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Morphological variation among different isolates of *Colletotrichum gloeosporioides* isolated from major fruit crops in Khandesh region of Maharashtra

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Abstract
Present investigation reveals the study of morphological variation among different isolates of *Colletotrichum gloeosporioides* isolated from various crops. Twelve diseased specimens were collected from different district of Khandesh region of Maharashtra and subjected to tissue isolation on PDA. Out of 12 specimens obtained from different hosts, 12 isolates from 3 isolate from pomegranate, 3 isolate from sweet orange, 3 isolate from banana and 3 isolates from guava from three different district of Khandesh region of Maharashtra. These 12 isolates from 4 major fruit crops were found to be pathogenic when inoculated on respective plant hosts. These 12 isolates were used for further study and further abbreviated as CgPD1, CgPJ 2, CgPN 3, CgSD 4, CgSJ 5, CgSN 6, CgBD 7, CgBJ 8, CgBN 9, CgGD 10, CgGJ 11 and CgGN 12. *Colletotrichum gloeosporioides* isolated differed significantly in all morphological traits except the type of mycelium. Mycelium of all isolates was septate. Maximum average mycelial growth rate of 12.85 mm / day was observed in isolates CgPD 1 which isolated from pomegranate fruit crops. The mycelial width of all 12 isolates ranged between 4.11 – 3.1 µ. In general, conidia were hyaline, cylindrical to allantoidal with round ends. The highest average conidial length was exhibited by isolate CgPJ 2 (11.75 µ) infecting pomegranate and it was at par with CgSD 4 (11.70 µ) while, shortest length of conidia were observed in CgGN 12 (6.88 µ) infecting guava and it was at par with CgGJ 11 (6.96 µ) and CgGD 10 (7.42 µ). There was significant variation in L X B ratio conidia of CgBD 7 were thin and narrow as the L X B ratio was maximum (4.70 µ) were from banana, while it was minimum (2.57 µ) in CgSD 4 these isolates were from sweet orange. Among the 12 isolates under study, 9 isolates were produced acervuli in the culture. Isolates CgPN 3, CgSJ 5 and CgGJ 11 never produced acervuli in culture. The average maximum length of the setae was (75.82 µ) recorded in isolate CgBJ 8 and it was followed by CgBD 7. The shortest setae were observed in CgBN 9 having average length of (27.03 µ) and it was on par with CgPJ 2, CgGN 12, CgSD 4 and CgGD 10. Out of 12 isolates, 9 isolates could produce setae as an integral part of acervulus. Acervuli of isolates CgPN 3, CgSJ 5 and CgGJ 11 were without setae. The average maximum length of the setae was (75.82 µ) recorded in isolate CgBJ 8 and it was followed by CgBD 7. The shortest setae were observed in CgBN 9 having average length of 27.03 µ.

Keywords: *Colletotrichum gloeosporioides,* conidia, mycelium, acervulus, setae, fruit crops

Introduction
The Khandesh region of Maharashtra majorly contributes for the production of fruit, vegetable, cereal, flower, medicinal and ornamental crops. The production of these agricultural crops has many problems particularly, fungal diseases. Among these, anthracnose or fruit rot caused by *Colletotrichum gloeosporioides* is most destructive disease and known to cause great losses to the fruit growers in terms of both quality and quantity. It causes anthracnose, die back, whither tip, shot hole, leaf blight and post-harvest rots in many economically important crops such as cereals, pulses, vegetables, fruits, spices and cash crops. *Colletotrichum gloeosporioides* cause typical disease symptoms known as anthracnose, characterized by sunken necrotic tissue, produced in lesions on petioles, leaves, mummified inflorescences, flower bracts and on fruits (Dodd *et al*., 1992) [4] and can acts as continuous sources of inoculums. The most significant damage of this fungus occurs upon its attack on fruiting stage (Baily *et al*., 1992). *C. gloeosporioides* is a ubiquitous pathogen causing substantial yield losses due to fruit decay and damage to vegetative parts in a variety of plant species (Freeman and Shabi, 1996) [6]. Anthracnose is the most prevalent disease that contributes significantly to pre and post-harvest

