

# ANALYSIS OF CYST AND CONE TOP MORPHOMETRICS OF INDIAN POPULATIONS OF MAIZE CYST NEMATODE

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**Abstract:** Hierarchical cluster analysis based on cyst and cone top morphometric means including cyst length, cyst width, cyst length to cyst width ratio, vulval slit length, vulval bridge length, vulval bridge breadth, under bridge breadth, length of fenestra, breadth of fenestra, distance from anus to fenestra and number of secondary bullae was used to learn more about cyst and cone top morphometric means and their relationships for six populations of *Heterodera zaeae* from Indore, Ludhiana, Delhi, Udaipur, Kanpur and Samastipur by using SPSS 13 for Windows computer software (SPSS Inc.). Values of proximity matrix based on cluster analysis of morphometrics and the dendrograms visually illustrated the grouping and relationships among populations. Intra specific variations in the different characters of the cone top structure revealed that Indore and Samastipur populations of *H. zaeae* were different as compared to other ones of *H. zaeae*. Cluster analysis of cyst morphometrics showed that the populations of *H. zaeae* from Kanpur and Delhi were different as compare to other four ones.

**Key words:** *Heterodera zaeae*, maize cyst nematode, morphometric variations, hierarchical cluster analysis

## INTRODUCTION

Maize is one of the top ranking cereals in the world agricultural economy both in terms of productivity and production. The world production in 2007 according to FAO was 766.23 million metric tons. India stands seventh in the world production of maize accounting for 15.5 million tons (USDA 2007). Although the economic loss caused by nematodes is estimated to be \$80 billion each year (Lilley *et al.* 1999), damage caused by them has not received adequate attention. *Heterodera zaeae*, the maize cyst nematode, is a major nematode pest of maize. *H. zaeae* was first described from 'Chapli' village in Udaipur district of Rajasthan, India by Koshy *et al.* (1970). It was later reported from Pakistan (Maqbool 1981), the Nile Valley, Egypt, (Abul and Ghorab 1981), USA (Sardanelli *et al.* 1981; Ringer *et al.* 1987; Eisenback *et al.* 1993) and Thailand (Chinnasri *et al.* 1995). It is found to be widely distributed now. In India it is widely distributed in the maize growing regions of the country causing good amount of damage to the maize crop (Srivastava and Sethi 1984). Besides maize, other plants like barley, wheat, rice and millets are good hosts for nematode (Srivastava and Swarup 1977; Ringer *et al.* 1987). Other good hosts reported for this nematode include Almond (Qasim and Ghaffar 1986), Tomato (Shahzad and Ghaffar 1986) *Capsicum annum*, *Corchorus capsularis*, and *Raphanus sativus* (Maqbool and Hashmi 1984) from Pakistan and vetiver reported from India (Lal and Mathur 1982). This nematode is widely distributed throughout many maize growing areas of the world.

Accurate identification is a prelude in efficient management designing. There are some numerical methods for analyzing the matrices of similarity to investigate the differences between populations of a nematode species. Multiple regression analysis, factor analysis, principal component analysis, and cluster analysis are some of the statistical techniques for the grouping individuals. Cluster analysis is a convenient method for organizing a large data set so information can be retrieved more efficiently and it can be easily understood without the need for complicated mathematical techniques. Data can nominally be summarized by a small number of groups of objects in a dendrogram generated by cluster analysis. The objective of this research was to learn more about cyst and cone top morphometric means and their relationships for six populations of *H. zaeae* from six regions in India.

