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CONTENTS

Report on severe infestation of root-knot nematode, *Meloidogyne incognita* on tuberous *Vigna vexillata* (L.)

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ABSTRACT: *Vigna vexillata,* a wild species closely related to the cultivated cowpea (*Vigna unguiculata* (L.) Walp), is used for its storage tuber like roots, protein-rich seed, forage and control of soil erosion. The stunted, chlorotic and wilted patches of *V. vexillata* were found at ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. The soil and root samples were collected with standard procedure to observe for nematode infestation. The severe infestation of root-knot nematode (RKN), *Meloidogyne incognita* was observed as roots of the infected plants were heavily galled with an average galling index of 4.3 (3.0-5.0). The soil population of J2 was also recorded well above the economic threshold level. The heavy root galling and presence of an extremely high population of J2 in soil (484-879 J2/ 200cc soil) indicates that *M. incognita* can be a potentially damaging pest to *V. vexillata* and can serve as a source of infestation to other hosts to be planted in same field. The present findings will awake the *V. vexillata* growers to consider growing *V. vexillata* under proper sanitation conditions. Soil samples from the proposed planting site should always be tested for the presence of nematode well before planting.

Key words: Cowpea, infestation, root-knot nematode, storage root, tuberous

Vigna vexillata (L.) A. Rich is an underutilized legume species belongs to the family Fabaceae. Two types of *V. vexillata* has been reported i.e., seed type and tuber (storage root) type. The tuber type which is resistant to several biotic and abiotic stresses (Amkul *et al.*, 2019) is a pantropical herbaceous legume occurring in Africa, Asia, Australia and America. It is a wild species closely related to the cowpea (*V. unguiculata* (L.) Walp) and is used for its storage tuber like roots, protein-rich seed, forage and for control of soil erosion (Garba and Pasquet, 1998). The tubers of *V. vexillata* have a protein content of about 15% which is roughly three times higher than that of potato and yam and six times than that of cassava (Chandel *et al.*, 1972). It is one of the underutilized legumes with potential for commercial exploitation. Tuber cowpea is one of the few species of the genus *Vigna viz., V. lobatifolia* and *V. marina* that produce tuberous roots. It is usually a vigorous twining or scrambling vine with large, showy purple or purplish-yellow flowers with fleshy tuberous roots from which the plants perenniate. In its range of occurrence, *V. vexillata* is collected from the wild habitat or cultivated for its tubers in African and Asian countries. Its fusiform roots are eaten raw or boiled by tribal people from Indian hills and are considered superior to sweet potato in flavours and nutrition (Arora, 2014). Among various domesticated species of *Vigna*, tuber cowpea is one of the least researched crops. Despite of its value, African collections have received more attention than southeast Asia.

Among the several factors that are responsible for the poor and unstable yield of food legume crops, biotic and abiotic stresses appeared to be the most important. Although several plant-parasitic nematodes are infesting legume crops, but root-knot nematodes (RKN), *Meloidogyne* spp. are considered as an important constraint in production of leguminous crops in the tropical and subtropical regions (Sharma *et al.*, 2005; Sikora *et al.*, 2005). However, limited information is available regarding the nature and extent of nematode disease problems in legume crops. With damage thresholds of 1-2 nematode(s) per gram of soil, *M. incognita* is one of the most destructive species worldwide. Infected plants show typical symptoms, which include root galling, stunting and nutrient deficiency symptoms. RKN are sedentary endoparasites of roots; they feed and develop within the galls. Infection of roots by RKN predisposes plants to infection by soil-borne

root-infecting pathogens and causes disease complex. Infestation of *M. incognita* in tubers of *V. vexillata* is reported here for the first time. Further research is required to explore the damage potential of *M. incognita* on *V. vexillata* tubers as well as its sustainable management.

MATERIALS AND METHODS

During a routine survey for nematode diseases, severe infestation of *M. incognita* was noticed on *V. vexillata*, (October 2020) grown at ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Stunted, chlorotic and wilted plants were observed in a scattered manner, which is a typical characteristic of nematode infestation because of their clustered distribution. The soil samples along with roots were randomly collected from the rhizosphere of infested plants. A total of 20 composite soil (4-5 cores) and root samples were collected from a depth of up to 20 cm from diseased plants. The second stage juveniles (J2) of the RKN were isolated from 200-cc of each sample by suspending them in water in a plastic bucket, passing the suspension through nested sieves (100 and 400 mesh), and finally separating from residue collected on 400 mesh sieve by placing on Baermann funnel for 24 hrs (Southey, 1986).