losses in cashew, pomegranate, guava, citrus and papaya. Under such circumstances, the nature of the *C. gloeosporioides* will be the decisive factor in the epidemic development. Therefore, investigation on the basic and applied aspects of population biology of *C. gloeosporioides* is the need of time. It is also necessary to understand how the existing population of the pathogen interacts with the emerging population of the host species and varieties. Similarly, behaviour of *C. gloeosporioides* under intensive management system in crops needs to be investigated in relation with fungicides commonly used as well as newly available. The different isolates of *Colletotrichum gloeosporioides* isolated from different host plants, shows variation in their morphological characters. The ambiguous taxonomic status of *Colletotrichum* species has resulted in inaccurate identification which may cause practical problems in plant breeding and disease management. Hence there is great potential for augmentation of morphological characteristics for a better classification and identification system, such data will allow the development of proper, objective and automated identification techniques for *Colletotrichum gloeosporioides*.
Considering all these points the investigation on morphological variability was carried out.

Materials and Methods
The present investigation was carried out during June 2018 to December 2018 at Department of Plant Pathology, College of Agriculture, Dhule, M.P.K.V. Rahuri, 413 722. The material used and methods and procedures followed to investigate the morphological variability within the isolates of *Colletotrichum gloeosporioides* were as follows.

Collection, isolation and pathogenicity
Collection of disease samples
Diseased specimens from different fruit crops in the form of infected fruits, were collected on the basis of symptoms from different districts of Khandesh regions in the Maharashtra state by personal visit (Table 1).

Isolation of the pathogen
Pomegranate, sweet orange, banana and guava fruits showing typical symptoms of anthracnose collected from different districts of Khandesh region in Maharashtra state during June - August 2018. Standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues along with healthy portions were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds and such bits were transferred to petridishes containing sterile water successively for three times and then bits were transferred into petridishes containing 15 ml of Potato Dextrose Agar medium and incubated at 28 °C for 7 days. Pure culture of the fungus was obtained by single spore isolation.

Single spore isolation
A loopful of well sporulated culture was taken with the help of inoculating needle and suspended in sterilized water blank, serial dilutions were made so as to obtain 50 spores/ ml and spore suspension was mixed with 2 per cent molten agar in the ratio of 1:15, poured into sterile petridishes and allowed to solidify. After solidification, petridishes were inverted on the stage of the microscope and examined under low power objective. Separated spores were located and marked them with glass marking pencil. Marked area was cut with the help of sterilized scalpel and transferred centre of petriplates containing Potato Dextrose Agar medium and incubated at 28 °C for 8 days. Such pure cultures were used for further studies (Table 2).

Pathogenicity
Pathogen inoculation was done in the laboratory by placing 5 mm disc to the Potato Dextrose Broth which was cut at periphery of the actively growing culture and incubated at 26 ± 2 °C for 7 days. The conidial suspension was prepared by adjusting conidial concentration to 4 x 10^6 cfu/ ml by adding sterile distilled water to the inoculum. Mature pomegranate, sweet orange, banana and guava fruits were brought from the orchard of College of Agriculture, Dhule. The fruits were throughly washed with sterilized distilled water using moist cotton and air dried. Inoculation of the fruits was done with the help of syringe by inoculating with suspension prepared from seven days old culture in sterile distilled water. The fruits were kept in moisture chamber for incubation to ensure successful penetration of the pathogen in to the host tissue. Observations were recorded for the appearance and development of symptoms. After symptom development, reisolation was done from the artificially infected fruits. The isolate obtained was compared with the original culture for confirmation.

Identification
Isolates fulfilling the pathogenicity test were tentatively identified on the basis of morphological and cultural characters with the help of available literature.

Variation in morphological characters
All isolates were grown on PDA for seven days at 28°C then to study the morphological variation within the isolates. Mounts of different fungal structures *viz.*, mycelium, conidia, acervuli and setae were prepared and observed under light microscope at magnification of 100 X, 40 X and 10 X. The filar micrometer was calibrated with the help of stage micrometer prior to measurements. Measurements of each structure was recorded from eight to ten slides. Twenty observation were taken for conidial measurements, while 10 observation were recorded for all other structures, and mean value were calculated. The observations on morphological measurements of different structures were further analysed collectively by agglomerative hierarchial cluster analysis (Aldenderfer and Blashfield, 1984) [1].

