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MORPHOLOGICAL VARIATION OF *BIPOLARIS* ISOLATE FROM THE WHEAT GERMPLASMS

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Abstract: Wheat is cultivated worldwide, especially at the eastern plains of south Asia, constitutes a very important source of food to a vast population. In India, it contributes nearly 31.50% of the total food grain basket. The investigation included the screening of 600 genotypes with a broad genetic base. A symptom of the different variant was collected. The pathogen was isolated from different genotypes from different plant parts; variations in the isolates were documented along with *in-vitro* pathogenicity testing. The morphological study of both culture and conidia was made on half-strength PDA and water agar, respectively. Singles spore cultures were made from all those isolates for the studies for pathogenicity. The results of the morphological study comes with the findings that the UBS-1 (Septation: 9.1±1.3, Length: 92.2 ± 25.7, Width:49.3± 37.8) and UBS-2 (Septation: 7.6±1.7,Length:85.3±19.1,Width:33.6±19.2) has higher in average septation.UBS-7,UBS-9,UBS-11,UBS-12 has triangular shape conidia. USB -9 and USB-14 has two different types of septation euseptate and distoseptate. The result of the pathogenicity shows that out of 14 isolates, UBS -1 and UBS-2 is the most virulent isolates in *in-vitro* condition and the remaining are moderately virulent and a virulent isolates. The finding comes with the variability of the pathogen depends on the host factor and environmental factor. Different degrees of host resistance lead to evolving new characters in the pathogen to adjust with the respective genotype and environment. From the cluster of different isolates, it was found that the potential virulent pathogen remains consistent *in-vivo*. In contrast, a virulent or moderately virulent isolate shows different degrees of virulence based on morphological and physiological characters in a different environment.

Introduction: Foliar blight symptoms are associated with more than one pathogen and are often difficult to differentiate in the naked eye; it is considered a disease complex, and its importance was highlighted previously in reports from India (Nema and Joshi, 1973), Bangladesh (Badaruddinet al., 1994) and Nepal (Devkota,1994). Spot blotch in wheat is caused by the fungi *Bipolarissorokiniana* (Sacc.) Shoem., in anamorph state (Saari, 1997). A key for distinguishing

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species of *Bipolarissorokiniana* was is described by Subramanian and Shoemaker (1971). In axenic culture, the mycelium is composed of hyphae interwoven as a loose cottony mass and appears as white or light to dark grey depending on the isolates (Kumar et al., 2002). These fungi are differentiated from the *Bipolaris* genus based on morphological features of conidiophores and conidia. On the leaf, lesions are due to anamorph *Bipolarissorokininana*, characterized by long multicellular spores, whereas the ascospores of *Cochliobolussativus* are formed in pseudothecia developed on the wheat residue.

Materials and methods:

Different symptoms were collected, and *Bipolaris spp*. were isolated from various types of symptoms observed at one location, and their pathogenicity was tested on highly susceptible genotype. The experiment was conducted in the University Research Field of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar District, West Bengal in the Rabi season, favourable for the wheat crop. The research field is situated at the latitude of 26˚19′ 86″ N and longitude of 89˚23′53″ E, at an elevation of about 43 meters above mean sea level. The crop is sown from November to December and harvested in March and April of the succeeding year. The trial was conducted in three consecutive wheat seasons, i.e. 2012-2013, 2013-2014 and 2014-2015.

The germplasm of different genotypes was collected through a Crop Research Program project from CIMMYT, Mexico, planted at the UBKV research field where 500 different germplasm with 2 replications was planted in winter 2012-2013. In the succeeding rabi season, 121 genotypes with two replication were planted with 31 entries from the first germplasm in 2013-2014. Again in the 2014-2015 rabi season, the germplasm was prepared with 8 susceptible checks and 47 resistant genotypes with differential response to the disease and planted in 3 replications.

Different types of symptoms (a typical symptom) present on the wheat leaf at the different growth stages were collected from the research plot by random selection in the paper bags to avoid contamination. The date and genotype number from the germplasm was recorded for future use. They were photographed and scanned for the record.

