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University of Calcutta, Kolkata 700 019, India

Variability in teliospore morphology of some smut fungi of Northern India

D. K. AGARWAL AND BAHRAM SHARIFNABI
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012

Variability in teliospores morphology of seven species of *Ustilago, Tilletia, Neovossia* and *Sporisorium* collected from various parts of Northern India were studied by using light and electron microscopy (TEM & SEM). The morphological data viz., length and width of teliospores and sterile cells, width and height of teliospore reticulum, meshes per teliospore diameter, thickness of gelatinoid sheath and length of apiculus were subjected to hierarchical cluster analysis and ANOVA analysis. UPGMA cluster analysis could divided the isolates into different groups, showed high level of variability in teliospore morphology of the studied smut fungi. The ultrastructure of the isolates was found to be substantially similar, As teliospore size is a variable character. It is imperative to use approaches involving DNA polymorphism for searching variability among isolates. *Pennisetum prieurii* Kunth collected from Delhi is recorded as a new host for the *Sporisorium penniseti.*

Key words : Teliospore morphology, variability, UPGMA clustering, *Ustilago, Neovossia, Tilletia, Sporisorium*

INTRODUCTION

The delimitation of the smut species is based on morphological characters of spores such as : spores single, in pairs or in more or less persistent spore balls, the colour, size, shape, structure of spore wall and spore surface ornamentation. The most valuable characters are spore measurements and the spore surface ornamentation. Unfortunately, most of the descriptions of spore surfaces are incomplete or erroneous, partly due to poor microscopy and facilities (Vánky, 1991). Modern high resolution light microscopy and SEM deliver exact and minutely detailed images.

Peterson *et al.* (1984) studied variability in teliospore morphology of Karnal bunt fungus from India and Mexico but did not find any significant differences among isolates of *Neovossia indica* for teliospore diameter.

High variability in the level of infection was observed in some varieties of wheat to common bunt of wheat in Czech Republic (Pospisil *et al.* 1999).

Variability in pathogenicity, morphological, cultural, physiological, biochemical and isozyme analysis characteristics of *N. indica* have been studied in India (Bonde *et al.* 1985 ; Singh and Shing. 1988 ; Sharma *et al.*, 1998 Singh *et at.* 1998)

Historically, smut fungi have been described, classified and identified based on the host, characteristics of the sori and teliospore. It is generally assumed that smut fungi are host-specific, but recent studies revealed that the determination of relationships between smut fungi is much more complicated (Shi *et al.*, 1996). In the present studies additional morphological characteristics have been undertaken to elucidate the existance of variablility among different isolates of smut fungi prevalent in Northern India.

* *Permanent addrsss : Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan 54156, Iran.*

MATERIALS AND METHODS

Collection of isolates

Twenty samples of *Ustilago tritici, 5 of U. nuda,* 14 of *U. cynodontis,* 20 of *Neovossia indica,* 11 of *Tilletia caries.* 7 of *Sporisorium sorghi* and 6 of *S. penniseti* were collected from various parts of Northern India viz., Punjab, Haryana, Himachal Pradesh, Uttar Pradesh, Delhi and it's surrounding places during February and May 1999. The teliospores of these samples were taken from infected heads and subjected to microscopic examination.

Microscopical studies

i) *Light microscopy* : One hundred dried teliospores of each sample were rehydrated in Shear's mounting fluid (Chupp, 1940) and observed at 2000X magnification with an Olympus BX50 microscope. Measurements for length and width of one hundred teliospores were taken with the help of ocular micrometer.

ii) *Scanning Electron Microscopy* : Air dried teliospores were dusted on small pieces of double sided adhesive tape, mounted on specimen stub, sputter-coated with gold-palladium under vacuum, ca. 20 mm for 4.5 min, 7.5 mA. The specimens were then observed and photographed in a LEO 435 VP SEM, operated at 15kv following the procedure of Vanky (1997).

iii) *Transmission Electron Microscopy* : The fixing of the teliospores for TEM was done as given by Vanky (1997). Teliospores were fixed with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.2 for one week. After six continuous transfers in 0.1 M Na—cacodylate buffer, the teliospores were postfixed in 1% osmium tetraoxide in the same buffer for 1 h in the dark then washed in distilled water, and stained in 1% aqucous uranyl acetate for 1 h in the dark. After five washings in distilled water, the material was dehydrated in acetone series embedded in Spurr's plastic and sectioned with a handy microtome. Semi thin sections were stained with new fuchsin and crystal violet, mounted in entellan and studied under light microscope. Ultra thin sections were taken by ultramicrotome with glass knives and were mounted on copper slot grids, post-stained with lead citrate for 5 min and examined under a CM10 PHILIPS electron microscope at 60 kv.

Data analysis

The data obtained were analysed by two methods viz. :

i) Analysis of Variance : The Analysis of Variance (ANOVA) for various studied characters of teliospores viz., length and width of teliospores and sterile cells, width and height of teliospore reticulum, meshes per teliospore diameter, thickness of gelatinoid sheath and length of apiculus was performed by using GLM procedure of PC SAS (SAS Institute, 1989). The mean comparison of the length and width of the isolates of *U. tritici* and *U. nuda* was separately done using Duncan's Multiple Range Test (DMRT).

ii) Ilicrarchical Cluster Analysis : The dissimilarity matrix was calculated from standardized morphological characters data using Euclidean measure of distance. Unweighted pair group method using arithmetic average (UPGMA) was selected to generate grouping (Sneath & Sokal, 1973).

RESULTS

***Ustilago tritici* (Persoon) Rostrup, *Overs.Kongel Danske Vidnesk. Selsk. Forh.* Medlemmers Arbeider 1890 : p.15, 1890.**

Sori borne in spikelets. 7-12 mm long, usually destroying all the floral parts entirely, dark olivaceous—brown, dusty (brown), leaving behind only the naked rachis consisting of teliospores. Teliospores mostly single, rarely forming very loose chains, olivaceous-brown, lighter on one side, globose to subglobose or ovoid, some elongate in shape, 5-12 µm (6.50 ± 0.91) × 4-9 µm (5.71 + 0.73) in diameter. SEM reveals teliospores surface as minutely echinulate with blunt spines evenly spaced without any intervening smaller spines or warts over a basically smooth teliospore surface.

The length and width of the teliospores in isolates

of *U. tritici* varied from 5.0-12.0 µm × 4.0-9.0 µm. The average mean length and width of teliospores was 6.50 µm and 5.71 µm respectively. The isolate Ut6, collected from Karnal, Haryana has the smallest teliospores having mean length 5.54 µm and width 5.13 µm. The largest teliospore size was observed in isolate Ut12 collected from Kanpur, U.P. having mean length of 8.41 µm and width of 7.21 µm (Table 1).

Table 1 : Comparison of the mean length and width of different isolates of *Ustilago tritici* using Duncan's Multiple Range Test (DMRT).