Results and Discussion
Isolation
In the present study, *C. gloeosporioides* was isolated in the laboratory on PDA. Isolations were made from fruit samples of pomegranate, banana, guava and sweet orange which were collected from different district of Khandesh region of Maharashtra. *C. gloeosporioides* initially produced white profuse cottony growth around the host tissue placed for isolation within 72 hrs of incubation which later turned gray with formation of acervuli in some of the isolates within next 72 hrs. These findings are in similar lines as those reported by Hasabnis (1984) [8], Korade *et al.*, (2001) [10].

Pathogenicity
To prove the pathogenicity of isolated pathogen's detached fruit inoculation technique was adopted for pomegranate, sweet orange, banana and guava fruits. The inoculation on detached fruit could infect the pomegranate, sweet orange, banana and guava fruits and cause disease symptoms. The

symptoms on fruit appeared within 3-6 days, whereas no symptoms appeared on the uninoculated fruits. Symptom development was rapid in banana fruits (72-90 hrs) followed by sweet orange (105 hrs). However, symptom initiation was comparatively late in pomegranate and guava (115 hrs).

Reisolations were made from every inoculated fruit to compare the test fungus. All isolates fulfilled the requirements of Koch's postulates. Therefore, all isolates fulfilled the pathogenicity test were carried and used for the further study.

These result are in confirmation with Benyahia *et al.* (2003) [3] [3], Fagan (1979) [5] and Prashanth (2007) [11].

Variation in the morphological characters

Mycelium width

Mycelium of all isolates was initially hyaline, thin, filamentous and profusely branched later turning brown to dark brown in colour. The mycelial width of all 12 isolates ranged between 4.11 – 3.1 µ. The isolate *Colletotrichum gloeosporioides* Pomegranate Nandurbar (CgPN 3) recorded maximum mycelium width (4.11 µ) followed by isolate CgSJ 5, CgPJ 2, CgSD 4, and CgPD 1 with mycelium width of 3 wid.95 µ, 3.80 µ, 3.74 µ and 3.57 µ width, respectively and are at par with each other. The lowest mycelium th was recorded in isolate *Colletotrichum gloeosporioides* Guava Nandurbar (CgGN 12) (3.1 µ) (Table 3).

Conidia

All isolates produced conidia in pure culture within 6 days after inoculation. In general, conidia were hyaline, cylindrical to allantoidal with round ends. Conidia of isolate CgPD 1 and CgPN 3 were narrow prominantly allantoidal, where as, isolate CgSD 4, CgSN 6, CgBJ 8 and CgGJ 11 produced almost bacilliform conidia. Conidia of CgPJ 2, CgBD 7 and CgBN 9 were more short and almost uniform in size with prominent round ends. Conidia of all other isolates were more or less identical in shape. The perusal of Table 4 revealed that the average length of conidia of *C. gloeosporioides* ranged between 11.75 – 6.88 µ and this difference was statistically significant.

The highest average conidial length was exhibited by isolate *Colletotrichum gloeosporioides* Pomegranate Jalgaon (CgPJ 2) (11.75 µ) infecting pomegranate and it was at par with CgSD 4 (11.70 µ) while, shortest length of conidia were observed in *Colletotrichum gloeosporioides* Guava Nandurbar (CgGN 12) (6.88 µ) infecting guava and it was at par with CgGJ 11 (6.96 µ) and CgGD 10 (7.42 µ). On the basis of C.D. value, three distinct groups have been formed as depicted in Table 5.

The significant variation was also observed in the breadth of conidia. The average breadth of conidia ranged between 4.55-2.83 µ. The maximum average breadth (4.55 µ) was recorded in *Colletotrichum gloeosporioides* Banana Dhule (CgBD 7) infecting banana and it was at par with CgBN 9 (4.4 µ) and CgCJ 5 (4.15 µ). The lowest average breadth (2.83 µ) was observed in *Colletotrichum gloeosporioides* Pomegranate Jalgaon (CgPJ 2) and it was statistically at par from CgPD 1 and CgPN 3. Maximum number of isolates (6) exhibited the breadth in the range of 3.18 - 4.0 µ.