## MATERIALS AND METHODS

During the course of investigation to assess the variability between populations of *Heterodera zaeae*, cysts of populations were collected from six maize growing localities namely Indore, Ludhiana, Delhi, Udaipur, Kanpur and Samastipur (Fig. 1). All the isolated populations were then cultured on maize grown in 10cm pots containing sterilized soil in the net house. Seventy-five days after raising pure culture the soil in the culture pots were processed using Cobb's sieving technique (Cobb 1918). Twenty and 100 mesh sieves were used for washing the soil. The cysts were collected and then processed as fol-

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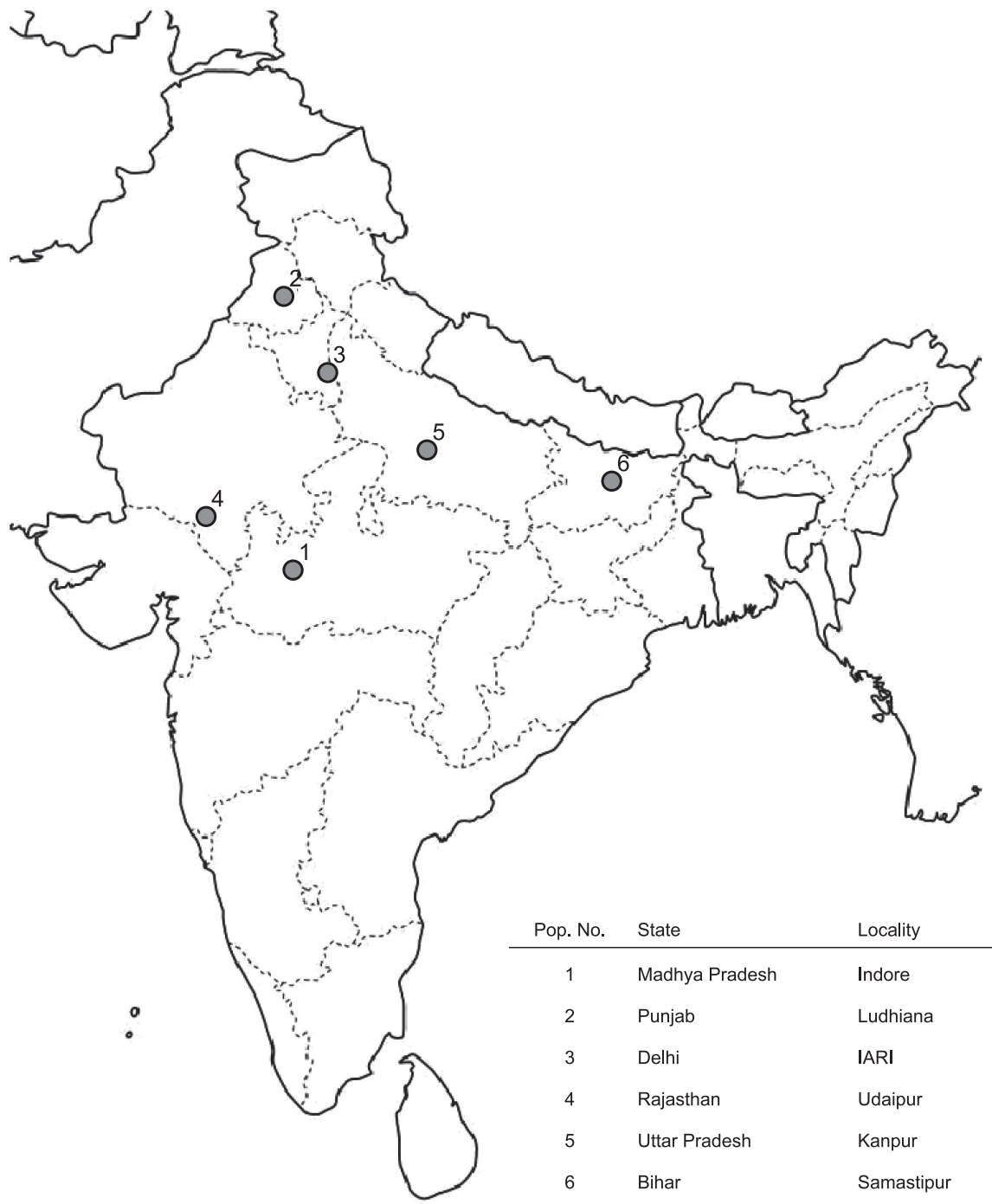


Fig. 1. List of localities from where populations of maize cyst nematode were collected

lows for detailed morphological studies. The cone tops of 20 cysts of each population were mounted in Canada balsam (Kornobis 1976). Identity of these populations as *H. zae* was confirmed as per the species descriptions given by Koshy *et al.* (1970).