Determination of variation in the pathogenicity due to different variants of the pathogen.

The collected diseased leaf symptoms samples are cut into square-shaped 25 mm 2 pieces with some parts of healthy tissue. The cut leaf samples are surface sterilized with Mercuric Chloride (0.1%) solution, washed thoroughly with a double /sterilized distilled water, and put on to sterilized blotting paper to remove the excess water. The leaf bits are placed under laminar airflow in a 9mm Petri dish containing PDA medium (PDA 200:10:20, containing half dextrose). It was incubated at 27±1˚C for 5 days to 7 days in an incubator. The isolated cultures were transferred in PDA slants for future use.

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After the crop harvest, wheat grain is stored in the paper bags separately, selected for isolation of the pathogen. 5 ml sterilized distilled water in a test tube was added with 25 seeds are shaken well to dislodge the conidia, diluted spore suspension of the pathogen was poured in the gridded Petri plate containing water agar medium. The number of spores is counted with the help of a microscope; then, it is left in the BOD for growth. The single conidia were picked under the microscope with the help of a sterilized needle, transferred to the PDA medium in Petri plate for further growth, and finally transferred to the slants for future use.

Characterization of the pathogen base on morphological and cultural characters

Culture characteristics of the pathogen

Morphological characters of the fungal pathogen are studied on a slide under a microscope fitted with photographic attachment and using image analysis software for taking the measurements after proper calibration. Septation of the conidia and conidiophores was observed and recorded along with the conidia's shape, size, and colour of the conidia.

Pathogenicity test in the healthy susceptible plant

The cut-leaf method determined in vitro pathogenicity on two susceptible cultivars, namely, Sonalika and CIANO T 79. The spore suspension was prepared from the mass multiplied culture on wheat grains to the concentration of 10^8 as measured by Haemacytometer. Cut leaves were put on folds of moist blotting papers, and the suspension of the spore was applied without causing any injury to the leaf. The development of symptoms was recorded periodically at an interval of 12, 24, 48, 72, 96 hours after inoculation. Percent leaf area infected was estimated based on a 0-9 scale, and speed of appearance of the disease was also recorded. Based on replicated data area under the disease progress curve was calculated according to the standard formula by Das *et al.* (1992) and Sharma *et al.* (2004).

Results:

Morphological variation

Disease infected wheat leaves were collected from the experimental plots having more than 600 genotypes of wheat. The pathogens were isolated from the infected leaves and grains at different phenological stages of growth. Isolates were then differentiated primarily based on morphological appearance and divided into fourteen different morphological types of conidia.

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Then detail morphological study of both culture and conidia was made on half-strength PDA and water agar, respectively. Singles spore cultures were made from all those isolates for the studies for pathogenicity.

Morphological characteristics of the culture and the conidia of the isolates are presented in table 1 with a brief description of the same.

Table: 1. Culture Characteristics of different isolates of *Bipolaris*sp. collected from wheat.

Isolate	Culture Characteristics	Conidia
USB-1	Colony colour is a light grey to blackish-grey at early stage uniform growth, no concentric rings observed. The mycelial growth is velvety in nature.	Conidia are ovoid to ellipsoidal and rarely fusiform in shape; conidia colour varies from brown to dark brown. The septation is euseptate type. Septation: 9.1±1.3 Length:92.2 ± 25.7 Width:49.3± 37.8
USB-2	Colony colour is a greenish-grey to blackish dark grey, with no consistent growth, no concentric rings observed. The mycelium is velvety in nature.	Conidia are ellipsoidal to straight cylindrical or slightly curved. Conidia are brown in colour. Conidia with euseptate type septation. Septation: 7.6±1.7 Length:85.3±19.1 Width:33.6±19.2
USB-3	Colony colour is a blackish dark grey with no consistent growth and no concentric rings. Continuous sub culturing change the colour of the PDA medium to light pinkish maroon.	Conidia are brown with cylindrical to ellipsoidal in shape or slightly oval. Euseptate type of septation was found. Septation: 5.5±1.1 Length:75.2±15.6 Width:29.6±9.7
USB-4	Colony colour was greenish-grey with white mixed, uniform growth, no formation of concentric rings. Mycelial growth observed was cottony.	Conidia are brown in colour, ellipsoidal shape to cylindrical shape. Type the separation was euseptate. Septation: 5.2±1.6 Length:98.2±37.9 Width:52.4±40.1
USB-5	Cottony colony, colour was greenish-grey with white mixed, uniform growth, no concentric rings observed.	Conidia are dark brown in colour, cylindrical curve shape to ellipsoidal shape was observed. Septation was euseptate type. Septation: 6.1±1.2 Length:80.7±16.52 Width:28.3±4.4
USB-6	Cottony colony, grey-white with consistent growth. A concentric ring was observed at the early stage of the	Conidia are brown cylindrical shape to ellipsoidal shape was observed with slightly oval at the ends. Conidia have a unique characteristic of having a three polar, middle cell protuberant when fully

