35	65	4	60	4	32.4	3.9	"
TVX 372	IITA	30	62	4	56	4	41.1	3.9	"
PARAQUAY	IAR&T	25	66	4	61	4	45.7	3.9	"
ACC 64343	NCRI	40	70	4	65	4	51.5	3.9	"
FAR 013CIT4'85	IITA	25	63	4	55	4	81.6	5.2	"
TV1836-015F	IITA	45	89	4	79	4	80.7	5.3	"
TUX 3236	IITA	42	80	4	70	4	88.4	5.4	"
IT 83D442CIT3'85	IITA	50	98	4	88	4	90.5	5.6	"
83D962CIT3'85	IITA	45	85	4	78	4	101.8	5.7	"
TUX3464 – 01E	IITA	30	72	4	66	4	88.8	5.8	"
82D513-CIT2'85	IITA	45	92	4	82	4	95.1	6.0	"
66-2-11	IAR&T	35	77	4	66	4	106.9	6.1	"
K59	IAR	30	82	4	71	4	102.7	6.3	"
IT82D951	IITA	45	86	4	73	4	110.4	6.4	"
ACC64298 (New Era)	NCRI	3	5	2	3	2	8.4	0.6	Resistant
ACC73001(Ife Brown)	NCRI	45	88	4	75	4	100.00	5.6	Susceptible
LSD (5%)		14.00	25.21	0.85	20.32	0.91	7.05	2.12	

RF: Reproduction factor = Final population density of eggs divided by initial population density (inoculum density);
RI: Reproduction index = Final population density of eggs expressed as proportion of that from the known susceptible cultivar Acc 73001 (Ife brown);
GI: Gall index, 0 = no gall, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 galls; EI: Egg mass index, 0 = no egg mass, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 egg masses;
[1] Resistant (GI ≤2, RF ≤1), tolerant (GI ≤2, RF >1), hyper susceptible (GI >2, RF <1), susceptible (GI >2, RF >1) (Canto-Saenz, 1983; Sasser *et al.*, 1984).
[2] IITA- International Institute of Tropical Agriculture, Ibadan, Nigeria; IAR &T- Institute of Agricultural Research and Training, Ibadan, Nigeria; IAR- Institute of Agricultural Research, Samaru, Zaria, Nigeria; NCRI-National Cereals Research Institute, Ibadan, Nigeria.

Table II. Per cent root galling, root galls per plant (GN), root gall (GI) and egg mass (EI) indices, egg masses per plant (EN), reproduction index (RI) and reproduction factor (RF) of *Meloidogyne incognita* host race 4 on cowpea cultivars. (Second experiment).

Genotype	Source	% galling	Root galls (GN)	GI	Egg masses (EN)	EI	RI	RF	Host reaction[1]
82D4532CIT'85	IITA[2]	3	8	2	6	2	6.7	0.6	Resistant
IT89KD – 289	IITA	2	6	2	4	2	8.2	0. 7	Resistant
IT 8D - 1228-10	IITA	4	6	2	35	4	21.4	2.3	Tolerant
835 - 860CIT2'85	IITA	15	40	4	35	4	22.4	2.3	Susceptible
IT90K 277-2	IITA	25	30	3	28	3	23.7	2.3	"
835-960 CIT285	IITA	20	49	4	40	4	21.0	2.4	"
82D-699CIT2'85	IITA	25	55	4	50	4	29.7	2.5	"
82D-812CIT3'85	IITA	18	28	3	25	3	26.6	2.7	"
81D-975	IITA	20	49	4	42	4	28.6	2.8	"
82D-952CIT2'85	IITA	25	54	4	51	4	38.9	2.9	"
84E1246CIT3'85	IITA	15	38	4	30	3	34.0	3.0	"
ISEYIN LOCAL	NCRI	20	49	4	40	4	60.0	3.3	"
835852-CIT2'85	IITA	25	51	4	45	4	70.9	3.4	"
81D897CITI'85	IITA	20	45	4	39	4	58.4	3.6	"
IAR 49	IAR	26	59	4	55	4	85.3	3.6	"
IT86D – 719	IITA	30	65	4	60	4	71.6	3.8	"
TVX1999-02E	IITA	25	64	4	55	4	63.5	4.0	"
ACC 131	NCRI	40	75	4	70	4	70.9	4.0	"
TVX304800	IITA	35	68	4	62	4	63.0	4.0	"
TVX330042E	IITA	40	82	4	75	4	52.9	4.0	"
ACC68002(NIG B7)	NCRI	45	95	4	90	4	65.2	4.0	"
TVX2939-090	IITA	35	73	4	65	4	63.0	4.1	"
TVX3336-04F	IITA	42	86	4	80	4	71.1	4.2	"
K 28	IAR	35	78	4	73	4	72.3	4.2	"
8ID-1137CIT1'85	IITA	30	92	4	80	4	102.4	4.3	"
IAR 48	IAR	30	65	4	60	4	70.1	4.4	"
83 D 235-CIT1'85	IITA	30	74	4	70	4	74.6	4.4	"
IFH101	IAR&T	35	95	4	89	4	76.2	4.5	"
82D927	IITA	40	85	4	80	4	70.1	4.7	"
TVX 3386 – 042E	IITA	25	63	4	60	4	65.4	4.7	"
ERUWA LOCAL	NCRI	28	60	4	55	4	58.1	4.9	"
KANO 1696	IAR	30	62	4	58	4	71.49	4.9	"
ACC 64386	NCRI	38	71	4	66	4	81.4	5.0	"
ACC64298 (New Era)	NCRI	5	4	2	2	1	7.4	0.7	Resistant
ACC73001 (Ife Brown)	NCRI	35	85	4	80	4	100.0	4.0	Susceptible
LSD (P = 5%)		10.00	21.00	0.72	18.75	0.80	11.00	1.54	