By using a counting dish, the second stage juveniles (J2) were counted under the stereoscopic microscope at 40X magnification.The root samples were washed thoroughly with a gentle jet of tap water and observed for the presence of root galls and egg masses. Severity of root galling on infested plants was assessed on a 0–5 rating scale according to the percentage of galled tissue, in which 0 = 0–10% of galled roots; 1 = 11–20%; 2 = 21–50%; 3 = 51–80%; 4 = 81–90%; and 5 = 91–100% (Barker, 1985). Twenty-five females, as well as other stages of nematodes, were dissected out from infested roots. Females were cut and their perineal pattern was used for species identification. Some specimens of each stage were fixed in formalin glycerin mixture (F:G) 4:1 solution, dehydrated by Seinhorst's rapid glycerin method (1959) and mounted on glass slides in anhydrous glycerin for microscopic observations. The species of RKN, *M. incognita* identified based on perennial pattern characteristics of mature females and morphological analysis of other stages, i.e., J2 and males (Eisenback *et al.*, 1981; Park *et al.*, 1998). Nematode J2s were also used for molecular characterization of RKN species. The genomic DNA was extracted from J2s using soil DNA extraction kit (Quagen) following manufactures protocol. DNA was subjected to PCR amplification using primers specific to Internal Transcribed Region (ITS) as described by Vrain *et al.* (1992). PCR products were subjected to agarose gel electrophoresis and visualized under UV. PCR products were purified and sequenced to know the gene sequences. The nucleotide sequence was analyzed and checked for sequence similarity in Genbank DNA database of NCBI using nucleotide Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

Based on the perineal pattern morphology and nucleotide sequences of ITS region, the population was identified as *Meloidogyne incognita*. PCR product of ~750 bp was visualized on 1 per cent agarose gel (Fig. 2). Sequence of ITS region was checked for its similarity with nematodes ITS sequences in Genbank database of NCBI, it was found similar with *M. incognita*. In soil analysis, all the 20 soil samples collected from the rhizosphere of infested plants yielded J2 of *M. incognita* in variable numbers (Table 1). The average population density was 687.5±123.72 J2/200cc soil (=3.44 J2/cc soil), which is much higher than the economic threshold level (ETL) of RKN i.e., 1-2 J2/cc soil. The entire roots of the infected plants were heavily galled in a unique pattern with gall index (GI) of 3-5 (4.3±0.79) encompassing 70-100% root systems (Fig. 1). The crops cultivated before *V. vexillata* may be critical to nematode infestation and play an important role in nematode population dynamics. Grasses can also be a suitable host for RKN; however, it is generally known that the highest population of RKN usually emanates from fields cultivated with vegetable crops such as tomato, brinjal, cucumber, okra and pepper. In this study, it was observed that the infested *V. vexillata* growing

Table 1: Population density of second stage juveniles (J2) of *Meloidogyne incognita* in soil and severity of root galling on *Vigna vexillata*

Sample number	No. of J2/ 200 cc soil*	Gall severity	
		Gall index†	Percentage of galled in infected roots tissues
1	695	4	90
2	755	5	100
3	824	5	100
4	547	3	70
5	676	4	80
6	731	5	100
7	769	5	100
8	484	3	75
9	743	5	100
10	494	3	70
11	584	4	90
12	754	5	100
13	879	5	100
14	784	5	95
15	821	5	100
16	675	4	85
17	669	5	95
18	601	4	80
19	832	5	100
20	433	3	70
Average	687.5	4.3	90.25
SD	123.72	0.79	10.89

*Number of J2/200 cc soil was average of three counting, †Severity of root galling was assessed on 0-5 scale, where in 0= 0-10% of root galled; 2= 21-50%; 3=51-80%; 4=81-90%; and 5=91-100%

Fig. 1: Photographs of roots of *Vigna vexillata*. A- Roots infected with *Meloidogyne incognita*, showing heavy root galling on entire root system. B- Non-infected healthy roots without galls.

a damaging pest of *V.vexillata* and can serve as a source of infestation to other host crops. Therefore, proper management strategies should be taken to minimize nematode damage in *V. vexillata* bed had a history of cucumber culturing (for the last three years), and before that, it was cultivated with vegetable crops (tomato, okra, bottle gourd etc.). This suggests that RKN may be derived from the previous crops known as RKN susceptible and continued to grow with susceptible crops without proper RKN management strategy.This might have led to the buildup of the high population density of *M. incognita*.