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	subculture.	mature, dividing to form three polar zone/apical bifurcate formation. Septation type is euseptate. Septation: 6±0.94 Length:102.6±16.1 Width:43.4±7.5
USB-7	Colony colour was grey, white mixed to greenish-grey, uniform growth, no concentric rings were observed. Mycelial growth was cottony.	Conidia are dark brown in colour, ellipsoidal to cylindrical ovoid. Conidia, when fully mature middle cell divide to form three polar zone formation. Type of septation is euseptate. Septation: 6.3±1.4 Length:104.5±46.2 Width:36.08±13.5
USB-8	Colony colour is greenish, dark grey with white mixed**.** Uniform growth with no concentric rings.	Conidia are dark brown, ellipsoidal to cylindrical curve. Septation type is euseptate . Septation: 5.4±1.7 Length:125.8±53.4 Width:47.1±25.7
USB-9	Colony colour is a dark white grey mixed with blackish-grey, consistent growth. Mycelial growth was cottony.	Conidia are dark brown or golden brown in colour, ellipsoidal to cylindrical. The colony has two types of conidia, one with two polar and another three polar. A unique characteristic in the middle cell protuberant when fully mature divides to form three polar zones. Conidium with two types of septation euseptate (parasitic stage) and distoseptate (saprophytic stage) was observed. Septation: 7.3±0.94 Length:151.7±46.08 Width:57.1±14.4
USB-10	Colony colour is a blackish grey, uniform growth; mycelial growth was velvety.	Conidia are dark brown in colour, ellipsoidal to cylindrical. Type of septation was euseptate. Septation: 5.7±0.67 Length:72±12.80 Width:25.6±7.5
USB-11	Colony colour is dark grey with white mixed; mycelial growth was cottony and uniform growth.	Conidia are dark brown in colour, ellipsoidal to cylindrical. The colony formed two types of conidia when the middle cell protuberant in fully mature conidia divides to form three polar zones with three ends and other conidia with two ends. Septation: 6.3±1.15 Length:77.12±19.46 Width:30.2±12.5
USB-12	Colony colour is a dark grey mixed with white, uniformed growth. Mycelial growth was	Conidia are dark brown pigmented, cylindrical shape to broadly ellipsoidal, slightly ovoid at ends. It has a unique characteristic of having three polar. In the

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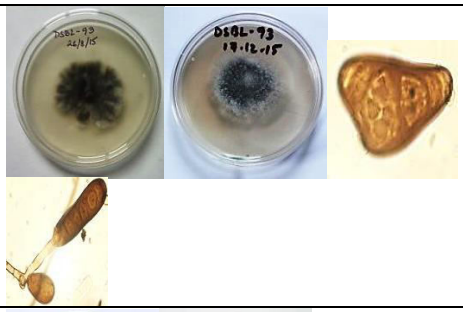
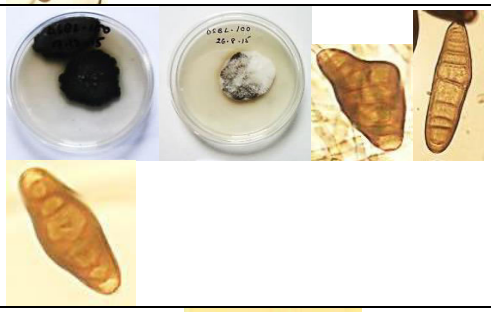
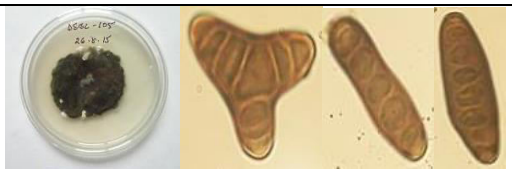
	cottony in nature.	centre of the conidia, it has a triangular cell with septation. Type of septation was euseptate type. Septation: 7.3±1.25 Length:82.6±14.9 Width:81.8±70.4,66.8±18.23,75.1±27.4

