RESEARCH ARTICLE

First report on association of *Meloidogyne graminicola* in twister disease of onion: An emerging problem in coastal tract of India

SURESH PATIL¹, V.B. NARGUND¹, GURUDATTH HEGDE¹, S. LINGARAJU¹ and A. SRINIVASARAGHAVAN^{2*}

¹Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580 005, Karnataka, India

²Department of Plant Pathology, Bihar Agricultural University, Sabour 813 210, Bihar, India

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ABSTRACT: Onion crop grown in west coast of sothern India has been seriously affected by the problem of onion twister disease charaterized by the symptoms viz., twisting of leaf and neck, slight to prominent galls in roots with root proliferation and scanty discoloration of root system. Survey carried out during *rabii*/summer of 2011-12 and 2012-13 revealed the prevalence of such disease in all the major onion cultivating areas of Karnataka (India). The highest disease index of 29.37 and 24.05 per cent was noticed Uttar Kannada district during 2011-12 and 2012-13 respectively. Aetiological investigations revealed the association of a nematode with symptomatic onion plants having root galls. Isolated nematode when inoculated, resulted in slender plants with typical abnormal elongation of leaf with proliferation and neck twisting during initial stage and at later stage whole plant appeared to be abnormal showing typical twisting symptoms. Artificially inoculated plant with typical symptoms were found to have severe galling with typical hooks like roots. At later stage discoloration and complete destruction of root system demonstrated the *Koch's* postulate for the involvement of isolated nematode in the disease. Identity of the nematode was confirmed as *Meloidogyne graminicola* by morphometric and perineal pattern studies. This is the first report of association of rice root knot nematode involved in onion twister disease.

Key words: *Meloidogyne graminicola*, onion, RKN, twisting

Onion (*Allium cepa* L.) is an important commercial vegetable crop cultivated worldwide (Berhanu and Berhanu, 2014). Onion being known for its excellent flavor, pungency and medicinal values is an indispensable vegetable in every kitchen plays a crucial role in internal as well as global market (Crowther *et al.*, 2005). India despite being 2nd largest producer of onion with approximately 19 per cent of world production often faces short supply of onion (Anonymous, 2012). Among the major factors affecting onion production diseases play an important role causing both pre and post-harvest losses. In the recent years, twister disease of onion is assuming an epidemic proportion in major onion growing areas especially the west coast of southern India causing heavy yield loss (Hegde *et al.*, 2012; Nargund *et al.*, 2013). The onion disease with similar kind of symptoms are first reported from Zaria, north Nigeria, in 1969 with 50-100 per cent estimated severity (Ebenebe, 1980) and later during 1970 in Sri Lanka (Kuruppu, 1999). In India, the disease was first reported by Sinha and Singh (1994).

The cause of the twisting symptoms in onion are mainly attributed to fungi viz., *Colletotrichum gloeosporioides* (Ebenebe, 1980; Farr and Rossman, 2010; Hill, 2008; Nischwitz *et al.*, 2008; Rodriguez-Salamanca *et al.*, 2012), *Colletotrichum coccodes*

(Rodriguez-Salamanca *et al.*, 2012) and *Fusarium oxysporum* f. sp. *cepae* (Kuruppu, 1999). Nargund *et al.*, (2013) also reported that, onion twister menace was observed to be caused by various plant pathogens viz., *Colletotrichum gloeosporioides*, *F. oxysporum*, *Sclerotium rolfsii* and also speculated a possible role of *Meloidogyne* spp. However, so far no definite conclusion has been made on the direct involvement of root knot nematode in onion twister diseases. The objectives of this study was to assess and quantify the possible role of root knot nematode in onion twister disease.

MATERIALS AND METHODS

An intensive roving survey was carried out during 2011-12 in major coastal tract and other onion growing districts in Karnataka to know the incidence and severity of onion twister disease. In each field 10 spots were selected in zig-zag manner starting from one end of field and noted the disease incidence and graded according to the 1-5 scale developed similar to that of purple blach (Bhargale and Joi, 1985) and calculated Disease Index (DI) by using the formula given by Wheeler (1969).