Cyst and cone top structure morphometric means including cyst length, cyst width, cyst length to cyst width ratio, vulval slit length, vulval bridge length, vulval bridge breadth, under bridge breadth, length of fenestra, breadth of fenestra, distance from anus to fenestra were measured from Camera-Lucida drawings. Morphometric characters of cysts and cone top observations for each of

the populations were analyzed by ANOVA (Analysis of variance). When the differences between groups were found to be significant, Tukey's test was used to determine the differences between means at prescribed level of  $\alpha = 0.05$ . Statistical values (ANOVA, mean, standard deviation, coefficient of variation, variance, minimum, maximum values) were calculated by the SPSS 13 for Windows computer software (SPSS Inc. Chicago, USA) (Tables 1, 2). On the basis of the coefficient of variation (CV%), the morphometric characters of cysts and cone top structures of mature cysts were assessed as least variable, moderately variable and highly variable. The scale

Table 1. Summary of descriptive statistics on cyst morphometric results

Character	Population	N	Mean Tukey's Test Results	SD	CV%	SE	95% Confidence Interval for Mean		Min.	Max.
							lower bound	upper bound		
Cyst Length	Indore	20	461.70 a	28.98	6.28	6.48	448.14	475.26	419.00	503.00
	Ludhiana	20	412.90 b	42.78	10.36	9.57	392.88	432.92	359.00	483.00
	Delhi	20	416.35 b	36.03	8.65	8.06	399.49	433.21	362.00	471.00
	Udaipur	20	430.50 ab	40.88	9.50	9.14	411.37	449.63	367.00	492.00
	Kanpur	20	406.55 b	35.67	8.77	7.98	389.86	423.24	351.00	482.00
	Samastipur	20	434.95 ab	22.50	5.17	5.03	424.42	445.48	397.00	467.00
	Total	120	427.16	39.01	9.13	3.56	420.11	434.21	351.00	503.00
Cyst Width	Indore	20	311.50 ab	47.28	15.18	10.57	289.37	333.63	238.00	367.00
	Ludhiana	20	320.40 ab	28.87	9.01	6.45	306.89	333.91	278.00	363.00
	Delhi	20	323.30 ab	30.39	9.40	6.79	309.08	337.52	278.00	372.00
	Udaipur	20	326.25 a	37.72	11.56	8.43	308.60	343.90	268.00	389.00
	Kanpur	20	304.40 ab	40.68	13.36	9.10	285.36	323.44	237.00	373.00
	Samastipur	20	292.65 b	30.48	10.42	6.82	278.39	306.91	250.00	359.00
	Total	120	313.08	37.63	12.02	3.44	306.28	319.89	237.00	389.00
L/W ratio	Indore	20	1.52 a	0.28	18.42	0.06	1.39	1.65	1.18	2.07
	Ludhiana	20	1.30 b	0.20	15.38	0.04	1.21	1.40	0.99	1.68
	Delhi	20	1.30 b	0.17	13.08	0.03	1.22	1.38	0.99	1.59
	Udaipur	20	1.34 ab	0.22	16.42	0.05	1.24	1.44	1.01	1.75
	Kanpur	20	1.36 ab	0.20	14.71	0.05	1.26	1.45	1.10	1.92
	Samastipur	20	1.50 a	0.16	10.67	0.04	1.42	1.58	1.24	1.81
	Total	120	1.39	0.22	15.83	0.02	1.35	1.43	0.99	2.07

