The Characterization of *Ditylenchus Gigas*, N.Sp in the Biskra Region

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***Abstract*—** Nematologists need proper identification of species for research, education, protection and other activities. Therefore, identification of nematodes must be more and more accurate. For this, the nematode strains must be identified using morphometric measurements. In Algeria the identification of the genus Ditylenchus is based only on the morphological aspect. For the first time in Algeria, 8 characteristic biometric criteria commonly used to define species of the genus were realized.

The results obtained give typical morphological characters for this nematode collected in a specific climatic zone with great potential for bean culture (Vicia faba) which would imply a new approach and research for a more appropriate integrated control.

Keywords: Ditylenchus gigas, Vicia faba, Nematodes, morphometrics Algeria.

I. Introduction

Ditylenchus gigas, is an obligate endoparasite mainly found in the stems and leaves of infested plants. It is considered in Algeria and globally as a limiting factor in the development of legume and especially bean crops (Esquibet et al., 1998, Sellami et al, 1998, Esquibet et al., 2003 in Saadi, 2008).

In Algeria, the presence of this nematode was reported for the first time by Debray and Maupas in 1896, this species is still considered the most formidable bean. is present in almost all bean plots in all areas of the country, it remains a serious threat to this crop (Di Vito et al., 1994, Sellami et al, 1996, Sellami et al, 1998, Saadi, 2008).).

The European and Mediterranean Plant Protection Organization (EPPO) has listed D. dipsaci as an A2 quarantine pest in many countries in this region (EPPO, 2009). This nematode easily withstands sub-zero temperatures in winter, and soil temperature up to 55 ° C in summer, and even for decades. It can be isolated even from completely dry plant material after moistening (Sturhan et al, 1991). This nematode of the stem and bulb is an endoparasite of the higher plants that feeds in the parenchymal tissues of the stem of about 500 plant species, causing growth retardation and swelling and significant deformities of the plants (Sturhan and al, 1991).

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It has been pointed out by many authors that D. gigas consists of a number of breeds and populations whose host preferences at different stages of biological reproductive speciation offer variances, representing a different species status (Sturhan et al 1991). After phylogenetic analysis of rDNA sequences of genes from different populations and breeds (Subbotin et al., 2005) confirmed that Ditylenchus dipsaci represented a complex of species, which comprises at least seven species, while other species are not not yet properly studied and described.

Only D. dipsaci sensu stricto and Ditylenchus gigas. Infecting the beans, known as the "giant race" of the stem nematode, is of economic importance as a plant parasite (Sikora and Greco, 1990) and (volvas et al, 2011).

The giant stem nematode breed was recorded as a serious bean disease in several European and African countries bordering the Mediterranean Sea and in Iran in 2013. In addition, several authors have noted that the giant breed is generally more damaging because it causes more severe symptoms in the bean field than any other D. dipsaci breeds and produces more infested seeds, despite the fact that it appears to have a limited range of hosts (Goodey 1941, Hooper 1971, Sikora et al. al, 1990, Sturhan et al 1991, volvas et al 2011, Tanha et al 2013). Several detailed studies conducted with a giant breed population have shown that this breed is also distinguished from the normal breed in its biology.

The normal race is thought to have a diploid number of 2n = 24 chromosomes, whereas the giant borer-parasitic breed is tetraploid at 2n = 48-60 (Sturhan, 1969, 1970, D'Addabbo et al., 1982, Sturhan et al, 1991 Volvas et al, 2011Tanha et al 2013).

Crossing experiments with diploid races D. dipsaci stricto sensu and the giant breed showed that F1 hybrids were formed, but they were sterile. These results led Sturhan (1983) to conclude that the giant breed should therefore be recognized as a distinct species.

Accurate detection and identification of Ditylenchus species as well as knowledge of the pathogenic variability of populations in the field is crucial for regulatory control of control, as well as for the reproduction and development of resistant cultivars (Mahfouz et al. 2014) In the present study, on nematodes isolated from Ditylenchus gigas individuals collected on areas with high potential for bean culture (Vicia faba) is a first unpublished and precise characterization of this

breed in Algeria using a morphological approach. metric. The new species of nematodes are described here as Ditylenchus gigas n. sp. Based on the analysis of several populations collected from beans in an arid region in southeastern Algeria (Biskra).