Establishing pathogenicity

Collection of root knot nematodes, maintenance and buildup of nematodes: The onion plants showing typical

*Corresponding author: sraghavan3628@gmail.com

Table 1. The details of the symptoms on different plant parts of onion observed under field condition during survey

Plant Part	Symptoms observed
Leaf	Twisting, curling and chlorosis of leaves. Circular to oval water-soaked areas and a zone of discolored tissue was formed around the spots. Further, dieback symptom was noticed in severely affected plants
Neck and bulb	Elongated neck with slender bulb, which were twisted abnormally
Root	Root knot, root discoloration and underdeveloped root system are important symptoms. In few cases, root galling was observed at tip and also intercalary which were whitish in early stage

twister disease symptoms were collected from field and the plants with prominent root galls were carefully removed from the soil and root portion was washed gently under running tap water. Egg masses were picked and kept for hatching in a Petri dish containing sterile distilled water. The Juveniles hatched after 24-36 hours were used to inoculate plants grown in sterilized soil. The inoculum was multiplied on onion plant roots grown in soil-sand mixture for about 100 days (Approximately 3-4 generations).

Inoculation and proving the pathogenicity: Onion (*Allium cepa*) cv. Arka kalyana was used in all experiments. Seeds were thoroughly surface sterilized with one per cent sodium hypochlorite for two minute and washed in sterile water, air dried and sown. Thirty days after planting in test pots, the suspension containing juvenile nematodes (500 juvenile/pot) were pipetted and spread uniformly over the surface of roots carefully exposed earlier. Later, roots were carefully covered with soil watered lightly to keep it moist. A similar treatment was given to un-inoculated check plants except that only water was used instead of the nematode suspension. Plants were kept under continuous monitoring for appearance of symptoms. The plants were allowed to grow for 60 days to give nematodes to complete 2 to 3 generations. The root systems of the de potted plants were washed to remove soil and were examined for presence of galls. For further confirmation nematode infection in roots, staining of root material was performed with acid fuchsin (Mc Beth *et al.*, 1941).

Identification of root knot nematode species: The galled root system was immersed in a beaker containing boiling cotton blue (0.1%) in lacto phenol and left overnight for clearing (Hooper, 1986). The roots infected by root knot nematode were washed. The females were dissected out from the well developed galls of the roots under the stereo binocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide.

Morphometric measurements: To study the variability within root knot nematode population, morphometric measurements were taken. Eggs were obtained by blending galled root segments in 1 per cent sodium hypochlorite solution, rinsed several times with tap water, and allowed to hatch in tap water for 48 hours. Juveniles were picked randomly in mass, placed in a drop of water on a glass slide and killed by gentle heat. The larvae were covered with a cover slip and 35 such larvae were selected per isolate and subjected to measurements viz., body length, width, stylet length and tail to terminus length.

Perineal pattern study: Mature females were picked-up from the galled roots and placed on a glass slide in a drop of water. The posterior end of females were cut with a sharp razor and cleaned. The glass slide with the female end (cut side down) was covered with a cover-slip and sealed. Ten female perineal patterns were analyzed per isolate. In total, 430 perineal patterns were evaluated in this study. The patterns were examined under a compound microscope at $\times 500$ and $\times 640$ and selective representative patterns were photographed.

RESULTS

Survey of the disease to gather information on the symptomatology, severity and distribution revealed, moderate to severe incidence of the twister disease of onion in different onion growing regions of Karnataka with maximum Incidence in Uttara Kannada district i.e., 29.37 and 24.05 per cent during 2011-12 and 2012-13 respectively (Table 1). Under Uttara Kannada district the maximum incidence of 55 per cent was recorded in Handigona village of Kumta taluk during both 2011-12 (Table 2). Root knot, root discoloration and underdeveloped root system are important symptoms observed. In few cases, root galling was observed at tip

Table 2. Mean percent disease index of twister disease of onion with root knot during 2011-12 and 2012-13 in different taluks of Uttara Kannada

Taluk	Village	PDI (%)	
		2011-12	2012-13
Kumta	Alvekodi	25.00	20.00
	Handigona	55.00	55.00
	Horbhagilu	50.00	8.00
	Kadekodi	10.00	16.00
	Mavinagundi	25.00	35.00
	Vannali	40.00	35.00
Mean		34.16	32.20
Honnarav	Haladipura	25.00	12.00
	Horbaga	20.00	8.00
	Karki	25.00	10.00
	Nagabana	20.00	15.00
Mean		22.50	11.00
Bhatkal	Jaali	30.00	32.00
	Shirali	20.00	20.00
	Tenginagundi	35.00	35.00
Mean		28.33	27.50
Ankola	Devanahalli	35.00	35.00
	Mirjan	30.00	16.00
Mean		32.50	25.50
Grand Mean		29.37	24.05