Isolate	Culture Characteristics& conidia	Isolate	Culture Characteristics& conidia
UBS-1		UBS-2	
UBS-3		UBS-4	
UBS-5		UBS-6	
UBS-7		UBS-8	
UBS-9		UBS-10	
UBS-11		UBS-12	

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	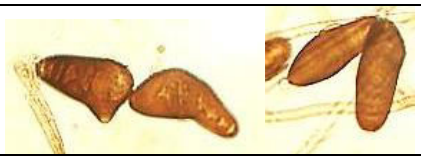		

From the morphological characterization, it was found there was a considerable variation in the isolates of *Bipolarissorokinina* on wheat in terms of shape, size, colour, septation and appearance of the culture on media. Their reaction on the wheat genotypes was also recorded by quantifying the number of lesions and size of lesions contributing to the disease (AUDPC). Most cultures were euseptate in nature with light brown to deep brown in colour with concolourous ends. Some isolates, namely UBS-7, UBS-12 distinctly showed different conidia morphology with triangular shapes than the typical cylindrical conidia shape. Again in some of the isolates, the conidia shape was neither triangular nor cylindrical. Still, it looked more like; however, it was much bigger than normal *Curvularia* spores (normal size of *Curvularia* 24.9 µm×10.3µm (Huang et al., 2004).

Pathogenic variation

The isolates collected from different plant parts and at different crop growth stages were tested for pathogenicity under *in-vitro* conditions. The pathogenicity test of the various isolates on the international check varieties Sonalika and Ciano T-79 was recorded through visual observation at a time scale. The leaf area infected due to inoculation was recorded at 8hrs, 12hrs, 24hrs, 48 hrs, 72 hrs, 96 hrs of the inoculated leaves. The result of which is presented in table 3 with their respective initiation of symptom production and magnitude.

Table2: **Pathogenic reaction of *Bipolaris* isolates on the susceptible host**.

CULTURE	Collected from	Percent Infection in leaf						AUDPC
		8 HRS	12 HRS	24HRS	48 HRS	72 HRS	96 HRS	
UBS-1	CRP-473	0.00	3.70	14.81	62.96	81.48	96.30	200^{A}
UBS-2	CRP-474	0.00	0.00	3.70	33.33	59.26	96.30	142.59^{CD}
UBS-3	CRP-449	0.00	0.00	0.00	11.11	22.22	22.22	44.44^{A}
UBS-4	CRP-396	0.00	0.00	0.00	11.11	11.11	22.22	33.33^{BC}
UBS-5	CRP-390	0.00	0.00	0.00	11.11	11.11	22.22	33.33^{AB}
UBS-6	DSBL-59	0.00	0.00	3.70	44.44	59.26	92.59	151.85^{D}
UBS-7	DSBL-93	0.00	0.00	0.00	11.11	22.22	29.63	48.14^{CD}
UBS-8	DSBL-94	0.00	0.00	0.00	3.70	18.52	29.63	37.03^{FG}
UBS-9	DSBL-100	0.00	0.00	0.00	11.11	22.22	62.96	64.81^{EFG}

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UBS-10	DSBL-101	0.00	0.00	11.11	59.26	88.89	100.00	203.70^{EF}
UBS-11	DSBL-118	0.00	0.00	0.00	7.41	22.22	59.26	59.25^{E}
UBS-12	DSBL-105	0.00	0.00	0.00	11.11	22.22	25.93	46.29^{FG}
UBS-13	VKM-1	0.00	0.00	11.11	33.33	44.44	70.37	118.51^{FG}
UBS-14	VLM-8	0.00	0.00	11.11	22.22	66.67	77.78	133.33^{G}