II. Materials and methods

A. Sampling and extraction of nematode populations

After prospecting on several sites of bean cultures in the regions of Biskra (located in the south-east of Algeria in an arid climatic zone) presenting the symptoms of nematode infection, namely brown and reddish necroses, which black and begin at the collar, and extend over the entire stem. Foliage and flower discolouration, plant malformation and distortion, and shortening of internodes (Stuteville et al., 1990, Caubel and Esquibet, 1995). Samples were taken from several plots. In the laboratory the stems are washed to remove soil and adhering debris. Subsequently the plant tissues are cut into pieces of ~ 2 cm with a pair of scissors and placed in the bottom of a Petri dish with a little water for immediate observation, after having previously torn the tissues of the plant with needles and forceps to release tissue nematodes. After 5-6 h, the nematodes that emerged are used for morphological observations.

B. Morphological examination

For diagnosis and identification, the nematodes were extracted by soft heat narcosis, and mounted in agar (Esser, 1986). Adult specimens were also treated in glycerol in permanent fixtures, according to the method of Seinhorst (1966), the morpho-diagnostic examination was carried out using a 35 mm camera mounted on a microscope composed Reichart (Reichart-Jung) equipped with an optical contrast contrast (DIC) differential. The measurements were calculated using software provided with the microscope (Leica Application Suite (LAS)) which allowed us to perform 14 morphometric measurements for each nematode.

III. Results and discussion

Giant specimens collected in the Biskra area cause severe swelling and deformation of the stem tissue in the bean, and lesions that turn reddish-brown and then black. The lesions of the stem wrapped and increased in length (from 0 to 8 cm2), often reaching the edge of an inter-node. Necrotic petioles were also evident, which can be confused with the symptoms induced by fungal leaf pathogens. In this advanced stage of infection, the seeds, which were also infected with the nematode, appeared darker, deformed, smaller in size and with speckles like on the surface. Serious infections often kill the main shoots, and stimulate the formation of secondary bars.

A. The morphometric results

Table I: Morphometrics (females and males) of Ditylenchus gigas n.sp extracted from Vicia faba in the region of Biskra

	Femelles	Mâles
N	20	18
L^b	1815.9 ± 82.9 (1653-1927)	1624.7 ± 107.6 (1462-1947)
Stylet length le stylet	12.2 ± 0.4 (11.5-12.9)	11.6 ± 0.5 (10.7-12.4)
V or T(%)	84.2 ± 1.0 (81-87)	73.4 ± 8.1 (62.3-85.7)
Pharynx (to end of gland lobe)	240.2 ± 3.2 (234-245)	212.7 ± 7.9(200-226)
Vulva-anus distance	230 ± 21.1 (201-264)	-
PUS	132 ± 14.5 (96-157)	-
Tail length	88.2 ± 6.5 (76.1 – 98.7)	82.2 ± 6.7 (72.6 – 91.2)
Spicule length	-	25.3 ± 1.5 (22.9- 27.7)
A	42.2 ± 2.2 (38.6 – 46.0)	42.2 ± 2.2 (39.2 – 46.8)
B	8.1 ± 0.4 (7.3- 8.6)	8.1 ± 0.6 (7.0 – 9.2)
b’	7.5 ± 0.3 (6.9 – 8.1)	7.6 ± 0.6 (6.7 – 8.9)
C	20.7 ± 1.8 (17.7 - 24.1)	19.9 ± 2.1 (16.4 – 23.5)
c’	4.8 ± 0.7 (3.4 - 6.6)	4.6 ± 0.6 (3.8 – 5.6)

1.The morphometric results Female:

The number of subjects analyzed in the Biskra region and 20 nematodes total length for females is 1815.9 ± 82.9µm. These averages are relatively larger than those obtained by

Volvas et al in 2011 in the three regions of the Mediterranean basin (Italy, Lebanon and Spain). The morphological and morphometric characteristics were in agreement with those published by Tanha et al 2013 and Volvas et al in 2011. *Similarly for the stylet length which is 12.2 ± 0.4 µm (see Figure 1 (a, b, h).*

The distance from the pharynx to the end of the lobe glans are in the order 240.2 ± 3.2 µm. From the head to the anus (excretory pore) the values are 162 ± 17.2 µm. For the ovular cyst ,. The distance from the vulva to the anus is in the order of 230 ± 21.1 µm (see Figure 1 (b)). Finally the length of the tail we found 88.2 ± 6..5 µm of Biskra (see Figure 1 (g)).

