



Morphometric Variations of Cyst Nematodes in Tamil Nadu

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Morphometric variations in *Heterodera cajani* samples collected from different districts of Tamil Nadu showed variability in respect of cyst length and width, cone and neck length, fenestral width, L/W ratio; fenestral and vulval slit length. Biochemical analysis also revealed high proportion of protein (0.453-0.799 µg/g) and low proportion of carbohydrate (0.045-0.098 µg/g) and lipid (0.014-0.066 µg/g) at varying levels among different populations of *Heterodera cajani* and *Globodera rostochiensis*.

Key words: Biochemical and morphometric variations, cyst nematodes, Tamil Nadu.

The pigeonpea cyst nematode, *Heterodera cajani* is a major cyst forming nematode of India and widely distributed in the states of Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu (Kaushal *et al.*, 2007). Swarup *et al.* (1964) first recorded the presence of *H. cajani* in pigeonpea fields at the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi under the name *Heterodera trifolii*. Subsequently Koshy in 1967 identified it as new species of *Heterodera* and named it as *H. cajani* after detailed taxonomic studies. The study on the morphometric variations is an important area of nematode taxonomy as it leads to the identification of new species races or pathotypes.

The second stage juveniles, cysts and eggs of *Heterodera cajani* were redescribed by means of measurement, drawings and microphotographs (Majid Ollia *et al.*, 2004). Kum Kum Walia and Bajaj (2000) studied the extent of variations in morphology and morphometry of two host races of pigeonpea and cluster bean in *Heterodera cajani*. The morphological variations in ten populations of *H. cajani* in relation to eggs, cysts and cone top structures were described by Abdollahi *et al.* (2007). In continuation, attempts were made to study the morphometric variations of *H. cajani* occurring in Tamil Nadu in the present study.

Materials and Methods

A random survey was undertaken in Tamil Nadu in the districts of Erode, Tiruchirapalli, Coimbatore, Villupuram, Pudukkottai, Thoothukudi, Madurai, Nilgiris, Dharmapuri, Krishnagiri and Salem and altogether 206 soil samples were collected from pulses, sesame, wheat, soybean, carrot, sugar beet, cruciferous vegetables, peas, potato, white clove, sugarcane, rice, maize and banana. The soil samples were collected at random at a depth of 15-30 cm from the rhizosphere region and shade dried. The dry soil samples were processed for the separation of *H. cajani* by Fenwick can method (Fenwick, 1940).

Soil samples were observed under a stereozoom microscope and cysts were picked out one by one using mounting needle and fine camel hair brush. Nematode samples were stored in eppendorf tubes and properly labeled for further studies. Dry cysts were soaked in water for 24 h. The moist cysts were placed on a glass slide and the posterior end was cut using a scalpel so that the fenestral area was at the centre. The internal content of the cysts were removed using a pick. The outer edge of the cone was trimmed with a scalpel and the vulval cone was placed in a slide containing molten glycerin jelly and properly oriented as the jelly solidified and cover slip (circular 10 mm) was placed and sealed with nail polish (Kornobis, 1976).

Morphometric variations of *Heterodera cajani* within and between populations

Cyst and cone top structures and morphometry including cyst length and width, L/W ratio, neck, cone, vulval slit and fenestral length, width of fenestra were measured under a stereozoom microscope with image analyzer using 'Biovis' software. Fenestral width was measured at the widest part and length from outermost posterior edges of semifenestrae. The cyst length, cyst width, L/W ratio, neck and cone and fenestral and vulval slit length, fenestral width were measured and the coefficient of variance worked out and the variability was classified as least variable (< 8%), moderately variable (8–12%), and highly variable (> 12%).

Biochemical analysis

Matured cysts collected from (1g each in eppendorf tube) different host plants were homogenized with 5ml ethanol and centrifuged at 3000 rpm. The supernatant was decanted and added with 3% chloroform-ethanol solution followed by centrifugation at 3000 rpm. Again the supernatant was decanted and mixed with 10ml trichloroacetic acid, and heated at 80°C. Then the contents were centrifuged to separate the supernatant liquid carefully with the help of micropipette in another

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tube. The extracts were used for the estimation of carbohydrate, lipid and protein.

