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Variability and Virulence Analysis of *Aspergillus niger* Isolates Causing Collar Rot of Groundnut

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ABSTRACT

Background: Collar rot of groundnut (*Arachis hypogaea* L.) caused by *Aspergillus niger* is a significant constraint in groundnut cultivation and responsible for huge economic losses in India including Rajasthan.

Methods: By surveying of eight major groundnut growing districts of Rajasthan, India, one representative *Aspergillus niger* isolate from each district was established (ANBK-01= Bikaner, ANCH-02= Churu, ANDA-03= Dausa, ANJP-04= Jaipur, ANJL-05= Jalore, ANJD-06= Jodhpur, ANNG-07= Nagaur and ANSK-08= Sikar) for studying variability in the pathogen as well as to know the response of groundnut varieties to the highly virulent isolate. The colony and spore characteristics were observed for cultural and morphological variability. For resistance response to the disease, ten varieties (M-13, RG-633-9, RG-382, Girnar-2, RG-604, RG-578, Gajraj 10, RG-510, RG-632-1 and RG-644) were evaluated in the field for two consecutive years against a highly virulent *Aspergillus niger* (ANJP-04) isolate.

Result: Our investigations cleared that all the isolates were showed cultural and morphological variability such as shape, colour and size of colony and size of conidia, conidiophores and columella. Isolate (ANJP-04) collected from Khejroli village of Chomu tehsil in Jaipur district showed maximum mycelial growth, conidia diameter, length and diameter of conidiophores and length and diameter of columella, early sporulation and found most virulent as it produced higher disease incidence (54.43%). Ten released varieties of groundnut in the field conditions, revealed that none of the variety was found completely free from the disease whereas RG-644, M-13 and RG-510 were found resistant while RG-604, Girnar-2, Gajraj-10 and RG-632-1 were found moderately resistant and rest were found susceptible to highly susceptible to the disease. Conclusively, it can be finalized that famers may cultivate these resistant varieties in areas where collar rot is a severe constraint. The conclusion of this study can also be utilized to screen varieties/genotypes of groundnut against highly virulent isolate for sustainability of breeding material to the disease effectively.

Key words: Groundnut, Collar rot, *Aspergillus niger*, Variability, Screening.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an important legume crop of tropical and sub-tropical areas of the world as described in 1753 by Linnaeus (Pattee and Young, 1982). It is a member of the genus *Arachis* in the sub tribe *Stylosanthinae* of tribe *Aeschynomeneae* of the family *Fabaceae*. It is the only species in the genus *Arachis* which is economically important and it forms underground fruits. It has a tap root system that is often covered with root nodules resulting from a symbiotic association with nitrogen-fixing bacteria (collectively called rhizobia). In association with these bacteria, it fixes about 80-160 kg N/ha per season (Alam *et al*., 1988) to incorporate substantial amount of nitrogen in the soil.

The groundnut oil is used as table oil and for the manufacturing of soap, margarine and other products such as sweets and butter. The shells are used as manure, animal feed, fuel and raw material for many products (Vankatanarayana, 1952). The residual oil cake contains substantial amounts of nitrogen, phosphorous and potassium and is used as a fertilizer particularly in organic production. It plays an important role in the dietary requirements of resources for poor families.

In our country, though groundnut is cultivated in one or more seasons (*Kharif* and *Rabi* and summer) but nearly 80 per cent of the annual acreage and production comes from *Kharif* crop (June to October) season. In India, the total coverage area under this crop is about 39.31 lakh hectares and production of 6.86 million tonnes with an average productivity of 1745 kg/hectare (Anonymous 2019). Rajasthan stands second position in terms of area and production. The cultivation of groundnut is well adapted to the conditions prevailing in Rajasthan and is cultivated in about 7.34 lakh hectares with annual production 1.612

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million tonnes and productivity of 2195 kg/hectare (Anonymous, 2019-20).

Several abiotic and biotic factors affect the growth and development of groundnut leading to qualitative and quantitative yield losses. Diseases that are most damaging and major limiting factors that cause huge economic losses in profitable cultivation in Rajasthan. The attack of several diseases primarily caused by fungi, take a heavy toll of the crop at all the stages of growth right from sowing to harvest and thereafter in storage.