Isolate	Length (µm)		Width (µm)	
	Mean	Rank	Mean	Rank
Ut1	5.940	I	5.270	JK
Ut2	6.050	HI	5.275	JK
Ut3	6.350	FG	5.670	EFGH
Ut4	6.690	CD	5.800	DE
Ut5	6.365	FG	5.775	DEF
Ut6	5.540	J	5.135	K
Ut7	6.470	DEF	5.940	D
Ut8	5.935	I	5.350	IJ
Ut9	7.710	C	6.170	C
Ut10	7.140	B	6.480	B
Ut11	6.420	EFG	5.575	GH
Ut12	8.410	A	7.215	A
Ut13	6.710	C	5.720	EFG
Ut14	6.730	C	5.700	EFG
Ut15	6.620	CDE	5.490	HI
Ut16	6.656	CDEF	5.635	EFGH
Ut17	6.200	GH	5.330	IJ
Ut18	6.375	FG	5.590	FGH
Ut19	6.435	EF	5.615	EFGH
Ut20	6.530	CDEF	5.590	FGH

ANOVA showed significant differences at 1% level among the isolates for both length and width of *U. tritici* teliospores (Table 2).

Based on DMRT data, results showed that there were 10 classes with respect to length and 11 with respect to width of teliospores. Ut12 is separated from all the other isolates by DMRT. The length and width data of the teliospores of various isolates of *U. tritici* was further subjected to cluster analysis using UPGMA on distance matrix based on Euclidean measure.

Results show that the cluster analysis separates isolates Ut 10 and Ut12 from all other isolats (Fig. 1). The remaining isolates were subdivided into 2 groups, one comprising of Ut1, Ut8, Ut2, Ut17 and Ut6 and the other comprising of the rest.

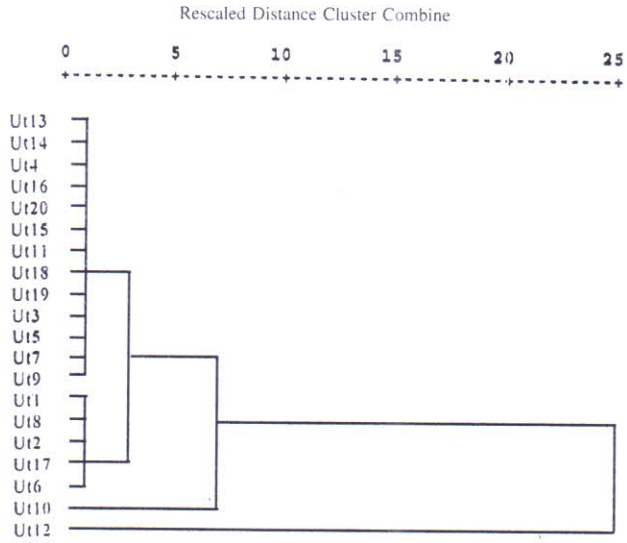

Fig. 1 : Dendrogram of *Ustilago tritici* isolates resulting from UPGMA cluster analysis based on length and width of teliospores.

Under TEM the epispore of *U. tritici* consists of electron dense outer layer and an electron transparent inner layer. The triangular spinous projection which is highly electron dense, are appended on the surface of an outer wall of an epispore. The inner layer exhibits uniform electron density and macro fiber arrangement, Protoplasm membrance is distinguishable from the cytoplasm. Nucleus is enclosed with a nuclear membrane and only one nucleus is recognizable per teliospore. Mitochondria are globose or oval and distributed in cytoplasm. From the results obtained it is conculded that the ultrastructure of all the isolats of *U. tritici* are quite similar in nature.

Specimens examined : On floral parts of *Triticum aestivum* L. Gurgaon, March 5, 1999, HCIO, 43325; Bathinda, March 1, 1999, Haryana, Coll., S. C. Chatterjee. HCIO, 43326; HAU, Hissar, March, 17, HCIO, 43328; Kaimri, Hissar, March 18, 1999, HCIO, 43329; DWR, Karnal, March 26, 1999, HCIO, 43330; Shimla, May 6, 1999, HCIO, 43334; HPKVV, Palampur, May 4, 1999, HCIO, 43331; PAU, Ludhiana, March 23, 1999, HCIO, 43342; PAU, Ludhiana, Pb., March 23 1999, HCIO, 43343; IARI, New Delhi, February 26, 1999, Coll., B. Sharifnabi, HCIO, 43344; Palampur, May 1999, HCIO, 43332; Shimla, March 25, 1999, HCIO,

43333: Kamarganj, Faizabad, U. P. March 15, 1999, HCIO, 43335; Kanpur, U. P. March 17, 1999, HCIO, 43336; Varanasi, U. P. March 17, 1999, HCIO, 43337; Pusa, Bihar, March 19, 1999, HCIO, 433339; RAU, Pusa, March 19, 1999, HCIO, 43340; Patna, Bihar, March 19, 1999, Coll., V. C. Sinha, HCIO, 43341; On seeds ot *T. durum* L., Pusa, Bihar, March 19, 1999, Coll., V. C. Sinha, HCIO, 43338; On *T. monococcum* L., Transsilvania, Sweden, June 1999, Coll., R. S. Romania, HCIO, 32240; On *T. vulgare* L., Surat Farm, Gujrat, January 1903, Coll., Kulkarni, HCIO, 7680; Palek, Burma, January 1919, Coll., F. J. F. Shaw, HCIO, 7693; Peshawar, March 1940, Coll., B. B. Mundkur, HCIO, 7660.

Table 2 : ANOVA of isolates of *Ustilago tritici* for length and width of the teliospores.

Source of Variance (S. V.)	Degree of Freedom (df)	Mean Square (MS)*	
		Length	Width
Isolates	19	32.461	22.365
Error	1980	0.526	0.337
Coefficient of Variation (%)	—	11.14	10.16

* All sources of variance are significant at 1% level.

***Ustilago nuda* (Jensen) Kellerman and Swingle, *Kansas Agric. Exp. Sta. Annual Rep.* p. 277, 1890.**

Sori in spikelets, usually destroying all the floral parts, about 5-9 mm long and 3-5 mm wide, dark olivaceous-brown, spores dusty, temporarily protected by a thin membrane but sori becoming dispersed and leaving behind only the naked rachis. Sori consisting of only teliospores.