Estimation of carbohydrate

Based on the method of Roe and Dailey (1966) the carbohydrate extraction was done by digesting sonicated samples in 3 ml of NaOH (1N). The test tubes were placed in a boiling water bath for 30 min. and shaken to disintegrate the tissues of nematodes. After completion of digestion, the tubes were kept at room temperature. Further 1N perchloric acid (6 ml) was added to the test tubes and shaken vigorously, allowed to stand for 15 min. and the content was centrifuged at 3700 rpm for 10 min to settle the debris. The precipitation of protein in the supernatant was collected. To the supernatant, ethanol (95%) containing 0.1% lithium chloride (5 ml), was added and mixed thoroughly. The tubes were allowed to stand overnight at room temperature for the precipitation of carbohydrate. Finally the precipitated samples were centrifuged at 3700 rpm for 10 min. The supernatant was discarded carefully and the precipitate containing carbohydrate was collected washed and allowed to stand for few hours to evaporate ethanol and finally dissolved in known volume of double distilled water.

The aliquots were suitably mixed with anthrone reagent (4 ml) containing 0.05% (w/v) anthrone, thiocerea 3% (w/v) and sulphuric acid (72%). The tubes were placed in water bath for 15 min., cooled to room temperature and optical density at 620 nm was measured with brown filter against blank.

Estimation of lipids

Total lipid content of the nematode was extracted using the method of Folch and Stanley (1957). The tissue samples were homogenized in a stopper test tube at 40°C for overnight. The samples were centrifuged at 3500rpm for 15 min and the residue was re-extracted in chloroform (2.1 ml). The supernatants were pooled together in a stopper test tube; Then 0.2 ml KCl (0.88%), was added, mixed thoroughly and allowed to stand for some time to separate the mixture into two phases. Suitable volumes of lower phase, containing most of the dried volume of lipid dissolved in chloroform, were taken for the estimation of lipid. The total lipid content was estimated by employing the method of Zollner and Krish (1962). The extracted lipids (1ml) and concentrated sulphuric acid (4 ml) were mixed in a test tube carefully on a cyclomixer. The test tubes containing samples were boiled for 10 min and then allowed to cool at room temperature. Required volume of (4 ml) of Zollner reagent (containing 13 mM Vanillin dissolved in 14M orthophosphoric acid) was added to the sample.

Optical density of both sample and control (solution only) was measured at 550 nm. The lipid content was calculated from a calibrated curve prepared using a standard lipid of known strength.

Estimation of protein

Total protein was extracted by simple homogenization followed by centrifugation of the tissue at 3700rpm. Further the total protein was estimated by the dye binding method of Bradford (1976) as modified by Spector (1978). The dye reagent was prepared by adding together the Coomassie Brilliant Blue G-250 (0.01% w/v), orthophosphoric acid (10% v/v) and ethanol 96% (5% v/v). For standard protein assay, the Bovine Serum Albumin (BSA), prepared in 0.1N NaOH was used as standard. The protein content was measured in the linear range of 1.0 to 2.1 ml (standard curve) of total assay volume at 595 nm.

Results and Discussion

Morphometric variations of Heterodera cajani within populations

Least variability in the cyst length of less than 8 per cent was noticed in four locations and among which the lowest variation of 1.85 per cent was noticed in Dasampatti followed by S.P.Koundanur (2.91%) population. Moderate variability was noticed in three populations and in seven populations the variability in cyst length was more than 12 per cent and the highest variation of 29.19 per cent was noticed in Unjapalayam population (Table 1).

Population from Vaikalpalam had the lowest variation of 6.77 per cent and moderate variability in the cyst width was noticed in Budapadi, Unjapalayam and Dasampatti population with the coefficient of variance ranging from 10.13 to 11.77 per cent. Ten populations showed highest variation ranging from 12.22 to 23.54 per cent and it was the highest with Paiyur population.

Least variation in the L/W ratio was noticed in two populations viz., Vaikalpalam (0.07%) and Seelampatti (0.29%) and moderate variability was noticed in three populations ranging from 10.49 to 11.66 per cent. The highest variability was noticed in ten populations ranging from 13.53 to 26.79 per cent and it was highest with Mottupatty population.