On the basis of this study it is concluded that the mean average size of conidia of *C. gloeosporioides* irrespective of isolates was 9.33 X 3.62 µ with a range of 11.75 - 6.88 X 4.55 -2.83 µ. The variation in conidial length and breadth was observed. Conidia of isolate CgPJ 2 from pomegranate were found to be larger with average dimensions of 11.75 X 4.55 µ followed by CgSD 4 infecting sweet orange (11.70 X 3.23 µ). Comparatively, small sized conidia were observed in CgGN 12 infecting guava (6.88 X 3.84 µ). The above findings with respect to conidial length and width are analogous with those reported by Gaikwad and Sawant (2005) [7].

Acervuli

Isolates CgPN 3, CgSJ 5 and CgGJ 11 never produced acervuli in culture. In rest of the isolates acervuli formation was noticed within 5-13 days after inoculation. The early development of acervuli after five days of inoculation was observed in CgPD 1, CgPJ 2, CgSN 6 and CgGN 12. The acervuli were formed profusely and regularly during the course of study with these isolates and therefore these isolates were reffered as profuse acervuli producers. The difference in acervulus measurements were statistically significant. Maximum length of acervuli was recorded in *Colletotrichum gloeosporioides* Guava Dhule (CgGD 10) and CgBJ 8 (160.73 µ and 134.15 µ, respectively) while, it was minimum (49.16 µ) in *Colletotrichum gloeosporioides* Sweet orange Nandurbar (CgSN 6) was statistically at par from CgBN 9 (51.44 µ). Isolate CgPN 3, CgSJ 5 and CgGJ 11 never produced acervuli in culture. Table 6 revealed that the size of acervuli ranges between 160.73 - 49.16 X 93.16 - 30.74 µ. The range of acervuli dimensions in the present study is comparatively narrow when compared to that of acervuli measurements reported earlier by Joshi (2008). The maximum width of acervulus was observed in *Colletotrichum gloeosporioides* Guava Dhule (CgGD 10) (93.16 µ), it was at par with CgBJ 8 (91.65 µ) while, minimum width was observed in *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) (30.74 µ) it was at par with CgSN 6 (33.29 µ). While studying the morphological variation within *C. gloeosporioides* isolates infecting custard apple. He has mentioned that the average size of acervuli ranged between 104.04 – 412.92 µm X 54.36 – 147.80 µm. These findings are in confirmity with Gaikwad and Sawant (2005) [7].

Setae

Out of 12 isolates, 9 isolates could produce setae as an integral part of acervulus. Acervuli of isolates CgPN 3, CgSJ 5 and CgGJ 11 were without setae. The overall shape of setae was erect, spine like, broader at base, tapering, becoming pointed towards apex. Stout, sword like setae were observed in CgPJ 2, CgBJ 8 and CgGN 12, whereas setae in acervuli of CgSD 4, CgSN 6, CgGD 10 were less erect, slightly wavy to curved with thin structure. Setae produced in CgPJ 2 and CgBJ 8 were completely different in morphology as they were extremely short, stout at base with blunt.

There was a considerable variation within the *C. gloeosporioides* isolates in the average length and average breadth at base of the setae (Table 7). The average maximum length of the setae was (75.82 µ) recorded in isolate *Colletotrichum gloeosporioides* Banana Jalgaon (CgBJ 8) and it was followed by CgBD 7. The shortest setae were observed in *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) having average length of 27.03 µ. Maximum breadth of setae at the base (3.95 µ) was observed in CgPJ 2 and it was at par with the isolates CgPD 1, CgBD 7, CgBJ 8 and CgGN 12. Significantly narrow based setae were observed in the acervuli of CgGD 10 (3.4 µ) and it was at par with CgSN 6, CgBN 9 and CgSD 4.

There was also significant variation in the number of setae per acervuli produced by different isolates. Maximum number of setae per acervulus were observed in *Colletotrichum gloeosporioides* Banana Dhule (CgBD 7) from banana and it

was followed by with CgPD 1 from pomegranate and CgBJ 8 from banana. Very less number of setae were observed in *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) with average setae per acervulus.