Teliospores mostly single, rarely forming very loose chains, olivaceous-brown, lighter on one side, globose to subglobose or ovoid, few elongate in shape, 5-50-10 µm (6.66 ± 0.75) × 5-8 µm (5.82 ± 0.68) in diameter. Light coloured side of the teliospore is thin and flattened while the slightly convex side is thickened. SEM reveals teliospores surface as minutely echinulate with sparse spines evenly spaced without any intervening smaller spines or warts over a basically smooth teliospore surface. The teliospore length and width of *U. nuda* isolates varied from 5.50-10.0 µm × 5.0 to 80.0 µm. The average mean length and width 6.66 µm and 5.82 µm respectively. The isolate Un5 collected from IARI, New Delhi revealed the smallest teliospores having mean length of 6.58 µm and width 5.73 µm. The largest teliospore size was observed in the isolate Un1 collected from Haralbagh, Almora having mean length of 6.93 µm and width of 6.20 µm (Table 3).

Table 3 : Comparison of the mean length and width of different isolates of *Ustilago nuda* using Duncan's Multiple Range Test (DMRT)

Isolate	Length (µm)		Width (µm)	
	Mean	Rank	Mean	Rank
Un1	6.930	A	6.205	A
Un2	6.655	B	6.030	A
Un3	6.600	B	5.520	B
Un4	6.650	B	5.630	BC
Un5	6.585	B	5.735	C

ANOVA showed significant differences at 1% level among the isolates for both length and width of *U. nuda* (Table 4).

Based on DMRT data, results showed that there were 2 classes and 3 with respect to width of teliospores. The variation in length and width of *U. nuda* teliospores was not significantly different. The data on length and width of the teliospores of various isolates of *U. nuda* was further subjected to cluster analysis using UPGMA on distance matrix based on Euclidean measures. The cluster analysis separated isolates Un1 from all other isolates which also has the largest teliospores size (Fig. 2). The remaining isolates were subdivided into major clusters, one comprising of only Un2 and the other of Un5 and Un4 and Un3.

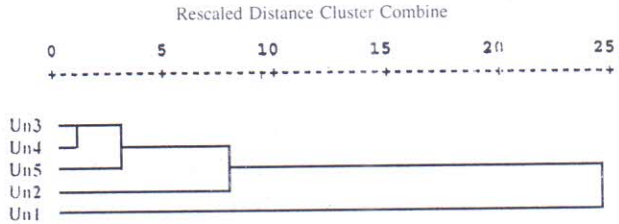

Fig. 2 : Dendrogram of *Ustilago nuda* isolates resulting from UPGMA cluster analysis based on length and width of teliospores.

TEM studies revealed that the cell wall of *U. nuda* has two distinct layers. The outer layer is electron dense and dark and the inner layer exhibits uniform electron density, which has corky texture with some

sort of cellular organisation. The spines which are highly electron dense, are appended on the surface of the outer layer. The inner layer is thicker under the thinner part of the outer layer. Protoplasm membrane is distinguishable from the cytoplasm. The only nucleus per teliospore is enclosed with a membrane. Mitochondria are globose or ovoid, distributee within the cytoplasm. From the results obtained it is concluded that, the ultrastructure of all the isolates of *U. nuda* is substantially similar in morphology.

Table 4 : ANOVA of isolates of *Ustilago tritici* for length and width of the teliospores.

Source of Variance (S. V.)	Degree of Freedom (df)	Mean Square (MS)*	
		Length	Width
Isolates	4	2.679	8.139
Error	495	0.555	0.405
Coefficient of Variation (%)	—	11.18	10.93

* All sources of variance are significant at 1% level.

Specimens examined : On floral parts of *Hordeum vulgare* L., Haval bagh, Almora, U. P., April 24, 1999, HCIO, 43299; Almora, U. P., April 24, 1999, Coll., V. C. Sinha, HCIO, 43300; HPKVV. Palampur, H. P., May 4, 1999, Palampur, H. P., May 5, 1999, HCIO, 43302; IARI, New Delhi, May 6, 1999, Coll., B. Sharifnabi, HCIO, 43303; Karnal, Hissar, Feburary 1937, Coll., M. Mitra, HCIO, 7534; Sonamarg Sceind Valley, Kashmir, August 1940, Coll., R. R. Stewart, HCIO, 7528; Agricultural Instt. Nariri, U. P., Feburary 1945, Coll., R. K. Saksena, HCIO, 10645; Botanical area, IARI, New Delhi, April 1955, Coll., R. L. Munjal, HCIO, 22144.

***Ustilago cynodontis* (P. Hennings) P. Hennings, *Bull, Harb. Boissier*, p. 114, 1893.**

Sori in inflorescence, destroying the spikelets and covering the inflorescence branches with a dark brown, dusty spore mass of 2-4 cm long, destroying all floral parts except the rachis. Sometimes the infection is localized to the basal parts of the inflorescence, bearing abortive spikelets in its distal parts. Infection is systemic in nature. Young sori often more or less hidden by enveloping leaf sheath, homogenous in nature consisting of teliospores only. Teliospores globose to subglobose, 6-9 µm (7.27 ± 0.61) × 5-8 µm (6.57 ± 0.62), yellowish-brown to light olivaceous-brown, smooth walled, Young teliospores borne in easily separable chains, connected by minute hyaline hyphal remnants. Under SEM, teliospores were showing apparently smooth wall bearing densely packed, very low warts.

Table 5 : Comparison of the mean length and width of different isolates of *Ustilago cynodontis* using Duncan's Multiple Range Test (DMRT).

Isolate No.	Teliospore Length (µm)		Teliospore Width (µm)	
	Mean	Rank	Mean	Rank
Uc1	7.475	BC	6.730	CD
Uc2	8.015	A	7.350	A
Uc3	7.600	B	6.930	B
Uc4	6.655	H	5.845	H
Uc5	6.995	F	6.270	G
Uc6	7.390	C	6.635	DE
Uc7	7.360	CD	6.650	DE
Uc8	7.220	DE	6.450	F
Uc9	7.100	EF	6.410	FG
Uc10	7.135	EF	6.500	EF
Uc11	7.465	BC	6.835	BC
Uc12	7.380	C	6.650	DE
Uc13	7.190	E	6.530	EF
Uc14	6.825	G	6.265	G

The length and width of the teliospores of *U. cynodontis* isolates varied from 6.0-9.0 µm × 5.0-8.0 µm. The average mean length and width was observed to be 7.27 µm and 6.57 µm respectively. The isolate Uc2 collected from Lucknow, U. P., has the largest teliospore having mean length 8.01 µm and width 7.35 µm. The smallest teliospore size was observed in the isolate Uc4 collected from Hissar, Haryana, having mean length of 6.65 µm and width of 5.84 µm (Table 5).

ANOVA showed significant differences at 1% level among the isolates for both length and width of *U. cynodontis* (Table 6).

Based on DMRT data, results obtained showed that there are 8 classes with respect to length of teliospores and another 8 with respect of width. Isolate Uc2 was separate from all other isolates by DMRT. The data on length and width of teliospores of different isolates was further subjected to cluster analysis using UPGMA on distance matrix based on Euclidean measures. The cluster analysis separated

isolates Uc2 and Uc4 from all other isolates (Fig. 3) which had the biggest and smallest teliospores size respectively. The remaining isolates were subdivided into two major groups, i) one comprising of Uc6, Uc12, Uc7, Uc1, Uc11 and Uc3 and the other ii) comprising of the rest of the isolates.