SD – Standard Deviation, SE – Standard Error, CV – Coefficient of Variation

Figures followed by the same letter are not significantly different ( $p = 0.05$ ) from each other

fixed for ranking least variable, moderately variable and highly variable was  $< 8\%$ ,  $8\text{--}12\%$  and  $> 12\%$  (Tables 1, 2). Tukey's test was used to determine the differences between means at prescribed level of  $\alpha = 0.05$  and results of Tukey's test are displayed in tables 1 and 2. Hierarchical cluster analysis of characteristics of cysts and cone top structure using squared Euclidean distance definition was calculated and dendrograms were produced using average linkage (between groups) (Fig. 2, 3).

## RESULTS

**Cyst:** Summary of descriptive statistics and ANOVA of the measurements of cyst is given in Table 1. The coefficient of variation (CV%) for length of cysts was low; it ranged from 5.17–10.36% signifying that it can be considered reliable character with taxonomic significance since the coefficient of variation for width of cysts was ranged 9.01–15.18% ranking moderately variable. The coefficient of variation (CV%) for L/W ratio shows range from 10.8–18.38, thus ranking this character under highly variable category, so this is not a reliable character. According to ANOVA for cyst length, cyst width and L/W ratio, differences between groups were found to be significant ( $F_{5;114} = 6.50$  ( $p < 0.001$ ), 2.48 ( $p = 0.036$ ) and 4.43 ( $p = 0.001$ ), respectively) so Tukey's test was used to determine the differences between means of all these characters at prescribed level of  $\alpha = 0.05$ .

According to Tukey's test results, highest cyst length was obtained in Indore population. The lowest for this

character was found in population from Kanpur followed by Ludhiana population which is at par with population from Delhi. Populations from Udaipur and Samastipur also were at par with each other having cyst length longer than Delhi population. On the basis of Tukey's test results for cyst length to cyst width ratio, population from Indore was at par with Samastipur population and the other four populations were at par with each other.

The dendrogram (Fig. 2) revealed that at the rescaled distance of 25 units all the populations were similar while at around 1 unit of such distance all the populations were distinct from each other. Two separate groups have been recognized. One includes Ludhiana, Delhi, Kanpur and Udaipur and the other group includes Indore and Samastipur. Hierarchical cluster analysis for the characteristics of the cyst showed that the Indore and Samastipur populations stood out from all other populations. It would be evident that Ludhiana, Delhi and Kanpur were very close to each other.

## Cone top

Summary of descriptive statistics and ANOVA of the measurements of cone top is given in table 2. The coefficient of variation (CV%) for vulval slit length and vulval bridge length was low; it ranged from 4.7 to 11.8% and 3.34–11.44, respectively, signifying that they can be considered reliable character with taxonomic significance since the coefficient of variation for length of fenestra was ranged from 4.98 to 15.69% ranking moderately variable. The coefficient of variation (CV%) for other characters