Amongst fungal diseases, collar rot of groundnut also known as seedling blight caused by *Aspergillus niger* van. Teighem is one of the important seed and soil borne diseases. Collar rot of groundnut prominently is distributed in countries with tropical and subtropical climates where high temperature prevails during the rainy season and it is present in all most the entire groundnut growing areas of the world and first reported by Jochem (1926) from Java. In Rajasthan, Bakhetia (1983) had reported disease incidence up to 50.00 per cent. Dighule *et al.* (2018) estimated yield losses in Maharashtra from 28.00 to 50.00 per cent due to collar rot of groundnut caused by *Aspergillus niger.* It is prevalent in almost all groundnut growing states of India *viz.*, Punjab, Gujarat, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Maharashtra, Rajasthan, Karnataka and Odisha (Dighule *et al.* 2018).

The disease results in an uneven germination and deformed seedlings and subsequently loss in plant population and yield. This disease appears in two phases, *viz.,* pre-emergence and post-emergence phases. In the pre-emergence phase, the seed may rot in the soil or be covered with sooty black masses of spores on germinating seeds; the emerging hypocotyls are rapidly killed by these spores. In the post-emergence phase, circular light brown lesions appear initially on the cotyledon and as these advances, hypocotyl or stem lesions become water soaked and show light brown discoloration. The seedlings then collapse and die due to the rotting of the succulent hypocotyls. The loss due to this disease was reported upto 40.00-50.00 per cent Chahal *et al.* (1974). According to Jain and Nema (1952) the first initiation of the disease was the appearance of a circular and brownish spots on the cotyledons. The discolored area rapidly became soft and rotten and spread on to the stems and hypocotyls which also become yellow, soft and rotten and collapse. The affected stems become shredded. Greyish white mycelia and black fructifications of the pathogen appeared on the surface of the affected parts. The primary source of the inoculum of collar rot pathogen has been shown to be mycelia and spores carried on the seeds and debris in the soil (Nema *et al.* 1955).

Variability in fungi regarding cultural, morphological, physiological and pathogenic characters plays a significant role to understand its nature and disease epidemic aspect. Vegetative growth of *A. niger* is very rapid on culture media with submerged mycelium. The hyphae are septate and hyaline more or less yellow in color. The colonies are black coloured and reverse usually colourless. Conidiophores mostly arise directly from substratum and are smooth, septate or non-septate, varying greatly in length and diameter. Conidial heads are fuscous, blackish-brown to purple-brown or in every shade to carbonous black, varying from small, almost columnar masses of a few conidial chains to the common globes or radiate heads. Conidia are globose to sub-globose, dark brown to black and rough-walled (Gilman, 2001 and Sharma 2012).

In general, testing of resistance is a continuous process because of evolution of new biotypes of the pathogen or it may be due to break down of resistance in host genotypes. Plant diseases management with the principal of host resistance is an economic and environmentally safe. Nathawat *et al.* (2014) evaluated five varieties against collar rot of groundnut caused by *A. niger* and only GG 2 was found tolerant to collar rot, while GG 5, GG 7, GG 20 and GG 37 were susceptible to highly susceptible. Palaiah *et al.* (2019) evaluated 64 groundnut germplasm and 33 varieties to identify the sources of resistance against collar rot (*Aspergillus niger*), stem rot (*Sclerotium rolfsii*) and dry root rot (*Rhizoctonia bataticola*) diseases. Among evaluated 33 groundnut varieties against these diseases, none of the varieties showed highly resistant and resistant.

In lieu of this, the present investigation was planned to observe prevalence of cultural, morphological and pathogenic variability in *A. niger* isolates, that may warranting the need for development of groundnut varieties with huge resistance to the prevailing highly virulent isolates.