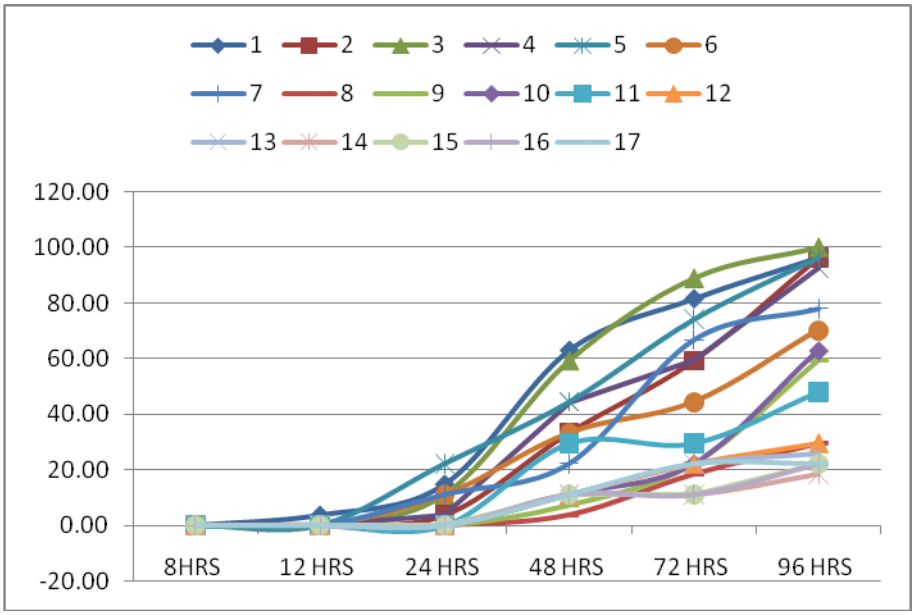

Fig1. Disease progress curve in susceptible host due to different isolates of *Bipolarissorokiniana.*

Results from the table1 indicate that UBS-1 initiated symptom production within 12 hrs of inoculation. Few isolates initiated symptom production by 4 hrs. The rest of the isolate initiated the disease after two days. Thus there was a difference in the leave area infected/covered by the disease. This finally culminated in AUDPC, which indicated disease progress throughout 96 hrs.

Morphological data of the conidia like the number of septa, length of conidia, the width of conidia was considered along with the in-vivo AUDPC for classification of the isolates into virulent and avirulent groups. This clearly indicated that UBS-1 and UBS-2 were the most virulent isolates collected in Cooch Behar. Other cultures like UBS-14, UBS-10, UBS-6 were among the virulent isolates.

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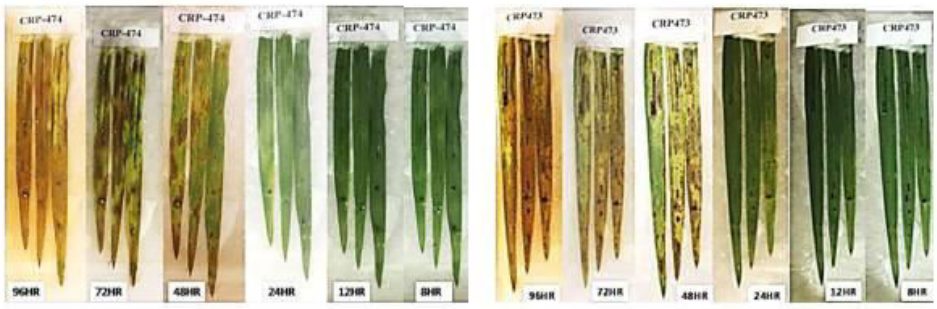

A. Virulent isolate in susceptible genotype

B. Moderately isolate in suceptible genotype

C. Avirulent isolate in susceptible genotype .

PLATE 1. Disease reaction of virulent , moderately virulent and avirulent isolates on susceptible genotypes.