It is, therefore, concluded that *M. incognita* can be

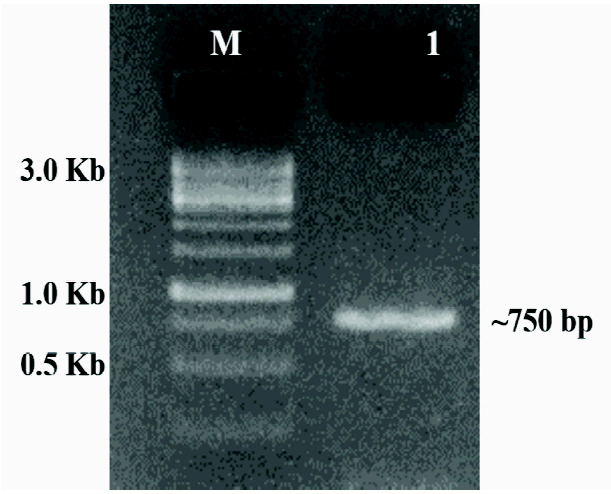

Fig 2: PCR amplification of ITS region of root-knot nematode population studied, Lane M: 1 Kb ladder, 1: PCR amplified ITS region of root-knot nematode, *M. incognita*

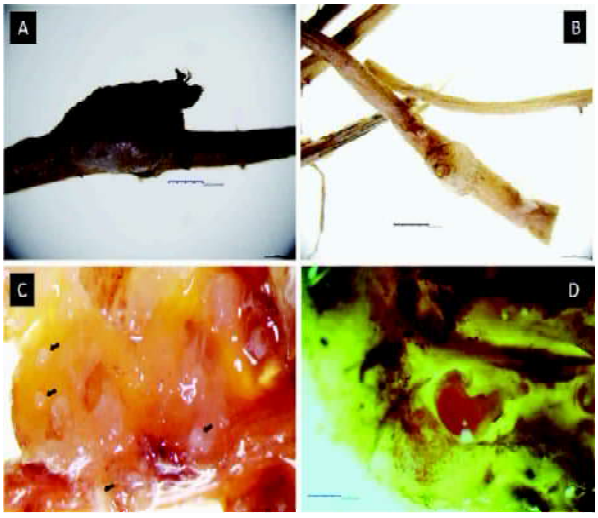

Fig 3: Microphotographs of *Meloidogyne incognita* infestation on *Vigna vexillata* roots

cultivation. Few eco-friendly and feasible strategies are suggested here to manage the nematode problem in *V. vexillata.* Growing a crop on which, the nematode can not reproduce is the way to control RKN. Unfortunately, crop rotation is not as easy for controlling RKN because of its vast host range, including weeds. However, with careful planning of crop rotation in combination with fallowing and soil solarization during peak summer can reduce RKN numbers below ETL. Annual crops that are useful in crop rotation for reducing RKN populations include wheat, barley, sudangrass, rapeseed, mustard, garlic and onion etc. Besides, certain marigold (*Tagetes* spp.) suppresses RKN when included in crop rotation. French marigolds (Nemagold, Petite Blanc, Queen Sophia, and Tangerine) are most effective. Incorporating cover crops as green manures suppress the RKN population by releasing nematotoxic compounds after decomposition (Chitwood and David, 2002), such as *Crotalaria*, castor bean, velvet bean, jack bean, sorghum-sudan, castor, and cereals have been successfully utilized as cover crops for RKN management (Viaene *et al*., 2006). Breeding and developing nematode-resistant plant cultivars are of much significance as an effective alternative to chemical nematicides. Very recently, some nematode-resistant lines/accessions of leguminous crops including cowpea (*Vigna unguiculata* L.) identified by Khan *et al.* (2017, 2018). Seeing the severe infestation of *M. incognita* on *V. vexillata*, soil contamination with nematodes needs to be considered for proper site selection to achieve a healthy product with better yield. Therefore, growers are advised to have the soil from the proposed area tested for nematodes before planting. For that, take soil samples with a hand shovel in a zigzag pattern across the selected plots from the top 20-30-cm of soil. Mix samples thoroughly and send to the nearest Nematology laboratory for nematode analysis.

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