Original Research Paper



First report on incidence of inflorescence blight and pod rot (*Choanephora infundibulifera*) on dolichos bean (*Dolichos lablab*, *L*.) and yard long beanin (*Vigna unguiculata sub sp. sesquipedalis*) India

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ABSTRACT

Inflorescence blight and pod rot caused by Choanephora sp. were recorded in two major vegetable crops viz.dolichos bean and yard long bean at a very high degree of severity. Usually, the disease occurs in mild proportions without causing economic loss during rainy season in Kerala (June to September) affecting vegetable crops like cowpea and bhendi. However, during the year 2016, the disease was noticed with a very high severity of more than 90 per cent on two crops grown at two different locations in Thrissur district of Kerala during October to January. None of the commonly used fungicides could control the disease. The pathogen was found to be luxuriantly growing on the inflorescences of dolichos bean and on the fruits of yard long bean. The first incidence of the disease was recorded during the last week of October, 2016. The disease spread was almost cent per cent on susceptible genotypes of dolichos bean and more than 90 per cent in variety 'Vaijayanthi' of yard long bean. A study was conducted exploring the pre disposing factors leading to the occurrence of the disease in epidemic proportions. Meteorological factors affecting the disease were studied and it was found that there is positive correlation of the disease severity with increasing atmospheric temperature and rainfall. Atmospheric temperature more than 30°C along with high humidity and rain fall during the preceding week are found to be the major pre disposing factors lead to the epidemic in both the crops. The pathogen was isolated and based on morphological characters, identified as Choanephora sp. Pathogenicity was proved by inoculation on healthy inflorescence stalks. Molecular characterization of the pathogen confirmed the identity as Choanephora infundibulifera. This is the first report of the pathogen on dolichos bean and yard long bean in India.

Keyword: Choanephora infundibulifera, Inflorescence blight and pod rot, dolichos bean (Dolichos lablab, yard long bean, Vignaunguiculata sub sp. sesquipedalis

INTRODUCTION

India is the largest producer and consumer of pulses in the world accounting for about 29 per cent of the world area and 19 per cent of the world's production (Singh et al., 2015). By 2050, the domestic requirements would be 26.50t., necessitating stepping up production by 81.50 per cent. The major production constraints of pulses are abiotic stresses, abrupt climatic changes and emergence of new species/strains of insect-pests and diseases. The commonly grown pulses are chickpea (Cicer arictinum L.), pigeonpea (Cajanus cajan (L.) Mill. blackgram or urdbean (Phaseolus vulgaris L.) and dolichos bean (Dolichos lablab L.).The dolichos bean also known as Indian bean is an important pulse-cum-vegetable crop traditionally grown in India. The green pods are rich in protein and fibre. It is a good source of the aminoacid, lysine, and it contains 20-28% crude protein. The crop is affected by many diseases including anthracnose caused by Colletotrichum lindemuthianum (Sacc. and Magn.), rust, Uromyces appendiculatus (Pers.) Fries and many others.

However, pod rot caused by Choanephora infundibulifera is causing direct yield loss up to 100 per cent. The disease occurs in mild proportions during rainy season in Kerala (June to September) affecting



vegetable crops like cowpea and bhendi. But, during October, 2016 the disease was noticed at a very high severity of more than 90 per cent on dolichos bean and yard long bean in Thrissur district of Kerala. None of the commonly used fungicides could control the disease effectively. However, in vitro evaluation of nine fungicides revealed that mancozeb, copper oxychloride, propiconazole, cosuit, taqat and carboxin completely inhibited the mycelial growth of the pathogen (George and Girija, 2015). In India, this is the first report of inflorescence blight caused by C.infundibulifera in dolichos bean. The disease was previously reported in Boerhavia diffusa (Singh et al., 2011) and Withania somnifera (Ashwagandha) (Saroj et al., 2012). Leaf rot of cauliflower caused by this fungus has been reported recently by Gogoi et al., (2016). On dolichos bean, the disease was found to have very high rate of spread by direct contact of infected inflorescences with adjacent healthy ones.