Table 6 : ANOVA of isolates of *Ustilago cynodontis* for length and width of the teliospores

Source of Variance (S. V.)	Degree of Freedom (df)	Mean Square (MS)* Length	Width
Isolates	13	11.434	12.346
Error	1386	0.271	0.278
Coefficient of Variation (%)	—	7.16	8.02

* All sources of variance are significant at 1% level.

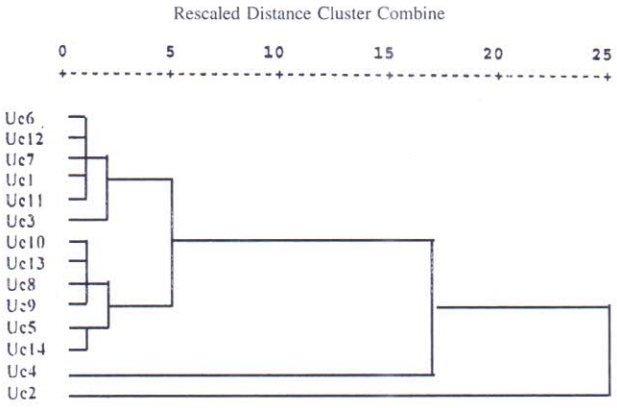

Fig. 3 : Dendrogram of *Ustilago cynodontis* isolates resulting from UPGMA cluster analysis based on length and width of teliospores.

TEM showed that the plasmalemma of spores, becomes undulate and a thin layer of electron dense material is formed against the plasmalemma, the electron dense layer is thicker adjacent to convex parts of the plasmalemma. The second layer of spore wall is formed between the plamalemma and surrounding electron dense layer. The wall of mature spores are smooth.

Specimens examined : In the inflorescence of *Cynodon dactylon* (L.), Pers., IARI, New Delhi, March 25, 1999, HCIO, 43304; NBRI, Lucknow, U. P., February 18, 1999, HCIO, 43305; Palampur, H. P., May 1999, HCIO, 43306; HAU, Hissar, March 18, 1999, HCIO, 43307; HAU, Hissar, March 17, 1999, HCIO, 43308, Durjanpur, Hissar, March 18, 1999, HCIO, 43309; HAU, Hissar, March 17, 1999,

Table 7 : Comparison of the mean of studied characters of *Neovossia indica* using Duncan's Multiple Range Test.

Isolate No.	Spore Length		Spore Width		Sterile Cell length		Sterile Cell Width		Meshes per spore		Reticulim width		Reticulim Height		Giatinoid sheath		Apiculus	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
Ni1	30.00	D	27.78	BC	21.96	CD	17.75	GHI	8.93	F	3.83	AB	4.11	BC	5.11	CD	0.58	C
Ni2	28.47	E	26.16	E	19.45	E	16.19	K	8.56	KI	3.87	A	4.31	A	5.45	A	0.61	C
Ni3	30.89	CD	24.92	F	21.38	DE	17.52	GHI	8.17	FGH	3.80	AD	4.22	AD	5.19	BC	2.26	A
Ni4	32/07	AB	26.60	DE	24.42	A	18.96	CDE	8.61	GHI	3.79	ABC	4.3	A	5.36	AB	2.45	A
Ni5	28.28	EF	25.32	F	20.63	EFG	17.08	IJ	8.67	GH	3.74	BC	4.12	BC	5.06	CDE	0.95	BC
Ni6	26/79	G	24.09	G	21.24	DE	18.01	FGH	8.44	I	3.78	ABC	4.02	CDE	5.10	CDE	1.26	BC
Ni7	26.86	G	23.34	G	18.21	I	15.65	K	8.53	hI	3.67	CD	4.08	BCD	5.02	CDEF	1.42	B
Ni8	27.36	FG	23.89	G	19.80	GH	16.41	JK	8.78	FG	3.61	D	4.00	CDE	4.99	CDEFG	1.03	BC
Ni9	30.83	CD	26.31	DE	21.37	DE	17.14	IJ	9.20	F	3.37	E	3.79	F	4.77	HI	1.18	BC
Ni10	31.76	ABC	26.20	DG	23.25	B	19.69	BC	9.22	DE	3.31	EF	3.62	G	4.62	I	1.17	BC
Ni11	32.37	AB	29.39	A	24.34	A	20.81	A	9.41	CD	3.19	FG	4.11	BC	5.11	CD	1.33	BC
Ni12	31.94	ABC	27.07	CD	23.18	B	20.00	B	9.56	BC	3.21	FG	3.82	F	4.81	GH	1.01	BC
Ni13	31.82	ABC	28.41	B	22.82	BC	18.61	EF	9.72	AB	3.17	G	4.07	BCD	5.09	CDE	0.75	BC
Ni14	32.76	A	30.07	A	21.51	DE	18.74	EF	9.77	AB	3.15	G	4.11	BC	5.07	CDE	1.13	BC
Ni15	30.20	D	26.36	DE	20.89	EF	17.56	GHI	9.37	CDE	3.27	EFG	34.91	DEF	4.91	DEFGH	0.94	BC
Ni16	31.93	ABC	28.14	B	22.88	BC	19.56	BCD	9.62	AB	3.18	G	3.96	CDEF	4.93	DEFGH	1.09	BC
Ni17	31.57	BC	27.97	B	20.53	EFG	17.79	GHI	9.69	AB	3.14	G	3.63	G	4.60	I	0.87	BC
Ni18	30.38	D	26.17	E	20.53	EFG	18.34	EFG	9.66	AB	3.18	G	3.91	DEF	4.90	EFGH	1.04	BC
Ni19	31.88	ABC	28.12	B	19.97	FGH	17.32	HI	9.80	A	3.15	G	3.87	EF	4.86	FGH	1.04	BC
Ni20	32.25	AB	29.29	A	22.10	CD	8.87	DE	9.72	AB	3.16	G	4.11	BC	5.10	CDE	1.04	BC

HCIO, 43310; B.R.I., Hissar, March 18, 1999, HCIO, 43311; IARI, New Delhi, March 10, 1999, HCIO, 43312; IARI, New Delhi, March 12, 1999, HCIO, 43313; PAU, Ludhiana, March 23, 1999, HCIO, 43314; DWR, Karnal, March 26, 1999, HCIO, 43315; PAU, Ludhiana, March 23, 1999, HCIO, 43316; HPKVV, Palampur, May 5, 1999, Coll., B. Sharifnabi, HCIO, 43317; Ghinsuroh farm, Bengal, December 1919, Coll., G. G. Ghosh, HCIO 7421; Karnal, Panjab, October 1953, Coll. A Khan, HCIO, 7430; Buglan, July, 1939, Coll., B. B. Mundkur, HCIO, 7423; N.S.W., Australia, October 1966, Coll., J. Walker, HCIO, 32023; Greece, July, 1976, Coll., K. Vanky, HCIO, 32587.