MATERIALS AND METHODS

The present investigations were carried out during *Kharif* 2019 and 2020 at the Agronomy Farm and Department of Plant Pathology, S.K.N. Collage of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Rajasthan. Jobner is situated at latitude of 26°5′ N, longitude of 75°20′ E and altitude of 427 meters above MSL (mean sea level). This region falls under semi-arid eastern plain (Agro Climatic Zone- III A) of Rajasthan. To ascertain the variability among the isolates of *A. niger*, cultural, morphological, pathogenic and varietal screening studies were conducted.

Cultural and morphological variability

PDA medium was used for cultural and morphological variability among eight isolates of *A. niger*. Twenty ml of sterilized PDA was poured aseptically in each sterilized glass Petri plate. On solidification of medium, plates were inoculated with 5 mm dia. mycelial disc obtained from the periphery of actively growing colony of four day-old cultures of different isolates and then incubated at 25±1°C in incubator. The cultural and morphological observations like colony growth, colour of colony sporulation and diameter of colony were recorded. Microscopic variation *viz.*, size of conidia, size of conidiophores and size of columella were taken with the help of fluorescens microscope from seven days old culture of pathogen.

Pathogenic variability

To test the pathogenic variability among eight isolates, apparently healthy surface sterilized groundnut seeds (variety RG-382) were taken. The seeds were treated on seven days old sporulating culture of each isolate of A. niger grown on media contained in Petri-plates. Inoculated four seeds were sown at five cm depth in 30 cm diameter earthen pots (pre-sterilized and having autoclaved soil) with three replications. One replication had five pots (20 seeds per replication). The un-inoculated apparently healthy seeds served as control. These pots were kept in cage house and watered as per requirement and observations on pre-emergence mortality and post-emergence mortality were recorded 10 and 30 days after sowing, respectively (Yadav and Ghasolia, 2020). Per cent disease incidence (PDI) was calculated by counting the diseased and the total number of plants per plot:

$$PDI = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

Screening of varieties

Ten varieties of groundnut (M-13, RG-633-9, RG-382, Girnar-2, RG-604, RG-578, Gajraj 10, RG-510, RG-632-1 and RG-644) received from RARI, Durgapura and SKN College of Agriculture, Jobner were evaluated against collar rot of groundnut under artificial inoculation condition in field during *Kharif* 2019 and 2020. Inoculum (ANJP-04 isolate) multiplied on sorghum grains was applied in furrows at the time of sowing @ 20 g/m row length to increase the disease pressure. An observation on PDI was recorded 45 days of sowing. On the basis of disease incidence, the varieties were categorized as per criterion followed by Nene *et al.* (1981) and slightly modified by Farooq *et al.* (2019) as immune (0 PDI), resistant (1-10 PDI), moderately resistance (11-20 PDI), moderately susceptible (21-30 PDI), susceptible (31-50 PDI) and highly susceptible (>50 PDI).

RESULTS AND DISCUSSION

During survey of eight major groundnut growing districts of Rajasthan for collar rot, the disease incidence was recorded 21.04% in Bikaner, 20.82% in Churu, 21.80% in Dausa, 28.85% in Jaipur, 23.71% in Jalore, 22.60% in Jodhpur, 20.15% in Nagaur and 24.94% in Sikar and one representative *Aspergillus niger* isolate from each district was established (ANBK-01 = Bikaner, ANCH-02 = Churu, ANDA-03 = Dausa, ANJP-04 = Jaipur, ANJL-05 = Jalore, ANJD-06 = Jodhpur, ANNG-07=Nagaur and ANSK-08=Sikar) for studying variability in the pathogen as well as to see the response of groundnut varieties to the highly virulent isolate.

Cultural and morphological variability

The cultural and morphological variability such as shape, colour and size of colony and size of conidia, conidiophores and columella were recorded in different isolates of *A. niger* by growing them on PDA medium. The results showed that all the eight isolates of *A. niger* were differ in their colony characters, colony diameter, sporulation and microscopic observations (Table 1).

