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Full Length Article

Hierarchical Cluster Analysis of Indian Populations of *Heterodera zeae* Based on Second Stage Juveniles and Egg Morphometrics

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ABSTRACT

Hierarchical cluster analysis, which identifies groups of samples that behave similarly or show similar characteristics, was used to describe and to make spatial and temporal comparisons of different Indian populations of *H. zeae* in heterogeneous environments. Cluster analysis based on egg and second stage juveniles' morphometric means were used to examine the morphometric relationships and create dendrograms for six populations of *Heterodera zeae*. Values of proximity matrix based on cluster analysis of morphometrics and the dendrograms visually illustrated the grouping and relationships of the populations. Intra-specific variations in the different characters of the J2s revealed that at the rescaled distance of 15 units, Indore population of *H. zeae* was different as compared to other populations of *H. zeae*. Cluster analysis of egg morphometrics showed that the populations of *H. zeae* from Kanpur and Indore were different as compare to other four populations. Cluster analysis of combined J2s and egg characters confirm the cluster analysis of the J2s morphometrics alone.

Key Word: *Heterodera zeae*; 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). The economic loss caused by nematodes is estimated to be US$80 billion throughout the world each year (Lilley *et al.*, 1999). *Heterodera zeae*, the maize cyst nematode, is a major nematode pest of maize. *Heterodera zeae* was first described from India by Koshy *et al.* (1970). It was later reported from Pakistan (Maqbool, 1981), Egypt (Abul & Ghorab, 1982), USA (Sardanelli *et al.*, 1981; Ringer *et al.*, 1987; Eisenback *et al.*, 1993) and Thailand (Chinnasri *et al.*, 1995). In India it is widely distributed in the maize growing regions of the country causing good amount of damage to the maize crop (Srivastava & Sethi, 1984). Besides maize, other plants like barley, wheat, rice and millets are good hosts of this nematode (Srivastava & Swarup, 1977; Ringer *et al.*, 1987). Other hosts reported for this nematode include Almond (Qasim & Ghaffar, 1986), Tomato (Shahzad & Ghaffar, 1986) *Capsicum annum*, *Corchorus capsularis* and *Raphanus sativus* from Pakistan (Maqbool & Hashmi, 1984) and vetiver (Lal & Mathur, 1982).

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 of 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. Cluster analysis of six populations of *Heterodera zeae* from Indore, Ludhiana, Delhi, Udaipur, Kanpur and Samastipur was done to investigate the morphometric relationships by generating dendrograms and to classify them on the basis of the similarity of the characteristics they possess.

MATERIALS AND METHODS

During the course of investigation to assess the variability between populations of *Heterodera zeae*, cysts of populations were collected from six maize growing localities namely Indore, Ludhiana, Delhi, Udaipur, Kanpur and Samastipur. The isolated populations were then cultured on maize grown in 10 cm pots containing sterilized soil in the net house. Seventy five days after raising pure culture

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Table I. Morphometric characters of second stage of juveniles of six populations of *H. zeae* [Mean ± S.D.; (range) C.V. %]