Of the fourteen populations, six populations showed the least variations in the neck length ranging from (2.45-7.19%) and it was lowest in Dasampatti population. Moderate variability was noticed in five populations ranging from 8.96 to 11.14 per cent. High variability was noticed in three populations and the highest was noticed with Vaikalpalam population (25.72%).

Least variability in cone length was noticed in four populations ranging from 3.69-7.90 per cent, moderate variability from 8.09 to 11.57 per cent was noticed in six populations and the highest variability was noticed in four populations. The highest variability in cone length was noticed in Vaikalpalam (20.31%).

Table 1. Variability of *Heterodera cajani* cyst characters within the populations

Character	Least variable (0-8%)	Moderate variable (8-12%)	Highly variable (>12%)	
Cyst length	Vaikalpalam (6.67)	Budapadi (11.07)	Unjalalayam (29.19)	
	S.P.Koundanur (2.91)	Arasampattu (10.52)	Seelampatti (14.36)	
	Dasampatti(1.85)	Ettayapuram (9.14)	Vamban (19.47)	
	Neikuppai(7.86)		Coimbatore (13.13)	
			Poovalur (15.89)	
			Mottupatti (13.85)	
			Paiyur (9.14).	
Cyst width	Vaikalpalam (6.77)	Budapadi (10.13)	Seelampatti (14.03)	
		Unjalalayam (10.85)	S.P.Koundanur (13.57)	
		Dasampatti (11.77)	Vamban (13.28)	
			Arasampattu(17.19)	
			Coimbatore (20.43)	
			Neikuppai (15.38)	
			Poovalur (19.36)	
			Mottupatti(20.59)	
			Paiyur (23.54)	
			Ettayapuram (12.22)	
L/W ratio	Seelampatti (0.29)	Budapadi (10.73)	Unjalalayam (17.94)	
	Vaikalpalam (0.07)	S.P.Koundanur (10.49)	Vamban (21.37)	
		Paiyur (11.66)	Arasampattu (18.96)	
			Dasampatti (13.53)	
			Coimbatore (29.93)	
			Neikuppai (14.68)	
			Poovalur (23.22)	
			Mottupatti (26.79)	
			Ettayapuram (19.13)	
	Fenestral width	Unjalalayam (5.25)	Vaikalpalam (11.87)	S.P.Koundanur(18.48)
Seelampatti (5.63)		Vamban (8.69)	Arasampattu (17.69)	
		Paiyur (9.24)	Dasampatti (17.82)	
			Coimbatore (22.22)	
			Neikuppai (18.18)	
			Poovalur (16.91)	
			Budapadi (12.43)	
			Mottupatti (17.85)	
			Ettayapuram (15.93)	
Vulval slit length		Unjalalayam (7.59)	Budapadi (9.02)	Vaikalpalam (14.16)
	Seelampatti (2.40)	Dasampatti (11.78)	Arasampattu (16.27)	
	S.P.Koundanur (6.52)	Ettayapuram (8.85)	Coimbatore (25.26)	
			Neikuppai (12.79)	
			Poovalur (16.40)	
			Mottupatti (15.44)	
			Paiyur (15.13)	
			Vamban (12.36)	
	Neck length	S.P.Koundanur (5.66)	Budapadi (10.41)	Vaikalpalam (25.72)
		Dasampatti (2.45)	Unjalalayam (10.80)	Neikuppai (13.40)
Coimbatore (4.09)		Seelampatti (10.68)	Mottupatti (12.41)	
Poovalur (5.57)		Vamban (11.14)		
Paiyur (6.15)		Arasampattu (8.96)		
Ettayapuram (7.19)				

Character	Least variable (0-8%)	Moderate variable (8-12%)	Highly variable (>12%)
Cone length	Unjapalayam (7.90)	Budapadi (10.11)	Seelampatti (18.82)
	Dasampatti (3.69)	S.P.Koundanur (9.11)	Vaikalpalam (20.31)
	Coimbatore (4.86)	Vamban (10.10)	Arasampattu (13.36)
	Paiyur (4.69)	Neikuppai (8.09)	Mottupatti (13.58)
		Poovalur (11.57)	
		Ettayapuram (8.35)	
Fenestral length	Seelampatti (4.18)	Vamban (11.26)	Budapadi (13.51)
	Vaikalpalam (5.91)	Paiyur (18.35)	Unjapalayam (19.0)
		Ettayapuram (11.48)	S.P.Koundanur (12.45)
			Arasampattu (17.12)
			Dasampatti (14.16)
			Coimbatore (26.42)
			Neikuppai (12.41)
		Poovalur (13.78)	
		Mottupatti (17.93)	