2. The morphometric results Male:

The number of males studied in the two regions are 20 nematodes the average total length of the male is 1524.7 ± 107.6 µm, all morphological characteristics relatively similar to those of the female, except in the reproductive system. Slightly narrower than the rest of the body (Tanha et al 2013 and Volvas et al in 2011). The length of the stylet is 12.2 ± 0.4 µm (see Figure 1 (a, c, h).) The pharynx at the end of the glans lobe are in the order 212.7 ± 7.9µm From the head to the anus the values are from 153 ± 13.8 and 160 ± 12.3µm, the length of the spicule raises it and 25.3 ± 1.5.

3. Synthesis of morphometric results

The gigas n.sp.se breed differs from all other Ditylenchus on several morphological characteristics. Major diagnostic character and body length.

Indeed, the results obtained on the length of the nematodes obtained 1815.9 ± 82.9 µm (1653-1927) for female nematodes 1624.7 ± 107.6 µm (1462-1947) for males clearly indicate higher values than those quoted by Volvas et al 2011 (Table: 03 and 04) at Noci, Bari Itali (1780 ± 97.4 µm (1561-1932) for females1557 ± 98.2 µm (1373-1716) for males) Other distinguishing parameter and distance from vulve- anus or results obtained by Tanha (2013) and 217 ± 21.0 µm (178-272) against 268 ± 32.8 µm (201-264).

Also for the stylet length or the results obtained by Volvas et al 2011 (Table: 03 and 04) and 12.0 ± 0.4 µm (11.5-13.0) for females and of 11.6 ± 0.4 µm (11.0-12.5) for females. males against nematodes from the Biskra region 12.2 ± 0.4 µm (11.5-12.9) for female subjects and 11.6 ± 0.5 µm (10.7-12.4) for male subjects.

TABLE II. MORPHOMETRY OF FEMALES AND MALES OF DITYLENCHUS GIGAS N. SP. OF VICIA FABA A (NOCI, BARI, SOUTHERN ITALY) (VOLVAS ET AL 2011)

	Les femelles	**Mâles**
	Moyenne ± SD (plage)	**Moyenne ± SD (plage)**
n	20	12
L [b]	1780 ± 97. 4 (1561-1932)	1557 ± 98.2 (1373-1716)
Longueur du stylet	12.0 ± 0 .4 (11.5-13.0)	11. 6 ± 0. 4 (11.0-12.5)
V ou T (%)	81.5 ± 1.0 (80-83)	72.4 ± 11.7 (57.6-100)
Tête au centre de métacorpus	77 ± 4.3 (65-83)	75 ± 7.8 (58-88)
Pharynx (à cardia)	206 ± 11.1 (191-228)	192 ± 25. 8 (143-218)
Pharynx (à la fin du lobe de la glande)	212 ± 11.8 (194-236)	198 ± 26 .4 (148-223)
Tête vers les pores excréteurs	158 ± 18.6 (108-179)	153 ± 14.8 (124-173)
Spermathèque longueur	127,5 ± 22,9 (98-176)	-
Vulva-anus distance	228 ± 20.1 (202-266)	-
PUS	120 ± 16. 4 (81-150)	-
Longueur de queue	88,5 ± 12,6 (69-103)	87.3 ± 6.5 (74-96)
Corps anal diam.	18.5 ± 2. 4 (15.0-21.5)	16.8 ± 1.3 (15. 0-19.5)
Longueur du spicule	-	25.4 ± 1.2 (23.5-28)
Gubernaculum	-	8.8 ± 0.8 (8 .0-10.0)
A	48.9 ± 3.9 (43.0-56.4)	56.7 ± 7.6 (34 · 3-63.0)
b	8.5 ± 0.5 (7.3-9.3)	8.3 ± 1.3 (6.7-10.7)
b '	8.3 ± 0.6 (7.3-9.2)	8.0 ± 1.3 (6.5-10.4)
c	20.0 ± 3.2 (16.8-27.6)	17.9 ± 1.2 (15.7-20.0)
c '	4.8 ± 0.5 (4.1-6.2)	5.2 ± 0.5 (4.5-6.5)

a All measurements are in µm unless otherwise indicated.