Character	Populations					
	Indore	**Ludhiana**	**Delhi**	**Udaipur**	**Kanpur**	**Samastipur**
Body length (μm)	411.85 ± 23.22 (366-443) 5.64 B	419.85 ± 21.25 (387-459) 5.06 AB	385.75 ± 17.26 (361-419) 4.47 C	388.15 ± 15.11 (354-407) 3.89 C	424.00 ± 13.65 (402-446) 3.22 A	395.35 ± 15.77 (371-420) 3.99 C
Body width (μm)	17.9 ± 1.29 (16-20) 7.23 C	18.5 ± 0.76 (17-19) 4.11 ABC	19.1 ± 1.21 (17-21) 6.33 A	19.3 ± 2.00 (17-22) 10.38 A	18.9 ± 0.72 (18-20) 3.80 AB	18.2 ± 0.77 (17-19) 4.22 BC
Stylet length (μm)	18.4 ± 2.30 (15-23) 12.52 C	21 ± 1.45 (19-23) 6.91 B	22.15 ± 1.46 (19-24) 6.6 A	20.85 ± 0.99 (19-22) 4.74 B	22.95 ± 0.76 (22-24) 3.31 A	22.4 ± 1.67 (19-25) 7.44 A
Head width (μm)	8.85 ± 0.88 (7-10) 9.89 B	9 ± 0.79 (8-10) 8.83 B	8.8 ± 0.62 (8-10) 7 B	8.95 ± 0.76 (8-10) 8.48 B	9.45 ± 0.51 (9-10) 5.4 A	8.8 ± 0.62 (8-10) 7 B
Head height (μm)	3.84 ± 0.69 (3-5) 17.91 C	5 ± 0.82 (4-6) 16.33 A	4.53 ± 0.51 (4-5) 11.33 B	4.32 ± 0.48 (4-5) 11.07 B	4.58 ± 0.51 (4-5) 11.08 AB	4.37 ± 0.50 (4-5) 11.34 B
Dorsal oesophageal gland opening (μm)	4.9 ± 0.79 (4-6) 16.08 A	4.9 ± 0.64 (4-6) 13.08 A	4.85 ± 0.81 (4-6) 16.76 A	5.1 ± 0.64 (4-6) 12.56 A	5.1± 0.79 (4-6) 15.45 A	4.8 ± 4.8 (4-6) 16 A
Head to excretory pore (μm)	86.6 ± 8.95 (73-102) 10.34 B	94.7 ± 3.69 (87-101) 3.89 A	89.65 ± 6.09 (82-101) 6.79 B	96.55 ± 5.85 (87-106) 6.06 A	95.6 ± 4.26 (88-103) 4.46 A	90.1 ± 5.18 (81-99) 5.75 B
Head to median bulb valve (μm)	68.45 ± 3.66 (63-75) 5.35 AB	68.15 ± 4.58 (60-74) 6.72 B	68.15 ± 3.66 (63-74) 5.37 B	69.15 ± 2.56 (65-73) 3.7 AB	71 ± 5.26 (63-78) 7.41 A	68.45 ± 2.11 (65-72) 3.09 AB
Head to oesophageal gland lobe (μm)	155.85 ± 14.72 (132-180) 9.44 A	153.8 ± 15.98 (126-174) 10.39 AB	150.85 ± 17.01 (127-179) 11.27 AB	144.3 ± 12.03 (124-163) 8.33 B	147.1 ± 15.76 (122-168) 10.71 AB	145.85 ± 12.20 (122-163) 8.37 AB

*Figures followed by the same letter are not significantly different (P 0.05) from each other

**Mean ± Standard Deviation; Range is given in parenthesis followed by coefficient of variability %

Table I. (Continue) Morphometric characters of second stage of juveniles of six populations of *H. zeae* [Mean ± S.D.; (range) C.V. %]