***Neovossia indica* (Mitra) Mundkur, *Sci. Monogr.* 12 : p. 18, 1938.**

Sori ovariicolous, only a few florets of the spike attacked, infected kernels showing little or no swelling, partially to completely destroyed, when partially attacked, infection restricted to the suture side destroying the embryo tissue; in severe attack the tissues along the suture and adjacent to endosperm are replaced by black teliospores. Spore mass black, foeted, dusty, held together by a pericarp.

Teliospores formed single at the ends of mycelial strands, mostly globose to subglobose, occasionally appendaged with mycelial fragment, 0-10 µm (1.16 ± 2.27) liver-brown to opaque, dark reddish to coppery brown, epispore with reticulated proliferations projecting into a winged margin at the circumference, 3-5 µm (3.43 ± 0.49) in width and 3-6 µm (4.00 ± 0.58) in height, lacunae roundish or irregular, 20-47 µm (30.52 ± 3.99) × 19-42 µm (26.78 ± 3.37) in diameter. Teliospores are embedded in a hyaline, gelatinoid sheath, which is 4-7 µm (5.00 ± 0.65) thick. Sterile cells present, variable, globose to subglobose, frequently lacrimiform, yellowish brown, 13-33 µm (21.52 ± 3.69) × 12.28 µm (8.66 ± 2.94) in diameter, with a small or robust appendage. The teliospores of twenty different isolates of *N. indica* were subjected to morphological characterisation for various parameters like teliospores length and width, sterile cells length and width, gelatinoid sheath thickness and length of apiculus. The mean length of teliospores varied from 26.76 µm for isolate Ni6 to 32.76 µm for Ni14. The teliospores of Ni14 had the maximum width of 30.07 µm. The range of length and width for sterile cells was observed to be 18.4 µm to 24.34 µm and 15.65 µm to 20.81 µm respectively. The teliospores did not show much variation as to the number of meshes per spore diameter as well as in hight and width of reticulation. The average thickness of gelatinoid sheath of all the isolates of *N. indica* was 4-7 µm. The data obtained for apiculus length varied as the samples examined consisted of both young and mature teliospores. In young teliospores the length of apiculus went up to 10 µm whereas in mature teliospores it was observed as very small or even absent (Table 7).

Table 8 : Correlations between the studied characters of the isolates of *Neovossia indica*

	Correlation Matrix								
	Sp. Length	Sp. Width	S.C. Length	S.C. Width	Mesh	Ret. Length	Ret. Width	Gelatin	Apiculus
Spore Length	1.000								
Spore Width	0.842	1.000							
S. Cell Length	0.679	0.513	1.000						
S. Cell Width	0.731	0.620	0.895	1.000					
Meshes	0.761	0.780	0.263	0.513	1.000				
Ret. Width	–0.675	–0.660	–0.250	–0.531	–0.951	1.000			
Ret. Height	–0.196	–0.049	0.023	–0.179	–0.488	0.588	1.000		
Gelatin	–0.220	–0.057	0.031	–0.173	–0.509	0.607	0.980	1.000	
Apiculus	0.084	–0.331	0.313	0.106	–0.349	–0.300	0.238	0.203	1.000

These morphological characteristics were further evaluated in terms of correlation between various parameters and the results are given as under (Table 8). From the Table 13 it is evidently clear that the width and height of the reticulum are negatively correlated with teliospore and sterile cells length

Table 9 : ANOVA of 20 isolates of *Neovossia indica* for the studied characters

SV	df	MS*								
		Length spore	Width spore	Length S. Cells	Width S. Cells	Meshes	Width reticu.	Height pecticu.	Gelatin sheath	Apicul. leng.
Iso.	19	374.641	354.152	263.541	177.022	24.120	8.303	3.776	4.541	21.258
Error	1980	12.537	8.078	11.263	7.049	0.475	0.170	0.308	0.388	5.036
CV (%)	—	11.60	10.61	15.59	14.67	7.49	12.02	13.84	12.45	194.00

* All sources of variance are significant at 1% level.

and width. The thickness of gelatinoid sheath is found to be negatively correlated with teliospores length and width. Whereas the other parameters showed a positive correlation with each other.

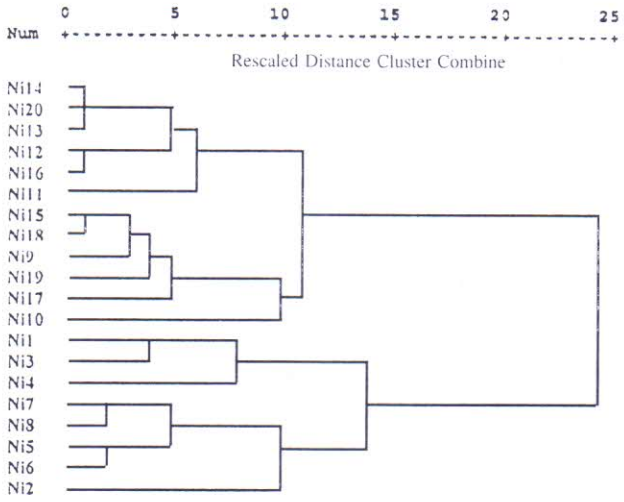

Fig. 4 : Dendrogram of *Neovossia indica* isolates resulting from UPGMA cluster analysis based on different characters of teliospores and sterile cells

The ANOVA showed significant differences at 1% level for and between the isolates of *N. indica* (Table 9). It is evident from Table 14 that the high coefficient of variance obtained for apiculus length is due to the fact that the observation varied from 0-10 µm owing to the presence of both young and mature teliospores.

The dendrogram obtained for morphological characters divided the *N. indica* isolates into two major clusters, i) Cluster "A" consisting of Ni14, Ni20, Ni13m Ni12, Ni16, Ni11, Ni15, Ni18, Ni19, Ni17 and Ni10 and ii) "B" cluster consisting of Ni1, Ni3, Ni4, Ni7, Ni8, Ni5, Ni6 and Ni2. It is evident that all the isolates from U. P. belonged to cluster "A" and "B" The major clusters "A" and "B" were further divided into sub groups there by showing the correlation as to the geographical distribution pattern (Fig. 4).

SEM studies of the teliospores showed prominent thick projections of outer wall layers which were irregularly arranged with blunt margins, giving rough look to the surface of the teliospore.