b All other abbreviations used are defined by Siddiqi (2000)

TABLE III. PRINCIPALES CARACTÉRISTIQUES MORPHOMÉTRIQUES DIAGNOSTIQUES D'AUTRES POPULATIONS DE DITYLENCHUS GIGAS N. SP. DE VICIA FABA CITÉ PAR VOLVAS ET AL 2011

	Beyrouth, vallée de la Bekaa, Liban	**Córdoba (province de Córdoba) Espagne**	**Ostuni (province de Brindisi), Italie**
	Moyenne ± SD (plage)	**Moyenne ± SD (plage)**	**Moyenne ± SD (plage)**
N	6	5	5
L [b]	1640 ± 52 (1560-1690)	1789 ± 101.8 (1657-1903)	1806 ± 84 · 4 (1687-1924)
Longueur du stylet	11.5 ± 0.6 (10.5-12 · 0)	12.5 ± 0.5 (12.0-13.0)	12 · 1 ± 0 · 5 (11 · 5-13 · 0)
V (%)	82.0 ± 1.0 (80-83)	81.8 ± 1.3 (80-83)	81 · 6 ± 0 · 9 (81-83)
PUS	94 ± 6.9 (85-101)	123 ± 19.0 (102-145)	119 ± 18.7 (98-148)
Longueur de queue	84.0 ± 5.6 (77-91)	95.0 ± 3.9 (89-99)	97.0 ± 5.7 (87-101)
Longueur du spicule	25.5 ± 0.8 (24.5-26.0)	25.0 ± 1.0 (24-26)	24.8 ± 0.8 (24-26)
Une	51.7 ± 2.6 (48.0-54.0)	44.3 ± 2.9 (40.4-47.7)	45.4 ± 2.1 (42.8-48 · 1)
b '	8.1 ± 0.5 (7.7-8.5)	8.8 ± 0.3 (8.4-9.0)	8.6 ± 0.3 (8.3-9.1)
C	19.5 ± 1.3 (17.5-21.0)	18.8 ± 0.6 (18.2-19.6)	18.6 ± 0.7 (18.0-19.4)
c '	5.1 ± 0.5 (4.8-5.4)	4.6 ± 0.3 (4.1-4.8)	5.0 ± 0.5 (4.4-5.6)

a All measurements are in µm unless otherwise indicated.

b All other abbreviations used are defined by Siddiqi (2000).

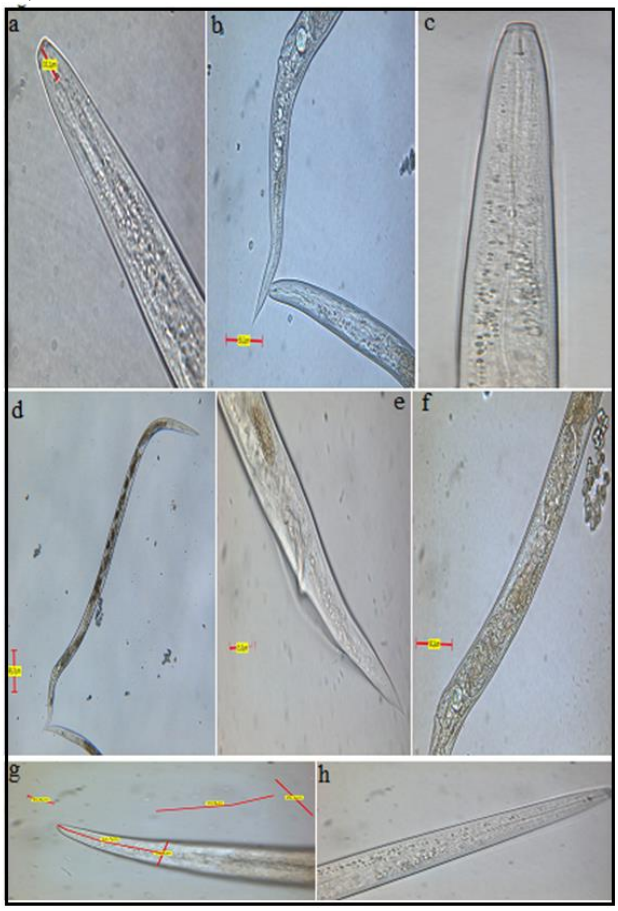

Fig. 1

at. measuring the stylus of D gigas b.posterior portion of female cC. metacorpus, stylet d. femalel Ditylenchus gigas e.posterior spicule of male portion, f Vulve, Spermatheca.g Measurement of Tail of nematode, metacorpus h, stylet