Numbers mentioned in parentheses are Coefficient of Variance

The highest variability in fenestral length was noticed with nine populations and the highest Coimbatore population (26.42%) ranked first in this regard. Three populations showed moderate variability ranging from 8.35 to 11.48 per cent and only two populations viz., Seelampatti and Vaikalpalam showed variability of 4.18 and 5.91 per cent.

High variability in fenestral width was noticed in eight populations and it was ranged from 15.93 to 22.22 per cent and the highest being in Coimbatore population. Three populations showed moderate variability of 8.69 to 11.87 per cent and two populations viz., Unjapalayam and Seelampatti showed the least variability of 5.25 and 5.63 per cent respectively.

Table 2. Morphometric variations of *Heterodera cajani*

The least variability in the length of vulval slit was noticed in three populations viz., Seelampatti, S.P.Koundanur and Unjapalayam which recorded 2.40 per cent, 6.52 per cent and 7.59 per cent respectively. Three populations recorded moderate variations with coefficient of variance values of 8.85 to 11.78 per cent. The highest variability was noticed in eight populations and the highest with Coimbatore population (25.26%).

Morphometric variations of *Heterodera cajani* between populations

Variations in respect of cyst length and width, L/W ratio; neck, cone and fenestral length, fenestral width and vulval slit length between the populations were worked out and furnished in Table 2.

between populations

Character	Populations									
	Erode	Pudukkottai	Villupuram	Dharmapuri	Coimbatore	Trichy	Salem	Krishnagiri	Thoothukudi	
Cyst length	Mean	465.52	491.83	503.46	453.06	477.71	461.01	504.93	420.47	503.42
	C.V.	16.05	19.47	10.52	1.85	13.13	11.87	13.85	12.04	9.14
Cyst width	Mean	310.63	342.52	295.84	342.90	317.60	315.11	305.83	329.42	323.53
	C.V.	13.15	13.28	17.19	11.77	20.43	16.64	20.59	23.54	12.22
L/W	Mean	1.50	1.45	1.74	1.33	1.57	1.49	1.71	1.29	1.58
	C.V.	11.92	21.37	18.96	13.53	29.93	19.25	26.79	11.66	19.13
Neck length	Mean	101.72	92.54	97.64	115.55	93.74	100.91	99.03	93.18	91.03
	C.V.	10.93	11.14	8.96	2.45	4.09	9.91	12.41	6.15	7.19
Cone length	Mean	63.69	57.82	64.04	77.44	55.22	68.92	65.30	55.32	56.38
	C.V.	10.74	10.10	13.36	3.69	4.86	9.44	13.58	4.69	8.35
Fenestral length	Mean	49.59	43.67	44.94	38.27	50.34	46.29	45.29	35.37	46.57
	C.V.	16.30	11.26	17.12	14.46	26.42	12.94	17.93	8.35	11.48
Fenestral width	Mean	35.13	30.33	34.07	31.3	37.21	36.22	34.70	25.11	35.36
	C.V.	11.36	8.69	17.69	17.82	22.22	18.20	17.85	9.24	15.93
Vulval slit length	Mean	44.91	37.78	43.53	34.64	41.83	41.70	44.16	37.27	47.78
	C.V.	11.65	12.36	16.27	11.78	25.26	13.87	15.44	15.13	8.85

Among the nine populations studied, only one population viz., Dharmapuri falls under the lowest variable (1.85%). Three populations viz., Villupuram (10.52%), Tiruchirapalli (11.87%) and Thoothukudi (9.14%) recorded moderate variation and four populations viz., Erode (16.05%), Pudukkottai (19.47%), Salem (13.85%) and Krishnagiri (12.04%) showed the highest variability in respect of cyst length.