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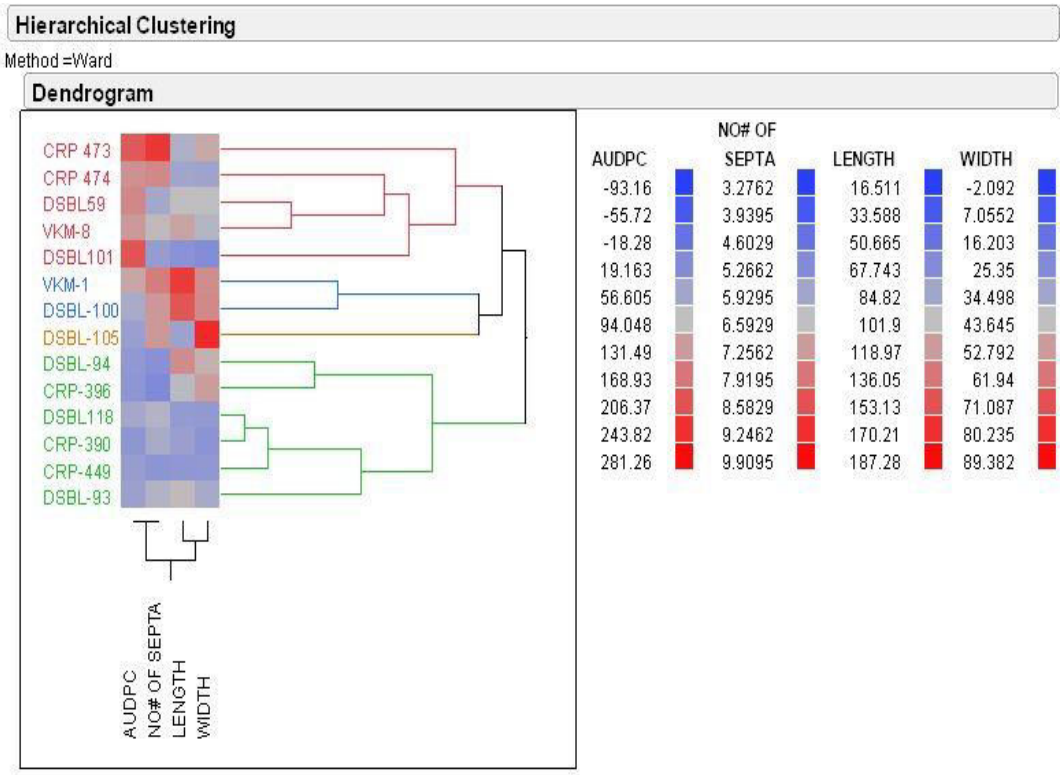

Fig3. Cluster diagram of the *Bipolaris* isolates using Ward's method data classification.

Discussion and Conclusion

Investigation with the isolates collected from spot blotch infected wheat genotypes indicated that there is a morphological variability that exists among the isolates. However, only morphological variations do not express the aggressive nature of *B. sorokiniana* (Maraite et al., 1998). *B. sorokiniana* being a hemibiotroph, pathogenic variability is crucial to work out a selection of resistant genotypes. Literature indicates a difference in disease-causing ability or aggressiveness of different variants on a given set of genotypes (Hetzler et al., 1991 and Maraite et al., 1998). Based on morphological appearance and pathogenic traits, isolates of *Bipolarissorokiniana* maybe grow in different categories (Chand et al., 2003). It was observed from the result that in addition to morphological differences, pathogenic variation also exists. However, meagre information about the difference in aggressiveness among the variants. A huge amount of variation from a single location is probably due to asexual recombination through the parasexual cycle (Tinline, 1962). After going through the results, it was also felt that varying host resistance also plays a role in changing the pathogen's virulence (Valjavec-Gratian and Steffenson 1997). The most virulent pathogen UBS-1 and UBS-2 isolated from CRP plots 473 and 474 through *in-vivo* studies also prove their virulence in the field with high AUDPC.

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