Character	Populations					
	Indore	**Ludhiana**	**Delhi**	**Udaipur**	**Kanpur**	**Samastipur**
Anal body width (μm)	11.55 ± 1.79 (8-14) 15.51 BC	12.6 ± 1.23 (10-14) 9.77 A	12.7 ± 0.98 (11-14) 7.71 A	10.8 ± 1.01 (9-12) 9.31 CD	12.2 ± 1.44 (10-14) 11.77 AB	10.1 ± 1.45 (8-12) 14.33 D
Tail length (μm)	40.45 ± 5.66 (34-51) 14 A	42 ± 5.55 (34-50) 13.22 A	42.8 ± 5.04 (36-52) 11.78 A	42 ± 5.75 (31-49) 13.69 A	42.15 ± 5.10 (34-53) 12.1 A	40.2 ± 4.06 (32-47) 10.1 A
Hyaline tail length (μm)	23.35 ± 1.27 (21-26) 5.43 B	23.35 ± 3.82 (19-30) 16.34 B	23.95 ± 2.44 (21-29) 10.18 AB	25.55 ± 2.37 (20-29) 9.29 A	24.7 ± 3.66 (19-30) 14.81 AB	25.8 ± 1.96 (23-28) 7.61 A
Hyaline tail width (μm)	6.85 ± 0.67 (6-8) 9.79 C	7.3 ± 0.86 (6-9) 11.84 ABC	7.45 ±1.05 (6-9) 14.09 AB	7.5 ± 0.51 (7-8) 6.84 A	6.8 ± 0.83 (6-8) 12.26 C	6.95 ± 0.69 (6-8) 9.88 BC
a = Total body length/ Maximum body width	23.12 ± 2.04 (19.85-26.63) 8.83 A	22.73 ± 1.48 (20.95-26.35) 6.52 AB	20.28 ± 1.63 (17.19-23.88) 8.05 C	20.32 ± 2.28 (16.64-23.76) 11.2 C	22.47 ± 1.29 (20.2-24.78) 5.74 AB	21.75 ± 1.18 (19.79-24) 5.43 B
b = Total body length / Head to oesophageal gland lobe	2.67 ± 0.32 (2.12-3.3) 12.06 B	2.76 ± 0.34 (2.24-3.48) 12.15 AB	2.58 ± 0.27 (2.15-3.01) 10.41 B	2.71 ± 0.23 (2.36-3.15) 8.35 B	2.92 ± 0.35 (2.48-3.53) 11.89 A	2.73 ± 0.27 (2.28-3.18) 9.89 AB
C = Body length/ Tail length	10.39 ± 1.71 (7.92-12.63) 16.45 A	10.15 ± 1.33 (8.22-11.94) 13.09 AB	9.14 ± 1.21 (6.94-11.11) 13.27 C	9.42 ± 1.43 (7.47-13.03) 15.22 BC	10.21 ± 1.35 (7.79-12.51) 13.26 AB	9.93 ± 1.05 (8.24-12.13) 10.57 ABC
C'= Tail length/ Anal body width	3.60 ± 0.87 (2.62-5.44) 24.1 BC	3.35 ± 0.44 (2.57-4.08) 13.04 C	3.40 ± 0.54 (2.64-4.73) 15.79 C	3.92 ± 0.66 (2.58-4.9) 16.89 AB	3.49 ± 0.54 (2.85-4.5) 15.44 C	4.07 ± 0.76 (2.92-5.63) 18.69 A

*Figures followed by the same letter are not significantly different (P 0.05) from each other

**Mean ± Standard Deviation; Range is given in parenthesis followed by coefficient of variability %

Table II. Morphometric characters of eggs of six populations of *H. zeae* [Mean ± S.D.; (range) C.V. %]

Character	Populations					
	Indore	**Ludhiana**	**Delhi**	**Udaipur**	**Kanpur**	**Samastipur**
Egg Length (um)	107.9 ± 6.24 (97-119) 5.78 A	101.2 ± 9.32 (83-112) 9.21 B	99.5 ± 6.87 (87-110) 6.91 B	99.15 ± 8.15 (85-111) 8.22 B	101 ± 10.13 (83-116) 10.03 B	101.75 ± 6.01 (93-110) 5.9 B
Egg Width (um)	45.1 ± 5.96 (31-52) 13.22 A	38.8 ± 4.86 (32-47) 12.53 B	40.85 ± 3.10 (37-47) 7.59 B	41.85 ± 5.77 (32-51) 13.79 B	39.1 ± 5.58 (30-48) 14.27 B	39.65 ± 4.17 (33-47) 10.52 B
Egg L / Egg W	2.44 ± 0.42 (1.87-3.45) 17.07 B	2.65 ± 0.42 (2.02-3.39) 15.73 A	2.45 ± 0.24 (2.11-2.84) 9.66 B	2.41 ± 0.37 (1.83-3.03) 15.26 B	2.62 ± 0.38 (2.07-3.23) 14.38 B	2.6 ± 0.33 (2.13-3.21) 12.59 B