Specimens examined : On seeds of *Triticum aestivun* L., Delhi, April, 1997, HCIO, 43262; HAU, Hissar, HCIO, 43263; HAU, Hissar, April 1998, Coll. J. C. Duhan, HCIO, 43264; HAU, Hissar, April 1998, HCIO, 43265; HAU, Hissar, April 1998, Coll. M. S. Beniwal, HCIO, 43266;

Table 10 : Mean comparison of the isolates of *Tilletia caries* for the studied characters

No.	Sp. Len.		Sp. Wid.		S.C. Len.		S.C. Wid.		Mesh		Ret. Wid.		Ret. Height		Gelatin	
	Mean	Rank	Mn	Rk	Mn	Rk	Mn	Rk	Mn	Rk	Mn	Rk	Mn	Rk	Mn	Rk
1	17.13	DE	15.75	DEF	14.05	BCD	12.36	B	5.26	G	4.73	A	1.20	B	1.83	BC
2	16.91	E	15.79	DEF	14.87	A	13.13	A	5.63	F	4.28	B	1.20	A	1.82	BC
3	17.39	CD	15.75	DEF	14.24	BC	12.43	B	6.25	DE	4.32	B	1.14	BC	1.79	CD
4	17.49	BCD	15.58	F	14.34	B	12.24	BC	6.22	DE	4.79	A	1.12	CD	1.79	CD
5	17.42	CD	15.87	CDE	13.92	CD	11.81	IJE	6.43	CD	4.30	B	1.06	DE	1.77	CDE
6	17.92	A	16.19	AB	13.79	DE	11.88	CD	6.61	BC	4.26	B	1.02	E	1.75	CDE
7	17.62	ABC	15.98	BCD	14.00	BCD	11.62	DEF	6.61	BC	3.86	CD	1.01	E	1.69	E
8	17.84	AB	15.58	F	13.66	DEF	11.47	EF	6.52	C	3.93	C	1.02	E	1.74	CDE
9	17.41	CD	16.07	BC	13.29	F	11.20	F	6.82	AB	3.69	D	0.97	F	1.71	DE
10	17.02	E	16.64	EF	13.29	F	11.43	EF	6.85	A	3.73	D	1.05	E	1.90	AB
11	17.57	ABC	16.39	A	13.50	EF	11.37	F	6.10	E	4.25	B	1.06	DE	1.92	A

HAU, Hissar, April 1999, Coll. E. Bizgir, HCIO, 43281; PAU, Ludhiana, May 1998, HCIO, 43267; PAU, Ludhiana, May 1998, Coll. S.S. Chahai, HCIO, 43268; Gurdaspur, Panjab, May 1998, HCIO, 43269; Muzaffarnagar, U.P., May 1998, HCIO, 43270; Jalandhar, Punjab, May 1998, HCIO, 43271; Yamunanagar, Haryana, May 1998, HCIO, 43272; Kashipur, U.P., May 1998, HCIO, 43273; Muzaffarnagar, U.P., May 1998, HCIO, 43274; Yamunanagar, Haryana, May 1998, HCIO, 43275; Roper, Punjab, May 1998, HCIO, 43276; Pantnagar, U.P., May 1998, HCIO, 43277; Batala, Punjab, May 1998, HCIO, 43278; Bilaspur, U.P., May 1998, HCIO, 43279; Muzaffarnagar, U.P., May 1998, Coll. E. J. Kumar, HCIO, 43280; On *T. vulgare* L., Saharanpur, U.P., August 1942, Coll. R. S. Kashi Ram, HCIO, 7858; Botanical section, New Delhi, June 1942, Coll. A. Khan, HCIO, 7862; Gujranwalla, Punjab, 1943, Coll. S. Ahmed, HCIO, 10031; Kalsi, Dehradun, April 1954, Coll. G. Lal, HCIO, 20650; Karnal, Punjab, 1930, Coll. M. Mitra, HCIO, 7865 (Type)*.

***Tilletia caries* (De Candolle) L.-R. and C. Tulasne, Ann. Sci. Nat. Bot. (III) 7 : p. 113, 1847.**

Sori in the ovaries, foeted, filling the ovaries with a reddish-brown to dark-brown teliospores, upto 7 mm long, assuming the shape of the normal caryopses, frequently larger in size usually giving some shade of brown, enclosed within the brittle pericap which easily ruptures to expose the dark-brown to reddish-brown spore mass, semi-agglutinated to pulverulent.

Teliospores globose, less frequently subglobose, occasionally ovoid, light yellow to reddish-brown 15-22 µm (17.42 ± 1.27) × 14-20 µm (15.87 ± 0.88) in diameter, wall reticulate, exospore adorned with rather shallow, polygonal reticulation. 3-7 µm (4.1 ± 0.73) wide and 0.5-2 µm (1.08 ± 0.24) height, variable in size, occasionally some what cerebriform, 4-8 µm (6.30 ± 0.92) meshes per spore diameter, gelatinoid sheath 1-2 µm (1.79 ± 0.28) thick.

Sterile cells few, globose to subglobose, hyaline to subhyaline, smooth, 10-17 µm (13.90 ± 1.39) × 10-16 µm (11.90 ± 1.46) in diameter. Under SEM, teliospores are reticulate with-wide wall.

The teliospors of eleven isolates of *T. caries* were subjected to morphological characterization for the parameters like, length and width of teliospore and sterile cells, height and width of reticulum and thickness of gelatinoid sheath. The mean length of teliospores varied from 16.91 µm for Tc2 and 17.92 µm for Tc6. The teliospores of Tc11 were observed to have the maximum width of 16.39 µm and the minimum in Tc4 and Tc8 of 15.58 µm. The length of sterile cells and width varied from 13.29 to 14.87

Table 11 : Correlations between the studied characters of the isolates of *Tilletia caries*

	Correlation Matrix							
	Sp. Length	Sp. Width	S.C. Length	S.C. Width	Mesh	Ret. Width	Ret. Height	Gelatin Sheath
Spore Length	1.000							
Spore Width	0.348	1.000						
S. Cell Length	–0.307	–0.330	1.000					
S. Cell Width	–0.480	–0.352	0.938	1.000				
Meshes/Sp. diam.	0.447	0.118	–0.635	–0.724	1.000			
Ret. Width	–0.093	–0.167	0.612	0.640	–0.740	1.000		
Ret. Height	–0.623	–0.386	0.770	0.886	–0.891	0.738	1.000	
Gelatin Sheath	–0.464	0.066	–0.097	0.089	–0.348	0.234	0.416	1.000

Table 12 : ANOVA of 11 isolates of *Tilletia caries* for the studied characters

SV	df	MS*							
		Length spore	Width of spore	Length of S. Cells	Width of S. Cells	Meshes	Width reticu.	Height recticu.	Gelatin sheath
Isolates	10	9.981	6.812	22.673	34.04	24.098	13.346	0.599	0.528
Error	1089	1.548	0.728	1.759	1.855	0.646	0.417	0.056	0.076
CV (%)	—	7.14	5.38	9.54	11.44	12.76	15.38	22.00	15.39

* All sources of variance are significant at 1% level.