MATERIAL AND METHODS

This experiment was conducted as a part of the monitoring of emerging diseases of vegetable crops under the All India Coordinated Research Project on Vegetable Crops. The disease was observed first as inflorescence die back on dolichos bean at Vellanikkara in Thrissur district. Later it was observed in yard long bean in a farmer's field at Kodakara which is about 30 km away from Vellanikkara and the symptom was pod rot. The symptoms were studied and the sporulation of the fungus present on the infected plant parts were observed under microscope. The stages of development of symptoms were studied by periodical observation of the infection in the field. The meteorological data during the crop period was obtained from the Department of Meteorology, College of Horticulture, Vellanikkara. The disease severity was scored at periodic intervals using 0-5 scale where 0 no infection, 1-1-19% infection, 2-20-39% infection, 3 - 40-59% infection, 4 - 60-79% infection and 5 -80-100% infection.

The fungus associated with the disease was isolated on potato dextrose agar (PDA) and the morphological characters were studied under microscope (Leica DM750). Healthy plant parts of dolichos bean and yard long bean were inoculated with purified culture of the fungus. On appearance of the symptoms, the pathogen was re-isolated from the infected plant parts and the characters were compared with those of the original isolate thus confirmed the pathogenicity of the fungus. Molecular characterisation of the pathogen was done at the Rajiv Gandhi Centre for Biotechnology for confirmation of the identity of the pathogen.

RESULTS AND DISCUSSION

Severe incidence of the disease was noticed in dolichos bean at College of Horticulture, Vellanikkara during the third week of October 2016. The percent disease incidence was more than 75 per cent (Figure.1). During December 2016, the disease was found in yard long bean at very high proportions in a farmer's field at Kodakara (about 30 km away from Vellanikkara). The disease affected the fruits of yard long bean causing very high yield loss.

Symptomatology and epidemiology

The symptomatology of the disease was studied and slight variation was observed between symptoms on the two crops. On dolichos bean, the disease appeared as inflorescence die back and blight. Initial infection began as wet rotting beginning from dried up petals remaining attached to the rachis. The petals remained attached to the inflorescence rachis and subsequently the infection spread rapidly in both directions on the rachis as brownish black discoloration and wet rotting. Luxurious growth of the fungus producing fructification was found on the petals and also all around the infected rachis when the infection progressed (Plate 1). The disease spread to the nearby plants at an alarming speed by direct contact to the rotting inflorescences with abundant sporulation on them. There was total loss of yield on susceptible varieties due to the disease. Fungicidal application was not advisable as the crop was in the harvesting stage. Moreover, peculiarity of the infected part prevented the spray fluid from reaching effectively on the infected area.





Plate 1. Inflorescence die back of dolichos bean caused by Choanephora infundibulifera.A- Initial symptom on dried up petals. B- Fructification on withered petals and inflorescence rachis. C- Discoloration of inflorescence rachis. D- Dieback of inflorescence

Std. Week	Max Temp (°C)	Rainfall (cm)	RH%	Windspeed (km/hr)	PDS
39	30.3	18.9	95	1.9	0
40	31.7	0	93	1.5	0
41	31.4	14.5	91	1.2	15.6
42	32.4	0.6	94	0.7	28.7
43	31.8	3.5	94	0.7	38.6
44	32.9	18.7	94	0.9	43.8
45	32.9	2.9	88	1	57.7
46	33.7	10.9	82	2.2	58.3
47	34.3	0	74	3	67.3
48	33.1	0.8	83	1.7	73.2
49	33.3	46.3	83	2.7	75.3
50	31.9	5.8	91	1.5	65.4
51	33.7	0	85	3.6	67
52	33.4	0	79	4.3	56.2
Corr. coeff*.	0.804*	0.056	-0.695*	0.421	

Table 1:	Effect of	major	meteorological	factors	on	inflorescence	dieback	of	dolichos	bean
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Meteorological factors influencing the pathogen were studied. It was found that, disease severity is positively correlated with atmospheric temperature and negatively correlated with relative humidity with no significant correlation torain fall and wind speed. However, the first incidence of the disease was noticed when there was more than 95 per cent RH and rain fall up to 18.9 cm during the preceding fortnight (**Table 1 & Figure 1**).