Fig. 1. Symptoms observed on root knot infected onion on variety 'Kumta' during survey (a) Onion Plant with typical twisting symptom (b) Terminal root galls in twisted onion plant (c) Reduced bulb size and root knots on onion plant affected by twister disease

Fig. 2(a). Different above ground symptoms observed on onion plants var. 'Kumta' inoculated with *M. graminicola*

Fig. 2(b). Root system with severe knotting at final stage on onion variety 'Kumta' inoculated with *M. graminicola*

and also intercalary which were whitish in early stage. The different symptoms of twister disease observed during survey are presented in Table 2 (Fig. 1).

Pathogenicity test: Fifteen days after Inoculation, plants showed stunted growth, yellowing of leaves with slight

abnormal elongation with twisting like symptoms (Fig. 2a). Such symptomatic plants when uprooted found to have root proliferation and slight root galling on bigger roots (Fig. 2b). Diagnostic microscopic examination of the galls revealed the presence of eggs, juveniles and

Fig. 3. Microscopic observation of the nematode isolated from inoculated roots and perineal patterns of *M. graminicola* a) Infective second stage juvenile b) Adult Male c) Adult Female d) Perineal pattern

females of nematodes in the vascular bundles of roots. The females were pyriform while, the males were filiform and in general outline (Fig. 3a). The presence of egg masses outside the gall was a commonly noticed in roots. The egg mass matrix was observed to be whitish, glistening and round which were exposed on the roots.

Morphometric measurements of larvae and perineal patterns: Average body length, stylet length and A, B and C values examined were 450.9 (425- 477) μm and 25.8 (22.3-30.2) μm . The perineal patterns were dorso-ventral, oval to almost circular in shape, moderate in height of arc, and no lateral incisures or gaps were observed. Tail tip was marked with prominent, coarse, fairly well separated striae that, sometimes forming an irregular tail whorl. These perineal patterns were similar to the pattern described for *M. graminicola* (Fig. 3).

DISCUSSION

The aetiology of onion twister disease was initially suspected as nematodes. However, Kuruppu (1999) isolated the fungus *F. oxysporum* from the diseased shallot plants and reported that thrips, mites, nematodes and other fungi could also cause similar symptoms. Similarly observations were made by Abawi *et al.* (1999) as above-ground symptoms on onions heavily infected with *M. hapla* are those of general stunting, uneven growth, smaller necks and bulbs. The diagnostic symptoms are found on roots as galls or root thickenings of various sizes and shapes. With respect to rice root-knot nematode, *M. graminicola*, infection in rice-onion cropping systems in the Philippines was reported. Gergon (2002) study showed that infected plants had short galled roots, smaller bulbs than normal. In India, Singh (2011) observed root-knot nematode to cause serious losses to green onion crop grown mostly in the field located near the Jamuna and Ganga river belt with the diagnostic symptoms *viz.*, roots as galls or root thickenings of various sizes and shapes. But, the involvement of *M. graminicola* being associated with typical twister symptom of onion was not discussed in any of the previous studies. In the present study, the involvement of rice root knot nematode in producing typical onion

twister disease has been confirmed by proving the pathogenicity by Koch's postulates. In the present study maximum severity in coastal Karnataka of Uttara Kannada is mainly attributed to season. It has an extreme climate, with temperatures in the range of 28°C to 36°C, soil type and cultivation practices. It is observed that, in areas *viz.*, Kumta, Honnaravara and Bhatkal the sweet onion are grown in sandy soils after harvesting of *kharif* paddy. Moreover, the farmers use seeds propagated from previous season without any seed treatment measures. Further on discussion with farmers during survey on cultivation practices, revealed that they use meagre amount of organic material as basal dose for onion crop. The paddy grown in *kharif* is also found to be suffered from *Meloidogyne graminicola*, which has wide host range. The present findings are in agreement with Tondok (2003), who reported that onion crop became less resistant to twister disease when exposed to high temperature. Subsistence farming practiced by the farmers in sandy soil with paddy-onion cropping system encourage soil borne pathogens like *Meloidogyne* spp. and *Gibberella fujikuroi* as they are having common host. Further, infected crop debris serves as source of inoculum for next season.

The distribution of *M. graminicola* in rice growing areas of different areas has been well documented worldwide (CABI, 2015). The information generated in this study will be of great significance in collating information to damage symptoms, biology, morphology, host range and management strategies.