In present study, all the eight isolates of *A. niger* were showed variations to each other in various growth characters like colour of colony (light blackish, blackish white, dark blackish and grayish black), colony color on reverse side of Petri plate (pale, colorless to dull white, yellow, pale white and white), initiation of sporulation (after 22 to 30 hrs of inoculation), margin of colony (circular, irregular and cut ends), diameter of colony at 4th (44.0 mm) and 7th day (90.0 mm) of inoculation, type of hyphae (branched and septate), length of conidiophores at 7th day of inoculation (220-400 µm), diameter of condiophores (6-10 µm) and diameter of conidia (2.8-5 µm).

The colony diameter of all the isolates was measured at fourth and seventh day after inoculation. Among these, ANJP-04 isolate (Jaipur isolate) showed maximum colony diameter (65.50 mm and 90.00 mm) followed by ANSK-08 isolate (Sikar isolate) (59.00 mm and 90.00 mm) while isolate ANBK-01 (Bikaner isolate) showed minimum colony diameter (44.00 mm and 85.70 mm) at fourth and seventh day of inoculation, respectively. Isolate ANJP-04 showed colony characters like dark blackish colony with black centre and round periphery and reverse side of Petri plate depicted yellow growth. In this isolate, sporulation started after 22 hrs of inoculation and size of colony was 65.50 and 90.00 mm at fourth and seventh day of inoculation which was fastest among all isolates.

Microscopic observations such as size, length and diameter of conidia, conidiophores and length of columella were varied among all the isolates under study. The isolate ANJP-04 showed maximum conidia diameter (3.5 to 5.0 µm), length (350-400 µm) and diameter (9-10 µm) of conidiophores and length (40 µm) and diameter (37 µm) of columella.

The isolate ANSK-08 was next to isolate ANJP-04 and it showed conidia diameter (3.3-4.7 µm), length and diameter of conidiophores (310-370 and 8-10 µm) and length (39 µm) and diameter (35 µm) of columella. It showed growth characters of colony as blackish growth with gray centre and reverse side pale growth with round margin, sporulation started after four days of inoculation and size of colony was (59.00-90.00 mm).

Variability with respect to present investigation, it is realistically apparent that all the eight groundnut isolates of *A. niger* were dissimilar to each other in opinion of cultural and morphological characteristics studied *viz.,* colony growth rate, colour and texture of colony, initiation of sporulation, size and diameter of conidia, conidiophores and columella. Based on the cultural and morphological characteristics, isolate ANJP-04 was designated as dark black and fast growing isolate, early sporulating with larger size and diameter of conidia, conidiophores and columella while ANBK-01 isolate as slow growing isolate, late sporulating

Table 1: Cultural and morphological variability in different isolates of *A. niger*

Isolate code no.	Colony characters*	Initiation of sporulation (hrs after incubation)	Size of colony at (mm)		Conidiophore*		Columella*		Dia. of conidia* (µm)	PDI in surveyed field
			4th day	7th day	Length (µm)	Dia. (µm)	Length (µm)	Dia. (µm)		
ANBK 01	Light blackish growth with gray centre and reverse side pale growth with circular margin	30	44.00	85.70	220-290	6-8	22	20	2.80-4.0	23.80
ANCH 02	Blackish white growth with gray centre and reverse side colorless growth with irregular margin	28	48.00	86.73	270-340	7-9	29	28	3.2-4.3	21.97
ANDA 03	Grayish black growth with black centre and reverse side white growth with irregular margin	27	49.33	88.50	290-350	8-9	32	30	3.2-4.5	24.77
ANJP 04	Dark blackish colony growth with black centre and reverse side yellow growth and had round periphery	22	65.50	90.00	350-400	9-10	40	37	3.5-5.0	32.38
ANJL 05	Dull white growth black with grayish centre and reverse side yellow growth with irregular margin	26	50.20	87.93	300-350	7-9	34	28	3.2-4.5	25.75
ANJD 06	Blackish growth with grayish centre and reverse side colorless to dull white growth with round margin	28	46.03	87.20	250-330	6-9	27	25	3.0-4.2	24.60
ANNG 07	Blackish white growth with grayish centre and reverse side pale white growth with irregular periphery	25	52.06	89.33	310-350	8-10	38	36	3.3-4.6	22.24
ANSK 08	Blackish growth with gray centre and reverse side pale growth with round margin	23	59.00	90.00	310-370	8-10	39	35	3.3-4.7	27.75