Table 2. Summary of descriptive statistics on cone top morphometric results

Character	Population	N	Mean Tukey's Test Results	SD	CV%	SE	95% Confidence Interval for Mean		Min.	Max.
							lower bound	upper bound		
Vulval slit length	Indore	20	38.20 bc	1.79	4.70	0.40	37.36	39.04	35	41
	Ludhiana	20	37.15 c	3.20	8.61	0.72	35.65	38.65	33	42
	Delhi	20	38.30 bc	4.52	11.80	1.01	36.18	40.42	32	46
	Udaipur	20	37.85 bc	2.60	6.87	0.58	36.63	39.07	34	43
	Kanpur	20	42.90 a	2.83	6.59	0.63	41.58	44.22	37	47
	Samastipur	20	40.20 ab	2.21	5.51	0.50	39.16	41.24	36	43
	Total	120	39.10	3.51	8.98	0.32	38.47	39.73	32	47
Vulval bridge length	Indore	20	46.40 ab	1.85	3.98	0.41	45.54	47.26	41	49
	Ludhiana	20	43.40 c	3.53	8.13	0.79	41.75	45.05	39	49
	Delhi	20	46.25 bc	5.29	11.44	1.18	43.77	48.73	38	53
	Udaipur	20	44.35 bc	2.98	6.72	0.67	42.96	45.74	37	49
	Kanpur	20	49.20 a	1.64	3.34	0.37	48.43	49.97	46	52
	Samastipur	20	45.85 bc	2.16	4.71	0.48	44.84	46.86	43	50
	Total	120	45.91	3.60	7.84	0.33	45.26	46.56	37	53
Vulval bridge breadth	Indore	20	5.50 cd	0.95	17.20	0.21	5.06	5.94	4	7
	Ludhiana	20	6.15 bc	0.59	9.55	0.13	5.88	6.42	5	7
	Delhi	20	6.85 ab	1.27	18.51	0.28	6.26	7.44	5	9
	Udaipur	20	7.40 a	1.39	18.81	0.31	6.75	8.05	6	10
	Kanpur	20	4.90 d	0.55	11.28	0.12	4.64	5.16	4	6
	Samastipur	20	5.80 c	0.52	9.02	0.12	5.56	6.04	5	7
	Total	120	6.10	1.25	20.43	0.11	5.87	6.33	4	10
Under bridge breadth	Indore	20	9.00 bc	1.12	12.49	0.25	8.47	9.53	7	11
	Ludhiana	20	8.60 c	0.50	5.84	0.11	8.36	8.84	8	9
	Delhi	20	9.85 b	1.35	13.69	0.30	9.22	10.48	8	12
	Udaipur	20	8.95 bc	2.06	23.06	0.46	7.98	9.92	6	12
	Kanpur	20	12.25 a	1.12	9.13	0.25	11.73	12.77	11	14
	Samastipur	20	8.05 c	0.83	10.26	0.18	7.66	8.44	7	9
	Total	120	9.45	1.84	19.49	0.17	9.12	9.78	6	14
Length of fenestra	Indore	20	44.05 c	2.44	5.54	0.55	42.91	45.19	40	47
	Ludhiana	20	45.60 c	7.16	15.69	1.60	42.25	48.95	36	56
	Delhi	20	50.25 a	4.25	8.46	0.95	48.26	52.24	42	56
	Udaipur	20	45.85 bc	3.70	8.08	0.83	44.12	47.58	41	53
	Kanpur	20	50.10 ab	5.93	11.83	1.33	47.33	52.87	41	59
	Samastipur	20	47.15 abc	2.35	4.98	0.52	46.05	48.25	43	50
	Total	120	47.17	5.11	10.83	0.47	46.24	48.09	36	59
Breadth of fenestra	Indore	20	26.60 b	4.55	17.09	1.02	24.47	28.73	20	34
	Ludhiana	20	29.00 b	2.81	9.69	0.63	27.69	30.32	22	33
	Delhi	20	33.55 a	5.64	16.82	1.26	30.91	36.19	23	40
	Udaipur	20	30.15 ab	2.74	9.08	0.61	28.87	31.43	24	35
	Kanpur	20	30.15 ab	5.83	19.35	1.30	27.42	32.88	21	38
	Samastipur	20	26.50 b	3.58	13.50	0.80	24.83	28.17	20	33
	Total	120	29.33	4.91	16.76	0.45	28.44	30.21	20	40
Distance from anus to fenestra	Indore	20	25.00 ab	3.09	12.35	0.69	23.55	26.44	20.33	29.53
	Ludhiana	20	23.99 ab	2.36	9.82	0.53	22.88	25.09	20.49	26.95
	Delhi	20	26.82 a	3.65	13.61	0.82	25.11	28.52	21.29	32.44
	Udaipur	20	23.11 b	2.43	10.54	0.54	21.97	24.25	20.19	27.88
	Kanpur	20	26.12 a	4.42	16.94	0.99	24.05	28.19	18.04	31.98
	Samastipur	20	19.85 c	2.51	12.66	0.56	18.67	21.02	15.07	23.82
	Total	120	24.15	3.86	15.98	0.35	23.45	24.84	15.07	32.44
Number of secondary bullae	Indore	20	5.90 b	1.25	21.23	0.28	5.31	6.49	4	8
	Ludhiana	20	4.15 c	1.04	25.06	0.23	3.66	4.64	3	6
	Delhi	20	3.40 c	1.23	36.21	0.28	2.82	3.98	2	5
	Udaipur	20	11.50 a	2.14	18.61	0.48	10.50	12.50	8	14
	Kanpur	20	1.50 d	0.61	40.47	0.14	1.22	1.78	1	3
	Samastipur	20	0.15 e	0.37	244.20	0.08	-0.02	0.32	0	1
	Total	120	4.43	3.87	87.23	0.35	3.73	5.13	0	14