μm and 11.20 to 13.13 μm, respectively. These did not show much variation as to the number of meshes per spore diameter as well as height and width of reticulation. The average thickness of gelatinoid sheath of all the isolates of *T. caries* was 1.69 to 1.92 μm (Table 10).

The morphological characteristics of the fungus were also evaluated in terms of correlation between various parameters (Table 11). It was observed that width and height of reticulum are negatively correlated with teliospore length and width. The thickness of gelatinoid sheath was found to be negatively correlated with length of teliospore and sterile cells. Recticulum width and height also showed negative correlation with meshes per spore diameter. The other parameters showed a positive correlation with each other.

The ANOVA showed significant differences at 1% level for and between the isolates of *T. caries* (Table 12).

The dendrogram obtained for morphological characters divided the *T. caries* into two major clusters i)''A'' cluster consisting of Tc3, Tc4, Tc1 and Tc2 and ii) ''B'' cluster consisting of Tc5, Tc7, Tc6, Tc8, Tc9, Tc11 and Tc10. The major clusters ''A'' and ''B'' were further divided into sub-groups. These did not show any correlation as to their geographical distribution.

SEM studies revealed the four distinct layers in the spore wall. The outer and inner wall layers have a fibrous texture while the middle layers, which gives the reticulated surface pattern, appears to be homogenous. The outer (W1) and inner (W3) layers are seperated by a relatively thin layer (partition layer). Large quantities of lipid are available in teliospores. These lipid bodies commonly have depressions and protuberances associated with them. Nuclei appear to be typical of other eukaryotic cells.

Specimens examined : On seeds of *Triticum aestivun* L., Palampur, H. P., May, 1998, Coll. K. Singh, HCIO, 43288; HAU, Hissar, April 1998 HCIO, 43289; HAU, Hissar, April 1998, HCIO, 43290; Thaska, Hissar, April 1998, Coll. E. Bazgir, HCIO, 43291; Baijnath, H. P., May 5, 1999, HCIO, 43292; Mandi, H. P., May 4, 1999, HCIO, 43293; Shimla, H. P., May 7, 1999, HCIO, 43294; May 7, 1999, Coll. B. Sharifnabi, HCIO, 43295; HPKV, Palampur, H. P., May 6, 1999, HCIO, 43296; HPKV, Palampur, H. P., May 6, 1999, Coll. S. K. Rana, HCIO, 43298; On *T. Vulgare* L., Shimla, September, 1908, Coll. D. Commissioner, HCIO, 7781; Australia, November 1918, HCIO, 7784; Cardiff, U. K. August 1943, Coll. S. J. Aughls, HCIO, 23863; Kapurthala, Pb., April 1962, Coll. B. L. Chona, HCIO, 26676; N. Sikkim, April 1962, Coll. J. N. Kapoor, HCIO, 27637.

***Sporisorium sorghi* Ehrenberg ex Link, *Linneus Specie, Plantarum* Ed. 4, 6 : p. 86, 1825.**

Sori in florets, 4-10 mm long, sometimes fusing the young florets into irregular forms, ovoid or cylindrical with, light brown bodies protruding from the glumes, covered by a well-developed peridium of interwoven hyphae overlaid by host tissue, which ruptures irregularly to expose the dark brown, powdery spore mass. Central columella composed of host tissues permeated by hyphae producing spores and sterile cells.

Teliospores globose, subglobose, ovoid to slightly irregular, 7-9 μm (7.75 ± 0.58) × 6-9 μm (6.99 ± 0.66) in diameter, light olive-brown. SEM shows teliospore wall verruculose with sparse to moderately dense minute wart. Sterile cells borne in irregular groups, globose to, subglobose, hyaline larger than teliospores, 10-20 μm (13.41 ± 2.50) × 8-18 μm (11.43 ± 2.08) in diameter, smooth walled.

Table 13 : Mean comparison of the isolates of *Sporisorium sorghi* for the studied characters

Isolate	Spore Length		Spore Width		S. C. Length		S. C. Width	
No.	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	7.670	AB	6.930	D	13.13	C	10.83	CD
2	7.710	AB	6.785	DE	12.42	C	10.83	D
3	7.835	A	7.115	BC	14.17	B	11.94	B
4	7.835	A	7.175	AB	13.13	C	10.95	CD
5	7.840	A	7.305	A	13.05	C	11.36	C
6	7.635	B	6.665	E	12.99	C	11.24	C
7	7.735	AB	6.960	CD	15.04	A	13.31	A

The length and width of the teliospores of *S. sorghi* isolates varied from 7-9 µm and 6 to 9 µm respectively. The average mean length and width of teliospores was 7.75 µm and 6.99 µm. The different isolates did not show much variation in length of teliospores. Ss5 collected from South Campus, Delhi University, had the largest teliospore size having mean length of 7.84 µm and width of 7.30 µm. The largest sterile cells were found in Ss7 which was collected from JNU Campus, Delhi having mean length of 15.04 µm and width of 13.31 µm (Table 13).

ANOVA showed significant differences at 1% level among the isolates for both length and width of *S. sorghi* teliospores and sterile cells (Table 14).

The length and width data of the teliospores and sterile cells of various isolates of *S. sorghi* was subjected to cluster analysis using UPGMA on distance matrix based on Euclidean measure. The cluster analysis separates isolate Ss7 from all other isolates. The remaining isolates were subdivided into two groups, one comprising of Ss4, Ss5 and Ss3 and the other comprising of Ss1, Ss2 and Ss6.

Table 14 : ANOVA of isolates of *Sporisorium sorghi* for the length and width of teliospores and sterile cells.

SV	df	MS*			
		Length spore	Width of spore	Length of S. Cells	Width of S. Cells
Isolates	6	0.733	5.020	77.997	90.175
Error	693	0.344	0.395	5.697	3.590
CV (%)	—	7.57	9.00	17.75	16.57

* All sources of variance are significant at 1% level.

Specimens examined : On floral parts of *Sorghum halepense* (L.) Pers., IARI, New Delhi, March 20, 1999, HCIO, 43318; IARI, New Delhi, March 20, 1999, HCIO, 43319; IARI, New Delhi, March 5, 1999, HCIO, 43320; Ridge Road, New Delhi, March 8, 1999, HCIO, 43321; South Campus, Delhi University, Delhi, March 22, 1999, HCIO, 43322; North Campus, Delhi, March 24, 1999, HCIO, 43323; JNU Campus, Delhi, March 23, 1999, Coll., B. Sharifnabi, HCIO, 43324; On *Andropogon sorghum*; Karnal, October 1942, Coll. C. B. Sahyay, HCIO, 7340; On *Sorghum vulgare* L., Larkhana, Sind, December, 1915, Coll., G. S. Kulkarni, HCIO, 7345; Bengal, October, 1945, Coll. J. C. Roy, HCIO, 10754; Or *S. halepense* (L.) Pers.: Sylhet, Assam, December, 1944, Coll., S. Chowdhary, HCIO, 10654.