*Figures followed by the same letter are not significantly different (P 0.05) from each other

**Mean ± Standard Deviation; Range is given in parenthesis followed by coefficient of variability %

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. Few mature cysts from each population were placed in fresh water at 37°C and the second stage juveniles (J2's) were allowed to emerge. After about a week the J2's were picked from the suspension and concentrated. The concentrated suspension for each population were killed separately in hot water bath and fixed in 2% formaldehyde. J2's were picked from the respective fixed nematode suspensions. Fixed juveniles were processed in glycerin following the method of Seinhorst (1959) and their permanent mounts were prepared in dehydrated glycerin. Identity of these populations as *H. zeae* was confirmed as per the species descriptions given by Koshy *et al.* (1970) and characteristics, which have demonstrated by Golden and Mulvey (1982).

J2s morphometric means including body length, maximum body width, stylet length, head width, head height, dorsal oesophageal gland opening, distance from the head to the excretory pore, distance from the head to the median bulb valve, distance from head to oesophageal gland lobe, tail length, hyaline tail length, anal body width, hyaline tail width, total body length to maximum body width (a ratio), total body length to head to oesophageal gland (b ratio), total body length to tail length (C ratio) and tail length to anal width (C' ratio) and egg morphometric means including egg length, egg width, and egg length to egg width ratio were measured from Camera-Lucida drawings. Morphometric characters of eggs including length, width and length to width ratio and J2s observations for each of the populations were analyzed by ANOVA (Analysis of variance). When the differences between groups were found to be significant, Duncan test was used to determine the differences between means at prescribed level of α=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) (Table I & II). Hierarchical cluster analysis of characteristics of eggs and J2s structure individually and combined characters of them was also calculated (Fig. 1-3).

RESULTS

J2s measurements. Summary of descriptive statistics and ANOVA of the measurements of J2s is given in Table I. According to ANOVA for body length, body width, stylet length, head width, head height, head to excretory pore, hyaline tail length, hyaline tail width, anal body width, a ratio, b ratio, C ratio and C' ratio, differences between groups were found to be significant ($F_{5;114}$ = 16.864, 4.008, 23.102, 2.436, 5.808, 9.027, 3.077, 3.135, 12.038, 10.492, 2.756, 2.594 & 4.081, respectively, $P<0.05$) so Duncan test was used to determine the differences between means of all these characters at prescribed level of $\alpha = 0.05$. The dendrogram (Fig. 1) 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. Delhi, Samastipur and Udaipur populations in one group and the other group includes the populations from Indore, Kanpur and Ludhiana. Hierarchical cluster analysis for the characteristics of the J2s showed that the Indore population was distinct from all other populations at the rescaled distance of 15 units. It would be evident that Samastipur and Udaipur were very close to each other.

Egg measurements. Summary of descriptive statistics and ANOVA of the measurements of egg is given in Table II. According to ANOVA for egg length and egg width, differences between groups were found to be significant ($F_{5; 114}$ = 3.206 & 4.422, respectively $P<0.05$) so Duncan test was used to determine the differences between means of these characters at prescribed level of $\alpha = 0.05$. According to Duncan test results, highest egg length was obtained in Indore population. The lowest for this character was found in population from Udaipur population, which is at par with other four populations. Widest egg belonged to the Indore population, whereas the population from Ludhiana had the narrowest, which is at par with other four populations. According to the dendrogram (Fig. 2) two separate groups have been recognized. One includes Indore population and the other group includes other five populations. Hierarchical cluster analysis for the characteristics of the egg showed that

the Indore population was considerably different from all other populations. It would be evident that Ludhiana, Delhi and Samastipur were very close to each other.