* Observed at 7th day of inoculation.

with small size of conidia, conidiophores and columella. Earlier researchers have isolated *A. niger* and proved pathogenic nature (Raper and Fennell (1965); Ramakrishna and Kolte, (1989) described various cultural and morphological characteristics (Gilman, 2001 and Sharma, 2012). Our findings are also in accordance with results of earlier investigators with different crops including groundnut (Diba *et al.,* 2007; Korat *et al.,* 2009; Mohapatra and Sahoo, 2011; Afzal *et al.,* 2013; Kumari and Singh, 2016; Mitra *et al*., 2021). They have noted similar growth characters of *A. niger* isolates and assigned into various groups like slow, average and rapid growing isolates. Majority of studied isolates depicted average growth rate of colony, sporulation and conidial characters.

Pathogenic variability

The virulence of all the isolates of *A. niger* was tested on variety RG-382 of groundnut by seed inoculation technique. All the isolates were found pathogenic to groundnut and produced characteristic symptoms of the disease. Among these, isolate ANJP 04 collected from Khejroli village of Chomu Tehsil of Jaipur district, was found to be most virulent as it produced highest disease incidence (54.43%) among all isolates, followed by isolates ANSK 08 (45.33%) and ANNG 07 (45.11%). The lowest disease incidence (32.81%) was examined with ANBK 01 isolate (Table 2). The overall mean disease incidence of eight districts was 40.76 per cent. Among these eight isolates, highly pathogenic was ANJP 04 isolate while ANBK 01 isolate was weak pathogenic. As per analysis of disease incidence recorded during survey, these two isolates *i.e*. ANJP-04 and ANSK-08 also caused maximum disease incidence at farmer's field. It means, these isolates with such peculiarities had greater virulence and responsible for higher disease production.

The present results are parallel to the findings of earlier researchers (Sharma, 2012; Kumari and Singh, 2016; Divya Rani *et al*., 2018). They have recorded pathogenic variability in different isolates of *A. niger.* Sharma, (2012) has been noted that dark brown or black conidiophores and conidia of *A. niger* with large condia (3.5 to 5.0 µm) are played an significant role in increased amount of pathogenicity in various species of plants. As the present investigation showed the occurrence of pathogenic variability in *A. niger* isolates that may warranting the need for development of groundnut varieties with huge resistance to the prevailing highly virulent isolates. So, only with a known highly virulent isolates used in resistance experiments, the success of the experiments can be guaranteed.

Screening of groundnut varieties

Ten varieties of groundnut were screened during *Kharif* 2019 and 2020 in artificially inoculated field conditions against collar rot disease. The observations (Table 3) on disease incidence on various varieties were recorded and categorized as per their reaction.

The results (Table 3) of first year data (*Kharif* 2019) revealed that none of groundnut variety was found completely free from the disease. Although, RG-644, M-13 and RG-510 were found resistant as these showed 7.62, 6.48 and 7.29 per cent disease incidence, respectively. RG-604, Girnar-2, Gajraj-10 and RG-632-1 were found moderately resistant as these showed 12.29, 15.07, 15.77 and 16.92 per cent disease incidence, respectively. Further, two varieties namely RG-633-9 (22.59 % incidence) and RG-578 (23.40% incidence) were found moderately susceptible

Table 2: Pathogenic variability in different isolates of *A. niger* causing collar rot of groundnut.

District	Isolate code no.	Per cent disease incidence
Bikaner	ANBK 01	32.81
Churu	ANCH 02	36.29
Dausa	ANDA 03	37.44
Jaipur	ANJP 04	54.43
Jalore	ANJL 05	39.33
Jodhpur	ANJD 06	35.40
Nagaur	ANNG 07	45.11
Sikar	ANSK 08	45.33
Overall mean		40.76

Table 3: Disease reaction of different varieties of groundnut against *A. niger* under field conditions.