None of the populations falls under least variation in respect of cyst width. Only one population viz., Dharmapuri showed moderate variation with 11.77

Table 3. Quantitative estimation of *Heterodera cajani* of Tamil Nadu districts for carbohydrate, lipid and protein

Details ($\mu\text{g/g}$)	Pudukkottai	Tiruchirapalli	Erode	Villupuram	Dharmapuri	Salem	Krishnagiri	Coimbatore	Thoothukudi
Carbohydrate	0.083	0.074	0.065	0.062	0.049	0.054	0.063	0.098	0.045
Lipid	0.039	0.031	0.033	0.023	0.025	0.063	0.025	0.014	0.066
Protein	0.724	0.453	0.678	0.566	0.754	0.587	0.799	0.676	0.768

and four populations showed moderate variability in neck length ranging from 8.96 to 11.14 per cent and Salem population only showed the highest variability of 12.41 per cent.

The length of cyst cone showed the least variability in three populations viz., Dharmapuri (3.69%), Krishnagiri (4.69%) and Coimbatore (4.86%) and moderate variability observed in four populations viz., Thoothukudi (8.35%), Tiruchirapalli (9.44%), Pudukkottai (10.10%) and Erode (10.74%). The two population's viz., Salem (13.58%) and Villupuram (13.36%) showed the highest variation.

None of the populations showed the least variability in respect of fenestral length. Three populations viz., Pudukkottai (11.26%), Thoothukudi (11.48%) and Krishnagiri (8.35%) showed moderate variability and the rest of the six populations showed high variability ranging from 12.94 to 26.42 per cent and the highest being with the Coimbatore population.

None of the populations showed the least variability in fenestral width and three populations viz., Pudukkottai (8.69%), Krishnagiri (9.24%) and Erode (11.36%) populations showed moderate variability and the rest of the six populations showed high variability ranging from 15.93 to 22.22 per cent and the highest being with the Coimbatore population.

None of the populations showed the least variability and moderate variability in the vulval slit length was noticed in three populations viz., Thoothukudi (8.85%), Erode (11.05%) and Dharmapuri (11.78%). The remaining six populations showed high variability from 12.36 to 25.26 per cent and the highest being with Coimbatore population.

The variations within the populations and between the populations of *Heterodera cajani* was worked out and the results showed that the Vaikalpalam

per cent and all the remaining eight populations showed high variation and the highest being with Krishnagiri population (23.54%).

None of the populations showed least variation and only two population viz., Krishnagiri (11.66%) and Erode (11.92%) showed moderate variability in respect of L/W ratio and all other populations showed the highest variability ranging from 13.53 to 29.93 per cent and the highest being in Coimbatore population.

Four populations viz., Dharmapuri (2.45%), Coimbatore (4.09%), Krishnagiri (6.15%) and Thoothukudi (7.19%) showed the least variability

population showed the least variability in respect of cyst length, cyst width, L/W ratio and the fenestral length but on the contrary the same population showed the highest variability in respect of neck length and vulval slit. In the same way, Seelampatti population showed the least variations in respect of L/W ratio, fenestral length, fenestral width and length of vulval slit but there was high variation in cone length and cyst width.

The Mottupatty population of *Heterodera cajani* showed high degree of variability in respect of all the morphometric values viz., cyst length, cyst width, L/W ratio; neck, cone and fenestral length, fenestral width and length of vulval slit. Coimbatore population of *H. cajani* also showed the highest variability in cyst length, cyst width, L/W ratio, fenestral length, fenestral width and length of vulval slit. However the population showed the least variability in neck and cone length.

With regard to morphometric variations, Villupuram population of *H. cajani* showed the highest variation between the populations in respect of cyst width, L/W ratio; neck, cone and fenestral length, fenestral width and length of vulval slit. The Coimbatore populations showed high degree of variability in the above and it is more than 20 per cent. The least variations was noticed in Dharmapuri populations.