The vulva anus distance at higher values for nematodes from Biskra populations respectively 230 ± 21.1 µm (201-264) than that by Tanha et al 2013 in Iran 217.0 ± 21.0 µm (178-272) and almost the same similarities for the tail. Similar observations for spicule length from the data cited by Volvas et al 2013 in Beka, Lebanon with 25.5 ± 0.8 µm (24.5-26.0) and Cordoba in Spain with 25.0 ± 1.0 µm (24-26). and finally in Ostuni in Italy with 24.8 ± 0.8 µm (24-26) against 25.3 ± 1.5 µm (22.9-27.7) in Biskra

In view of Tables 3 and 4 the collected parameters cited by Volvas et al 2011 and Tanha et al 2013 demonstrate the set of characters studied approach and define the individuals collected on Vicia faba in Biskra as Ditylenchus gigas.

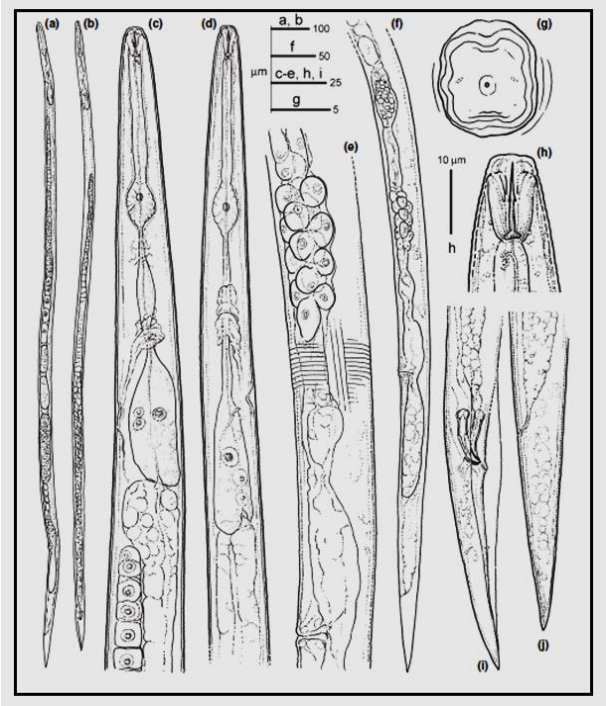

Fig. 2

: Schemas of the different parts of Ditylenchus gigas.n.sp, (a) female (b) male all the body (b)

IV. CONCLUSION

Stem and bulb nematodes in a Ditylenchus species complex are endoparasites, which attack a wide range of crops and cause stunting and swelling, causing significant economic

losses (Madani et al 2015). It is considered in Algeria and at the global level as a limiting factor in the development of legume and especially bean crops (Esquibet et al., 1998, Sellami et al, 1998 and Esquibet et al, 2003).

Several authors have suggested that D.dipsaci is a set of races (comprising at least 7 races) and in particular, that the "giant race" should be considered as a distinct taxon. In Algeria The Ditylenchus genus is traditionally identified with general typological morphology because in this population, adult nematodes were considerably larger than those currently observed, making breed recognition a very difficult task (Sturhan et al, 1991). This breed has damage that differs from that caused by D. dipsaci stricto sensu in a heavier distribution across the main stem, leaves and pods, and in the highest percentage of infected seeds, which could be of concern (Hooper 1984 in Esquibet, 1998)

The description of nematode species is particularly. The giant race of Ditylenchus dipsaci is based on morphometric and morphological records, which was obtained for the first time in Algeria with these types of measurements from populations of D. dipsaci collected on V. faba in the region of Biskra and which corroborate with those obtained in southern Italy and Spain and Lebanon, Vovlas et al. (2011) and finally in Iran in 2013 (Tanha et al 2013).

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