***Sporisorium penniseti* (Rabenhorst) Ershad, *Iran. J. Plant Path.* 30 : p. 18, 1994.**

Sori in ovaries, clongated, 3-5 mm long, destroying them entirely. All the ovaries of the panicle are infected, at first concealed by the enveloping glumes and covered by a delicate membrane, which easily ruptures to reveal the blackish-brown powdery sporemass, surrounding a well-developed and prominent columella. Spore balls broadly oval or irregularly globose, dark-brown, with 15-25 teliospores.

Teliospores globose to subglobose, irregular, cinnamon-brown, 8-13 µm (10.32 ± 1.08) × 7-12 µm (9.03 ± 0.94) in diameter, Episore thick, sterile cells were not observed. SEM studies revealed the teliospores surface as verrucose in nature bearing minutely dense, regular to irregular warts.

The length and width of the isolates of teliospores of *S. penniseti* varied from 8-13 µm and 7 to 12 µm respectively. The average mean length and width of teliospores was observed as 10.32 µm and 9.03 µm respectively. The isolates Sp3, collected from Buddha Park, New Delhi, has the biggest teliospores having mean length 10.72 µm and width 9.33 µm. The smallest teliospores size was observed in the isolate Sp6 collected from JNU Campus, Delhi having mean length of 9.36 µm and width of 8.29 µm (Tables 15 and 16).

Table 15 : Comparison of the mean length and width of different isolates of *Sporisorium penniseti* using Duncan's Multiple Range Test (DMRT)

Isolates No.	Length (µm)		Width (µm)	
	Mean	Rank	Mean	Rank
Sp1	10.71	A	9.27	A
Sp2	10.61	AB	9.36	A
Sp3	10.72	A	9.33	A
Sp4	10.37	BC	9.00	B
Sp5	9.36	D	8.29	C
Sp6	10.16	C	8.95	B

ANOVA showed significant differences at 1% level

among the isolates for both length and width of teliospores (Table 16).

Table 16 : ANOVA of isolates of *Sporisorium penniseti* for length and width of teliospores

Source of Variance (S.V.)	Degree of Freedom (df)	Mean Square (MS)* Teliospore Length	Teliospore Width
Isolates	5	26.905	16.374
Error	594	0.970	0.765
Coefficient of Variation %	—	9.54	9.68

* All sources of variance are significant at 1% level.

Based on DMRT data, results showed that there were 5 classes with respect to length and 3 with respect to width of teliospores. The length and width data of the telispores of various isolates of *S. penniseti* was subjected to cluster analysis using UPGMA on distance matrix based on Euclidean measure. The cluster analysis separates isolates Sp5 from all other isolates, this isolate possessed the smallest teliospore size. The remaining isolates were subdivided into 2 groups, comprising of Sp6 and another of Sp4, Sp2, Sp3, and Sp1 this cluster also were subdivided into clusters. The host plant *Pennisetum prieurii* Kunth collected from Delhi is recorded as a new host (*Matrix nova*) for the fungus *Sporisorium penniseti.*

Specimens examined : On floral parts of *Pennisetum prieurii* Kunth, Buddha Park, New Delhi, September 19, 1998, HCIO, 43282; September 20, 1999, HCIO, 43283; Buddha Park, New Delhi, September 20, 1999, HCIO, 43284; Ridge Road, New Delhi, October 5, 1999, HCIO, 43285; South Campus, Delhi University, Delhi, October 5, 1999, HCIO, 43286; JNU Campus, Delhi, October 10, 1999, Coll. B. Sharifnabi, HCIO, 43287; On *Pennisetum caliarie* (L.) Link, Delhi, August 1938, Coll. A. Khan, HCIO, 7749.

DISCUSSION

The variability within species and generation of smut fungi is a product of genetic recombination during sexual reproduction. The progeny from a single generation may differ from each other and from the parent in virulence, host specificity and other characteristics (Fischer and Holton, 1957).

In the present investigation the ultrastructure of the isolates was substantially similar but the clustering of the isolates based on various parameters divided them into different clusters. The clustering, however, did not bear any correlation with the geographical distribution.

Physiological specialization in *N. indica* was first reported by Mitra (1931). He observed differences in the size of teliospores collected from Karnal, India and Peshawar, Pakistan and speculated two races. Mitra (1935) regarded two different collections of *N. indica* as two physiological forms differing in teliospore size.

Munjal (1970) differentiated seven physiologic races on the basis of serology and their host reaction. The pathotypes could not be differentiated on the basis of the morphological studies as the shape and colour of teliospores in the samples resembled a lot. Four pathotypes in 21 collections of *N. indica* from Punjab and Himachal Pradesh. India have been reported (Aujla *et al.,* 1987).

Singh and Singh (1988) reported that since teliospore size is influenced by environmental variation there are no large differences in size of the teliospores from different locations and varieties, hence it can not be used as a differentiating character for the collections of *N. indica.* Observations of Bansal *et al.* (1984) indicated the average size of teliospores from various samples of wheat varieties collected from Punjab, Haryana and Uttar Pradesh differed significantly but the teliospore size from Himachal Pradesh and Jummu and Kashmir did not differ significantly within themselves in comparison to the type specimen, Apparently there is no difference in teliospore size between the type specimen and the samples studied. He concluded that the teliospore size is a slightly variable character and is influenced by the environmental factors.

Peterson *et al.* (1984) did not find any significant differences among isolates of *N. indica* from India and Mexico for teliospore diameter.

Sharma *et al.* (1998) carried out pathogenicity test and isozyme analysis and grouped 5 distinct groups

using pathogenicity and 2 groups based on the analysis of esterase and acid phosphatase isozymes. Singh *et al.* (1998) studied the biochemical variability of different isolates and found that the isolates varied considerably in their cellular fatty acid. Lipids analysis could not distinguish between the strains, but autoradiography allowed the strains to be distinguished clearly.

SEM studies of teliosprcs of *N. indica* by Aggarwal *et al.* (1998) showed that episporium surface looked rough and the stretchability of perisporium did not keep pace with the advancing maturity. Khanna and Payak (1968) also reported that teliospore projections (exospore ornamentation) under light microscopy were truncate with flattened to occasionally curved tips that sometimes develop tears or become forked. From the results obtained, it is concluded that the ultrastructure of the different isolates of studied smut fungi are found to be substantially similar. The present investigation points to the fact that the cluster analysis, indicates that variability exists among different isolates of smut fungi between and within different geographical regions of Northern India. It points towards the fact that further investigation are needed to closely elucidate the variability spectrum of these important pathogens in relation to their pathogenicity and DNA polymorphism.

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