The existence of biotypes/host races of different cyst nematode species have been reported from India. Physiological, molecular and morphometrical studies have been carried out to reveal the intra-specific variations within cyst nematodes. Mathur *et al.* (1974) used various differentials (cereals and grasses) to indicate the presence of five biotypes of *H. avenae*. Swarup *et al.* (1979) used cereals as differentials to compare six populations of *H. avenae* and reported two biotypes. Three races of *H. zaeae* have been reported from Haryana using maize and vetiver as differentials (Bajaj and Gupta, 1994). Hisar

population was found to multiply both on maize and vetiver whereas Ambala and Sonipat population multiplied only on maize and vetiver respectively. Ringer *et al.* (1987) reported that population of *H. zea* from USA, India and Egypt differed in their ability to reproduce on certain hosts. Srivastava and Sethi (1984) compared populations from Pusa Bihar, Delhi and Udaipur in Rajasthan for their virulence and ability to reproduce and multiply on different cultivars of maize and found that they varied significantly in their ability to reproduce and multiply on these hosts. They also found the population from Pusa Bihar to be highly virulent. This virulence in one population of the same species is of special interest. This may be explained by looking at the genotypic and phenotypic variability in these populations.

Use of cone top structure to differentiate species and genera of cyst nematodes was reported by Mulvey (1972). He studied the posterior ends of the cysts of 39 species of *Heterodera* and arranged them into 5 major groups based on variations in cone top structure and cysts. Abdollahi *et al.* (2007) studied the morphometric variations in ten populations from India and showed similarity of Coimbatore and Ghaziabad populations based on cone top structures. The coefficient of variance values in respect of cyst length and cyst width was between 6 to 23 per cent and 9 to 36 per cent respectively. The same pattern was also noticed in the present study.

Kum Kum Walia and Harish Bajaj (2000) compared two races of *H. cajani* morphologically and morphometrically and the two races were differentiated in vulval cone structures. The morphometric studies are considered as one of the major tools for identification of species or races within the population. In the present study, highest variations have been noticed between the populations of *H. cajani* collected from Coimbatore district and the coefficient of variation of cone structures was more than 20 per cent. It clearly indicates the possibility of the existence of closely related species or races in these areas.

Knowledge of variability in any plant pathogen in different geographical populations is important for planning suitable management schemes. Hence the variability discovered through this study will pave a way for more detailed studies. This could also serve as a base line for further population genetic studies.

Biochemical analysis of cyst nematodes

The biochemical analysis of *H. cajani* carried out with nine populations revealed that the protein was in the highest proportions ranging from 0.453-0.799 µg/g followed by carbohydrate (0.045-0.098 µg/g) and lipid (0.014-0.066 µg/g). Not much variations were noticed between the populations except a marginal increase in the carbohydrate content in the Coimbatore population.

The carbohydrate, lipid, protein content of *H. cajani* in nine populations were carried out and

almost uniform pattern in respect of carbohydrate was noticed in all the populations (Table 3). The lipid and protein content of *H. cajani* in all the nine populations also showed similar trend as above.

The carbohydrate, lipid, protein content of *Globodera rostochiensis* from three populations were carried out. Almost uniform pattern in respect of carbohydrate was noticed in all the populations (Table 4).

Table 4. Quantitative estimation of *Globodera rostochiensis* of Nilgiris district for carbohydrate, lipid and protein

Parameters (µg/g)	Nanjanad	Vijayanagaram	Kotagiri
Carbohydrate	0.068	0.051	0.053
Lipid	0.137	0.066	0.077
Protein	0.718	0.674	0.753

The lipid and protein contents of *Globodera rostochiensis* recorded in all the three populations from Nanjanad, Vijayanagaram and Kotagiri were 0.137, 0.066 and 0.077 µg/g; 0.718, 0.674 and 0.753 µg/g respectively.

The same trend was noticed with the *G. rostochiensis* in respect of carbohydrate, lipid and protein. The lipid profile in the Nanjanad population was slightly higher than Vijayanagaram and Kotagiri populations. Biochemical analysis of female nematodes of *Meloidogyne* spp. was carried out by Nidhityagi *et al.*, (2010) to find out the species of root knot nematodes in vegetables. The present study was carried out to know variations in the profiles of carbohydrates, protein and lipid which is useful for differentiating the species, sub species or races. The present study was carried out to know variations in the profiles of carbohydrates, protein and lipid which is useful for differentiating the species, sub species or races.

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