These results were also recorded by Prashanth (2007) [11], Rajesh (1999) [12] and Sutton (1980) [13].

Table 1: List of diseased samples isolates of *C. gloeosporioides* collected from different hosts in different districts of Khandesh regions in Maharashtra.

Sr. No	Name of fruit crop	Host Sample	Village	Tahsil	District
1	Pomegranate	Fruit	AC, Dhule campus	Dhule	Dhule
2		Fruit	Chunchale	Chopda	Jalgaon
3		Fruit	Borad	Shahada	Nandurbar
4	Sweet orange	Fruit	Pimpalner	Sakri	Dhule
5		Fruit	Dharangaon	Dharangaon	Jalgaon
6		Fruit	Koparli	Shahada	Nandurbar
7	Banana	Fruit	Dhondaicha	Shindkheda	Dhule
8		Fruit	Adawad	Chopda	Jalgaon
9		Fruit	Ashta	Nandurbar	Nandurbar
10	Guava	Fruit	Ner	Dhule	Dhule
11		Fruit	Kingaon	Chopda	Jalgaon
12		Fruit	Sarangkheda	Shahada	Nandurbar

Table 2: Cultures of *C. gloeosporioides*

Sr. No	Isolate No.	District	Host
1	CgPD 1	Dhule	Pomegranate
2	CgPJ 2	Jalgaon	
3	CgPN 3	Nandurbar	
4	CgSD 4	Dhule	Sweet orange
5	CgSJ 5	Jalgaon	
6	CgSN 6	Nandurbar	
7	CgBD 7	Dhule	Banana
8	CgBJ 8	Jalgaon	
9	CgBN 9	Nandurbar	
10	CgGD 10	Dhule	Guava
11	CgGJ 11	Jalgaon	
12	CgGN 12	Nandurbar	

Table 3: Variation in mycelium width of *C. gloeosporioides* isolates

Isolates	Mycelium width (µ)		
	R I	R II	Mean
CgPD 1	3.66	3.49	3.58
CgPJ 2	3.88	3.72	3.8
CgPN 3	4.04	4.19	4.12
CgSD 4	3.81	3.67	3.74
CgSJ 5	3.89	4.02	3.96
CgSN 6	3.03	3.52	3.2
CgBD 7	3.32	3.47	3.40
CgBJ 8	3.21	3.3	3.26
CgBN 9	3.42	3.45	3.44
CgGD 10	3.25	3.39	3.32
CgGJ 11	3.4	3.49	3.45
CgGN 12	3.2	3	3.1
SE ±			0.10
CD @ 5%			0.30

Table 4: Variation in conidial measurements of *C. gloeosporioides* isolates (in µ)

Conidia length				Conidia breadth			Length : breadth ratio		
Isolates	RI	RII	Mean	RI	RII	Mean	RI	RII	Mean
CgPD 1	11	10.5	10.75	2.62	3.21	2.92	3.25	3.47	3.36
CgPJ 2	12.2	11.3	11.75	2.77	2.89	2.83	2.89	3.13	3.01
CgPN 3	10.4	10.1	10.25	3.15	3.05	3.1	2.66	2.97	2.82
CgSD 4	11.6	11.8	11.7	3.26	3.19	3.23	2.4	2.73	2.57
CgSJ 5	9.4	9.7	9.55	4.2	4.1	4.15	3.5	3.62	3.56
CgSN 6	10.3	10.8	10.55	3.5	3.7	3.6	3.65	3.67	3.66
CgBD 7	10	9	9.5	4.42	4.68	4.55	4.7	4.7	4.7
CgBJ 8	8.5	8.6	8.55	3.56	3.64	3.6	4.2	4.3	4.25
CgBN 9	8.4	7.9	8.15	4.3	4.5	4.4	4.1	4.2	4.15

CgGD 10	7.71	7.12	7.42	3.62	3.88	3.75	2.73	2.47	2.6
CgGJ 11	6.93	6.99	6.96	3.41	3.53	3.47	2.83	2.99	2.91
CgGN 12	6.82	6.94	6.88	3.88	3.8	3.84	3.01	3.07	3.04
SE +			0.26			0.11			0.10
CD @ 5%			0.79			0.35			0.30