SD – Standard Deviation, SE – Standard Error, CV – Coefficient of Variation

Figures followed by the same letter are not significantly different ( $p = 0.05$ ) from each other

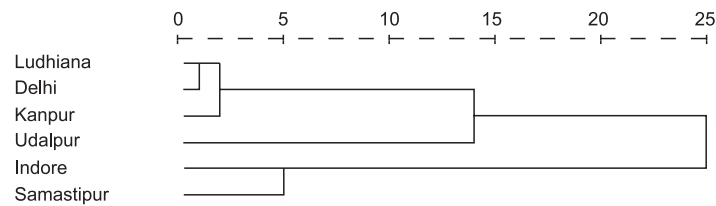


Fig. 2. Relationship among different populations of *H. zae* on the basis of hierarchical cluster analysis of characteristics of cyst

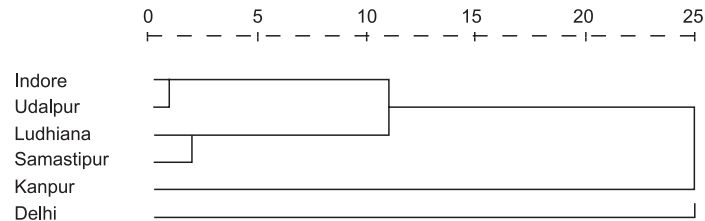


Fig. 3. Relationship among different populations of *H. zae* on the basis of hierarchical cluster analysis of characteristics of cone top

were high, thus ranking these characters under highly variable category. According to ANOVA for vulval slit length, vulval bridge length, vulval bridge breadth, under bridge breadth, length of fenestra, breadth of fenestra, distance from anus to fenestra and number of secondary bullae, differences between groups were found to be significant [ $F_{5;114} = 10.07$  ( $p < 0.001$ ),  $7.96$  ( $p < 0.001$ ),  $18.55$  ( $p < 0.001$ ),  $28.05$  ( $p < 0.001$ ),  $5.93$  ( $p < 0.001$ ),  $7.26$  ( $p < 0.001$ ),  $12.48$  ( $p < 0.001$ ) and  $208.11$  ( $p < 0.001$ ), respectively] so Tukey's test was used to determine the differences between means of all these characters at prescribed level of  $\alpha = 0.05$ .

According to Tukey's test results, longest vulval slit and vulval bridge were obtained in Kanpur population. The shortest for these characters were found in populations from Ludhiana and Udaipur. Tukey's test on length of fenestra revealed that populations from Delhi and Kanpur have longest fenestra which were at par with each other. The other four populations were at par with each other for this character. The dendrogram (Fig. 3) revealed that at the rescaled distance of 25 units all the populations were similar while at around 1 unit of such distance all the populations were distinct from each other. Three separate groups have been recognized. Delhi and Kanpur populations in two separate groups and the other group includes the other four populations. Hierarchical cluster analysis for the characteristics of the cone top showed that the Kanpur and Delhi populations stood out from all other populations. It would be evident that Indore and Udaipur were very close to each other.