Combined of J2s and egg characters. The dendrogram (Fig. 3) showed that there are two separate groups. One includes Ludhiana, Kanpur and Indore and the other group includes Udaipur, Samastipur and Delhi. Hierarchical cluster analysis for the combined characteristics of the egg and J2s conforms the hierarchical cluster analysis of the J2s morphometrics and showed that the Indore population stood out from all other populations. It would be evident that Udaipur and Samastipur populations were very close to each other.

DISCUSSION

The present results indicate considerable variation between maize cyst nematode collected from areas that are not geographically isolated, as seen in the populations from Indore and Udaipur that have a low similarity index. Hierarchical cluster analysis based on average of J2s morphometric characters, generated two clusters for populations of *H. zeae* from India, whereas the number and shape of clusters based on eggs morphometrics where different. Thus choosing the correct variables is critical in discriminate analysis. Formation of a separate cluster by the Indore population is of major concern, which shows this population is completely different from the rest of the populations.

The analysis of genetic variability in species and populations of *Heterodera* has been addressed in host preference studies (Andersen & Andersen, 1982), isozyme analysis (Nobbs *et al*., 1992), protein analysis (Podzol & Noel, 1984; Ferris *et al.*, 1989) and ribosomal DNA (Subbotin *et al.*, 2000). The existence of biotypes/host races of different cyst nematode species have been reported from India (Walia & Mehta, 2003). 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 & grasses) to indicate the presence of five biotypes of *Heterodera avenae*. Swarup *et al.* (1979) used cereals as differentials to compare six populations of *H. avenae* and reported two biotypes. Three races of *H. zeae* have been reported from Haryana using maize and vetiver as differentials (Bajaj & 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. zeae* biotypes have also been reported from Egypt (Khair *et al*., 1989). Ringer *et al.* (1987) reported that population of *H. zeae* 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 Rajastan 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 an 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.

Fig. 1. Relationship among different populations of *H. zeae* on the basis of hierarchical cluster analysis of characteristics of J2s

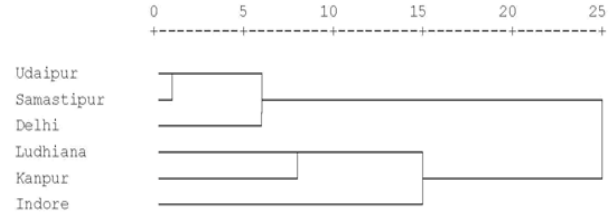


Fig. 2. Relationship among different populations of *H. zeae* on the basis of hierarchical cluster analysis of characteristics of eggs

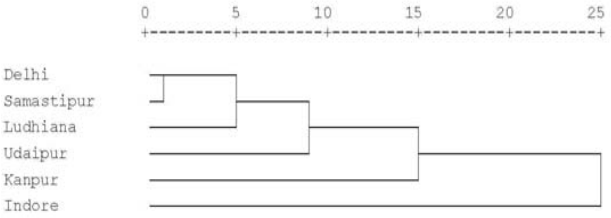
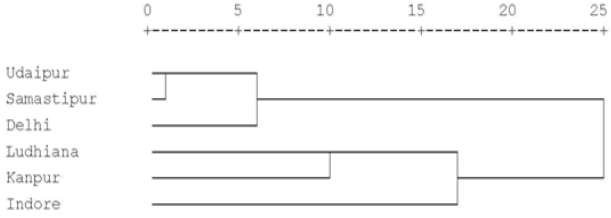


Fig. 3. Relationship among different populations of *H. zeae* on the basis of hierarchical cluster analysis of characteristics of eggs and J2s



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 punctuations 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 & 2007) used morphometric characters of different stages to show the intra-specific variations of ten Indian populations of *Heterodera cajani*. In their study the Indore population was different from the other studied populations.

This study has evaluated cluster analysis as a method for grouping and distinguishing *H. zeae* 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 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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