Table 5: *C. gloeosporioides* isolates grouped on the basis of conidial length

Conidia with higher length	11.27- 13.29 µ	CgPJ 2 - *Colletotrichum gloeosporioides* Pomegranate Jalgaon CgSD 4 - *Colletotrichum gloeosporioides* Sweet orange Dhule
Conidia with moderate length	11.26- 9.24 µ	CgPD 1 - *Colletotrichum gloeosporioides* Pomegranate Dhule CgPN 3 - *Colletotrichum gloeosporioides* Pomegranate Nandurbar CgSJ 5 - *Colletotrichum gloeosporioides* Sweet orange Jalgaon CgSN 6 - *Colletotrichum gloeosporioides* Sweet orange Nandurbar CgBD 7 - *Colletotrichum gloeosporioides* Banana Dhule
Conidia with shorter length	9.23 -7.21 µ	CgBJ 8 - *Colletotrichum gloeosporioides* Banana Jalgaon CgBN 9 - *Colletotrichum gloeosporioides* Banana Nandurbar CgGD 10 - *Colletotrichum gloeosporioides* Guava Dhule CgGJ 11- *Colletotrichum gloeosporioides* Guava Jalgaon CgGN 12 - *Colletotrichum gloeosporioides* Guava Nandurbar

Table 6: Variation in acervuli measurement of *C. gloeosporioides* isolates (in µ)

Acervulus length				**Acervulus width**		
Isolates	**RI**	**RII**	**Mean**	**RI**	**RII**	**Mean**
CgPD 1	88.48	91.01	89.75	74.12	76.38	75.25
CgPJ 2	59.60	63.12	61.36	48.93	42.15	45.54
CgPN 3	0.00	0.00	0.00	0.00	0.00	0.00
CgSD 4	69.31	67.33	68.32	47.52	50.33	48.93
CgSJ 5	0.00	0.00	0.00	0.00	0.00	0.00
CgSN 6	48.81	49.52	49.17	34.18	32.39	33.29
CgBD 7	54.40	55.10	54.75	43.88	40.12	42.00
CgBJ 8	133.62	134.69	134.16	89.35	93.95	91.65
CgBN 9	51.09	51.80	51.45	31.09	30.39	30.74
CgGD 10	161.17	160.30	160.74	93.65	92.67	93.16
CgGJ 11	0.00	0.00	0.00	0.00	0.00	0.00
CgGN 12	71.91	73.71	72.81	51.14	49.70	50.42
SE+			0.78			1.45
CD @ 5%			2.41			4.47

Table 7: Variation in setae measurement of *C. gloeosporioides* isolates (in µ)

Setae length (µ)				**Setae breadth at base (µ)**			**No.of setae /acervulus (µ)**		
Isolates	**RI**	**RII**	**Mean**	**RI**	**RII**	**Mean**	**RI**	**RII**	**Mean**
CgPD 1	68.31	69.46	68.89	3.99	3.80	3.90	14.70	14.88	14.79
CgPJ 2	48.39	47.22	47.81	3.88	4.03	3.96	11.70	10.83	11.27
CgPN 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CgSD 4	58.49	60.81	59.65	3.72	3.43	3.58	9.80	10.77	10.29
CgSJ 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CgSN 6	61.37	62.11	61.74	3.67	3.33	3.50	14.55	13.52	14.04
CgBD 7	71.04	70.59	70.89	3.73	3.81	3.77	18.8	18.88	18.84
CgBJ 8	76.14	75.5	75.82	3.82	3.89	3.86	14.1	14.26	14.18
CgBN 9	26.34	27.72	27.03	3.57	3.47	3.52	4.95	5.01	4.98
CgGD 10	59.72	60.67	60.2	3.48	3.32	3.40	11.4	10.47	10.94
CgGJ 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CgGN 12	54.27	54.82	54.55	3.93	3.67	3.80	13.11	13.2	13.16
SE+			0.51			0.09			0.28
CD @ 5%			1.56			0.27			0.86

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