## DISCUSSION

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 cere-

als as differentials to compare six populations of *H. avenae* and reported two biotypes. Three races of *H. zae* 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. Three *H. zae* biotypes have also been reported from Egypt (Khair *et al.* 1989). Ringer *et al.* (1987) reported that population of *H. zae* 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.

Though host range remained the main criteria for the designation of *Heterodera* species for several years, a few workers also laid stress on morphological differences. Franklin (1951) summarized the diagnostic characters used by earlier workers, including the size and shape of the cyst, the presence or absence of regularly arranged punctations on the cyst wall, sub-crystalline layer, egg-sac or yellow cyst phase, the pale color of *H. humuli*, the anal fenestration of *H. punctata*. Color of the cyst, presence or absence of sub crystalline layer and patterns on cyst wall are other characters that can be used at the genus and species levels. The shape of cysts is of taxonomic value in identification of cyst forming nematodes. Thorne (1928) described the grass cyst nematode, *H. punctata* from Canada on the basis of spherical shape of the cyst. 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. Walia and

Bajaj (2000) compared the pigeonpea and cluster bean races of *H. cajani* morphologically and morphometrically. These two races were differentiated in vulval cone structure and male morphology. However, the mean values for these characters were overlapping and well within the range of the species. Abdollahi *et al.* (2006 and 2007) used morphometric characters of different stages to show the intra-specific variations of ten Indian populations of *H. cajani*. In their study the Indore population stood out from all other studied populations.

In present study, the Kanpur and Delhi populations exhibited variations from other populations in vulval slit length, vulval bridge length, underbridge breadth, length of fenestra, breadth of fenestra and distance from anus to fenestra of cone top structure, which were on the higher side. The dendrogram also shows this variation within the populations. These are however considered as intra-specific variations. Assessment of the morphometric evaluation of different populations revealed that vulval slit length, vulval bridge length and length of fenestra were found most stable in all the six populations. Other characters were found to be highly variable.

This study has evaluated cluster analysis as a method for grouping and distinguishing *H. zaeae* populations by morphometric parameters. It is obvious that some morphological characters are useful in identification but we have used morphometric data to clarify the relationships within this group, making it easier to classify populations. Our results show that cluster analysis is suitable for studying within-species variability but it should be mentioned that the cluster analysis is only based on morphometric data. So, it does not reflect phylogenetic relationships. Variability discovered through this study is encouraging and will pave way for more detailed studies looking at the host range of each of the populations. This could also serve as a base line for further population genetic studies to look at dispersal behavior of this nematode.

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## POLISH SUMMARY

### ANALIZA MORFOMETRII CYSTY I SZCZYTU STOŻKA WULWALNEGO INDYJSKICH POPULACJI CYSTY NICIENIA KUKURYDZY

W celu lepszego poznania morfometrii cysty i szczytu stożka wulwalnego sześciu populacji *Heterodera zae* z rejonów Indore, Ludhiana, Delhi, Udaipur, Kanpur i Samastipur (India) zastosowano hierarchiczną analizę grupową używając oprogramowania SPSS 13 dla Windows

(SPSS Inc.). Analiza obejmowała długość i szerokość cysty oraz stosunek tych wartości, długość szczeliny wulwy, długość i szerokość mostu wulwy, szerokość mostu dolnego, długość i szerokość okienka, odległość odbytu od okienka oraz liczbę wtórnych bulli. Wartości macierzy oparte na analizie grupowej morfometrii i dendrogramach ilustrują grupowanie się i związki pomiędzy populacjami. Wewnątrzgatunkowe zmienności w strukturach szczytu stożka wulwalnego różnych populacji wykazują, że populacje *H. zae* z Indore i Samastipur różnią się od pozostałych, a analiza grupowa morfometrii cyst wskazuje na różnice pomiędzy populacjami *H. zae* z Kanpur i Delhi, a czterema pozostałymi.