Name of variety	Per cent disease incidence*			Host reaction**
	2019	2020	Pooled	
Girnar-2	15.07 (22.84)	16.56 (24.01)	15.82 (23.43)	MR
RG-633-9	22.59 (28.38)	25.14 (30.09)	23.87 (29.24)	MS
RG-382	54.51 (47.59)	55.85 (48.36)	55.18 (47.97)	HS
RG-510	7.29 (15.66)	8.07 (16.50)	7.68 (16.09)	R
RG-604	12.29 (20.52)	15.22 (22.96)	13.76 (21.77)	MR
RG-578	23.40 (28.93)	24.66 (29.77)	24.03 (29.35)	MS
Gajraj-10	15.77 (23.40)	17.96 (25.07)	16.87 (24.25)	MR
RG-632-1	16.92 (24.29)	18.14 (25.21)	17.53 (24.75)	MR
RG-644	7.62 (16.02)	8.07 (16.50)	7.85 (16.27)	R
M-13	6.48 (14.75)	7.33 (15.71)	6.91 (15.23)	R
SEm±	0.80	0.86	0.91	
CD (p=0.05)	2.47	2.64	2.80	
CV (%)	7.62	7.54	6.31	

*Av. of three replications, Figures given in parenthesis are angular transformed as: Degrees {Asin [Sqrt (% value/100)]}.

**Categorization-immune (I): PDI=0, Resistant (R): PDI= 1-10, Moderately resistant (MR): PDI=11-20, Moderately susceptible (MS): PDI=21-30; Highly susceptible (HS): PDI=>3.

whereas RG-382 (54.51% incidence) was found highly susceptible to the disease. The results of second year (*Kharif* 2020) also depicted a similar trend of results as in *Kharif* 2019.

The results of two years pooled data (Table 3) revealed that none of groundnut variety was found completely free from the disease while RG-644, M-13 and RG-510 were found resistant as these showed 7.85, 6.91 and 7.68 per cent disease incidence, respectively. RG-604, Girnar-2, Gajraj-10 and RG-632-1 were found moderately resistant as these showed 13.76, 15.82, 16.87 and 17.53 per cent disease incidence, respectively. Further, two varieties namely RG-633-9 (23.87% incidence) and RG-578 (24.03% incidence) were found moderately susceptible whereas RG-382 (55.18% incidence) was found highly susceptible to the disease. Our results are in agreement with the findings of Bhatia and Gangopadhyay, (1996); Nathawat *et al.,* (2014); Kumari *et al*., (2016). Bhatia and Gangopadhyay (1996) have been evaluated of 600 groundnut germplasm for three consecutive years, among these US 12A, US71 and GR 3 were found free from collar rot. Other three entries, *viz.*, Shulamith, Lambuy and U-4-47-7 showed highly resistant reaction. Seventeen entries were resistant, 11 moderately resistant and majority of the entries showed susceptible to highly susceptible reaction. Nathawat *et al.* (2014) evaluated five varieties against collar rot of groundnut and only GG 2 was found tolerant to collar rot, while GG 5, GG 7, GG 20 and GG 37 were susceptible to highly susceptible. Kumari *et al*. (2016) have also been screened 14 varieties of groundnut against collar rot. Among these, five varieties were found moderately resistance (RG-425, CSNG-19-1, SNG-69, GG-21 and RG-559-3), six were susceptible (RG-578, RG-378, RG-582, M-13, Girnar-2 and SNG-123) and three were found highly susceptible (RG-382, RG-510 and Chitra).

CONCLUSION

Based on the cultural, morphological and virulence analysis, it can be concluded that *Aspergillus niger* isolate ANJP 04 was designated as highly virulent as it fast growing in nature, early sporulating with larger size and diameter of conidia, conidiophores, columella and it produced maximum (54.73%) per cent disease incidence. Out of ten, three varieties namely, RG-644, M-13 and RG-510 were found resistant and famers may cultivate these resistant varieties in areas where collar rot is a major limiting factor. The findings of this study can also be utilized to screen varieties/genotypes of groundnut against highly virulent isolate for sustainability of breeding material to the disease effectively.

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