PDS Percent disease severity, RH – Relative humidity Fig 1. Effect of major meteorological factors on inflorescence dieback of dolichos bean



Plate 2. Pod rot of yard long bean A- Infection spreading from dried up petals to the pod. B –Lamb's tail symptom

The symptoms of pod rot of yard long bean initiated as rotting of the dried up petals at the base of the pod which spread to the pods and caused wet rot. The infection extended to the entire length of the pod which was subsequently covered with sporangiophores bearing pin head like sporangia producing the typical lamb's tail symptom (**Plate 2**). Similar symptoms on cowpea have been reported by Wilson and Jose(1965), Kangatharalingam (1979) and George and Girija, (2015).





Plate 3. A,B Sporangiophores, sporangia and sporangiospores of Choanephorainfundibulifera. C-Sporangiospores showing slightly slanting longitudinal striations. D –Dehiscing sporangia

Characterization and identification

The pathogen was isolated on PDA which produced white mycelium. Sporangial production was not observed when the plate was sealed using parafilm but when it was removed there was abundant production of sporangia as pin head like structures (**Plate 4**). Slides were prepared by mounting a colony fragment in lactophenol. The fungal structures were observed under a microscope (Leica DM750). The morphological and cultural characters of the fungus were recorded and compared with published descriptions of soft rot-fungi (Kwon et al. 2001; Kagiwadaet al. 2010). The sporangiospores appeared oval and light brown in colour. Slightly slanting striations were observed on the surface of the sporangiospores (sporangiola). Park et al. (2016) has observed characteristic longitudinal striations on the sporangiola of Choanephorainfundibulifera. However, in the present study the striations were slanting to the longitudinal axis of the sporangiola (**Plate 3**).



Plate 4. Culture of Choanephora infundibuliferaon PDA A – Absence of sporulation when the plate is sealed with parafilm. B – Abundant production of sporangia when not sealed (in slide culture).



Kirk (1984) reported that, a fungus that produced cottony, white mycelium and black spore masses was consistently isolated from diseased tissues of plants. The fungus was identified as Choanephora sp. based on morphology and colony characteristics using standard monographs and taxonomic keys. The mycelium was non-septate, unbranched and hyaline. Secondary vesicles bore conidia that were smoothwalled, light brown, about 2.4 µm in diameter and typically ovate. Sporangiophores were non-septate, 5-13 mm high, straight but not swollen and roughened at the base. Sporangia were numerous in culture measuring 20-45 x 150-200 µm in diameter, bearing few to many sporangiospores, that were ellipsoid in shape and brown to pale brown in colour, measuring 15-21 x 10-13 µm. Koch's postulates were fulfilled by re-isolating the fungal pathogen, which was identified as Choanephora sp. Earlier, Choanephora cucurbitarum causing wet rot

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on Capsicum annuum has been reported in India (Prabhavathy& Reddy, 1995). The sporangia were multisporous, subglobose in shape, non columellate and dehisce into two half releasing the spores. Choanephoracucurbitarum (Mucorales) is an important plant pathogen causing soft rot of floral parts and fruits of many plants, including summer squash, pumpkin, pepper and okra (Agrios, 2008).

Molecular characterisation was done for species level identification and confirmation of the identity of the pathogen and it was confirmed that, that the fungus causing inflorescence rot and die back of dolichos bean and the pod rot of yard long bean is Choanephora infundibulifera as there was cent percent similarity between the query sequence and the sequence of Choanephora infundibulifera available in the website with accession number KX980520.1.

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