REFERENCE

- Abawi GS, Widmer TL, Ludwig JW and Mitkowski NA (1999). Biology and management of *Meloidogyne hapla* on carrots, onions and lettuce in New York. *J. Nematol.* 31: 521.
- Anonymous (2012). "A Report on Competitive Assessment of Onion Markets in India" Competition Commission of India.
- Berhanu MA and Berhanu GA (2014). Constraints of Onion (*Allium cepa* var. *cepa* L.) Yield Production and Food Preference to Shallot (*Allium cepa* var. *aggregatum*) in the Case of Bibugn Woreda, Amhara Regional State, Ethiopia. *Food Sci. Qual. Manage.* 32: 41-45

- Bhangale GT and Joi MB (1985). Screening of onion cultivars for resistance to purple blotch and thrips. *J. Maharashtra Agric. Univ.* 10: 355-356.
- CABI datasheet, <http://www.cabi.org/isc/datasheet/33243>, accessed on 14th September, 2015
- Crowther TA, Collin H, Smith B, Tomsett A, O'Connor and Jones GM (2005). Assessment of the flavor of fresh uncooked onions by taste-panels and analysis of flavor precursors, pyruvate and sugars. *J. Sci. Food Agric.* 85: 112-120
- Ebenebe AC (1980). Onion twister disease caused by *Glomerella cingulata* in northern Nigeria. *Plant Dis.* 64: 1030-1032.
- Farr DF and Rossman AY (2010). Fungal databases. Systematic mycology and microbiology laboratory, ARS, USDA. Retrieved from <http://nt.ars-grin.gov/fungaldatabases/>, August 6, 2010.
- Gergon EB (2002). Occurrence, disease cycle, effects on yield, and management of root knot disease of onion (*Allium cepa* L.) caused by *Meloidogyne graminicola* Golden and Birchfield. Laguna, Philippines: Philippines University, Los Banos College, Ph.D. Thesis.
- Hegde GM, Rajkumar GR and Gouda Jaware (2012). Integrated disease management of twister disease of onion. Dharwad, Karnataka, India: University of Agriculture Sciences: Extension Bulletin.
- Hill JP (2008). Compendium of onion and garlic diseases, 2nd ed. St. Paul MN, USA: The American Phytopathological Society.
- Hooper DJ (1986). Extraction of nematodes from plant material. In : *Laboratory Methods for Work with Plant and Soil Nematodes* (Ed. Southey JJ), Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office. London, UK, pp. 51-58.
- Kuruppu U (1999). First Report of *Fusarium oxysporum* causing a leaf twisting disease on *Allium cepa* var. *ascalonicum* in Sri Lanka, *Dis. Notes Louisiana State University, Baton Rouge*. 83: 695.
- Mc Beth CW, Taylor AL and Smith AL (1941). Note on staining nematodes in root tissues. *Proceeding of Helminthological Soc., Washington*. 8: 26.
- Nargund VB, Gurudath H, Nayak GV, Benagi VI, Suresh P, Dharmatti PR and Ravichandran S (2013). Management of twister disease in sweet onion - a strategy for livelihood improvement and welfare of mankind. In: *Proceedings of International Symposium on Human health effects of fruits and vegetables*, University of Agricultural Sciences, Dharwad, Karnataka, India: 205.
- Nischwitz C, Langston DR, Jr Sanders FH, Torrence R, Lewis KJ and Gitaitis RD (2008). First report of *Colletotrichum gloeosporoides* causing 'twister disease' of onion (*Allium cepa*). *Plant Dis.* 92: 974.
- Rodriguez-Salamanca LM, Enzenbacher TB, Derie ML, du Toit LJ, Feng C, Correll JC and Hausbeck MK (2012). First Report of *Colletotrichum coccodes* causing Leaf and Neck Anthracnose on Onions (*Allium cepa*) in Michigan and the United States. *Plant Dis.* 96: 769.
- Singh RK (2011). Occurrence of root-knot disease in green onion in Allahabad. *Ach. Phytopathol. Plant Protec.* 44: 101-104.
- Sinha JN and Singh AP (1994). Severe curls or twister disease of onion in India. *Indian Phytopath.* 47: 29.
- Tondok E (2003). The causal agent of twister disease of Shallot. M.Sc. (Agari.) Thesis, University of Goettingen, Germany.
- Wheeler BEJ (1969). An introduction to plant diseases, John Wiley and Sons Ltd., London, p. 301.