RF: Reproduction factor = Final population density of eggs divided by initial population density (inoculum density).
RI: Reproduction index = Final population density of eggs expressed as proportion of that from the known susceptible cultivar Acc 73001 (Ife brown).
GI: Gall index, 0 = no gall, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 galls; EI: Egg mass index, 0 = no egg mass, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 egg masses.
[1] Resistant (GI ≤2, RF ≤1), tolerant (GI ≤2, RF >1), hyper susceptible (GI >2, RF <1), susceptible (GI >2, RF >1) (Canto-Saenz, 1983; Sasser *et al.,* 1984).
[2] See text and Table I for details of the source acronyms.

curred in the GN and percentage root galling (15) when the hyper susceptible genotype was compared with the resistant cultivars. The remaining 62 genotypes were susceptible to *M. incognita* as they showed RF of 2.3-6.4, GI 3-4 , EN 25-90 and EI 3-4. The susceptible Acc 73001 (cv. Ife Brown) had RF of 4.0-5.6, GN of 85-88, GI of 4, EN of 75-80, EI of 4 and root galling of 35-45%, which were significantly larger values than those of the resistant and tolerant genotypes (Tables I and II).

DISCUSSION

The genotypes Acc 64298 (cv. New Era), (Olowe, 1976), VITA 3 (Singh *et al.,* 1975) and IT89KD-288 (Florini, 1997) had been previously reported resistant to *M. incognita*, and Acc 64298 had also shown resistance to races 1, 3 and 4 of *M. incongita* but was susceptible to race 2 and *M. javanica* (Olowe, 1976). In addition, the genotype IT89KD-288 had been found resistant to aphid and bruchid insects (Singh, 1993) and the cultivar VITA 3 to virus, leaf hoppers, aphids and pod bugs (Singh *et al.,* 1975).

The hyper susceptible cultivar IAR 339-1, even though it allowed only a little reproduction of the nematode, is of no practical importance because it suffers severe damage and may eventually succumb. The tolerant cultivars, although they may yield satisfactorily, would leave large population densities of nematodes in the soil that could be detrimental for the crops succeeding in the rotation, thus nullifying the role of rotation in nematode control.

Resistant cultivars have been found to increase and stabilize yield of the crop in a similar manner to treating high yielding susceptible cultivars with nematicides. Growing resistant cultivars may also increase the yield by 19-69% or even up to five times that of highly susceptible cultivars (Duke and Hamitton, 1979; Kinloch *et al.*, 1988; Young and Hartwig, 1988). Unfortunately, none of the tested resistant cultivars were acceptable to the farmers and local market because of colour, taste, long cooking time, indeterminate growth habit (climbing) and late maturity. However, the nematode resistance sources of these genotypes could be used in breeding programmes designed to improve the good but susceptible elite cultivars, which could then also be utilized in rotation schemes to suppress nematode population density. Likewise, growing the resistant cowpea cv. Iron in rotation with soybeans is known to reduce soil population densities of *Meloidogyne arenaria, M. incognita, Heterodera glycines* Ichinohe (race 4), *Paratrichodorus christie* (Allen) Siddiqi, *Pratylenchus brachyurus* (Godfrey, Filipjev *et* Schuurmans Stekhoven and *Helicotylenchus dishystera* Cobb in green-house trials (Rodriguez-Kabana *et al.*, 1988a, b).

The use of resistant cowpea cultivars blends with the traditional cultural control. It is non-polluting, easy and no extra capital investment is required over the normal production practice other than the probable higher cost of the seed. The practice of using resistant cultivars will permit the scope of crop rotation to be widened to include preferred low or high value cash crops, thereby guaranteeing the best use of land suited for crop production and maximizing economic returns.

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