

## Simple Technique for Screening of Gray Mold Disease in Castor

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### ABSTRACT

*Castor is an important non-edible oil seed crop. It is affected by many fungal pathogens, among them gray mold of castor is one of the destructive diseases caused by Botryotinia ricini. The present study was aimed to develop screening technique against gray mold of castor under in-vitro conditions. Spore suspension of Botryotinia ricini was sprayed on detached spike of different germplasm source (detached spike technique). Inoculated spikes were maintained at 27°C temperature and 85 % relative humidity in glass house for 7 days. Out of the twenty, few germplasm sources like RG-1139, CI-1, CI-2 showed better resistant to the gray mold disease. Out of the twenty, few germplasm lines CI-1, CI-2, RG-1139 were showed least susceptibility to gray mold disease when compared to susceptible check DCH-519(95 %).*

**Key words:** Gray mold, Green house, Detached spike technique

### INTRODUCTION

Castor is an important non-edible oil seed crop. Castor is known to suffer from many fungal and bacterial diseases at different crop growth stages. The crop is most commonly affected by wilt, gray mould, root rot, seedling blight, Cercospora leaf spot, powdery mildew Alternaria blight, and bacterial leaf spot. Among them, gray mold of castor is one of the most destructive diseases of castor is gray mold, caused by the fungus *Botryotinia ricini*. India is the world's largest producer of castor and its derivatives contributing to almost 65 per cent share. Gujarat, Rajasthan and Andhra Pradesh are the major castor growing states in India. In India castor occupied about 8.39 lakh ha area and production is estimated about

10.62 lakh MT reported<sup>11</sup>. In Telangana State, the area of castor cultivation has come down drastically from 3.92 lakh ha during the year 2000-01 to 1.30 lakh ha during the year 2014-15 as the farmers are reluctant to grow the crop due to huge yield losses caused by the gray mold. In India, the disease first occurred in Karnataka<sup>2</sup> and appeared in epidemic form during 1987 in Andhra Pradesh. Castor oil is commercially very valuable and obtained from seeds, which contain 50-55% oil, and plays a vital role in Indian vegetable oil economy<sup>5</sup>. This oil is considered as an option for biodiesel production in several countries. In Brazil, governmental policies promoted castor as a biodiesel feedstock to bring benefits to small farmers<sup>4</sup>.

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The disease is confined to few states of India viz., Telangana, Andhra Pradesh, Tamil Nadu, Karnataka, Odisha, Rajasthan and Gujarat. Gray mold is regarded as troublesome only in Andhra Pradesh and Tamil Nadu, in the South, where the weather conditions are more favourable for disease development<sup>6</sup>. Castor gray mold was first reported in the USA in 1918, following pioneering investigations by H.E. Stevens and F. W. Patterson, who promptly suggested that the causal organism of castor gray mold was an unknown *Botrytis* specie<sup>9</sup>. This fungus had caused serious losses of castor crop in the summer of 1918 mainly in Florida and others southern States, where it was responsible for losses up to 100% of castor yield<sup>8</sup>. In Brazil, the disease was first reported in the São Paulo state in 1932. However, it was only in 1936 that any attention was given to the disease due to the serious losses which occurred that year<sup>10</sup>. Currently, gray mold is present in almost all Brazilian states and its importance has grown at the same time that the crop cultivation has been intensified, mainly in those regions where the weather conditions are favourable for disease development, including the Southern and South-eastern Brazilian states<sup>7</sup>. Yield losses of up to 100% are quite frequent when highly susceptible cultivars are planted. Conversely, in Bahia the major castor producer in Brazil gray mold is not a problem because the weather conditions are usually not favorable for disease development.

## MATERIALS AND METHODS

### Isolation of *Botryotinia ricini* and Detached spike technique:

The pathogen *Botryotinia ricini* Whetzel used in the present study were isolated from

infected samples collected from (DOR), experimental fields of Directorate of Oilseeds Research Hyderabad, India. *Botryotinia ricini* was isolated using Oat meal agar (OMA) medium. The culture thus obtained was maintained on OMA slants at 4°C. Preparation of conidia suspension of *Botryotinia ricini* on OMA media. The pathogenic isolates has been maintained on modified Oat Meal Agar (OMA) reported<sup>13</sup>. Sterile water (50ml) was added to the culture plates and the surface was scraped lightly with a sterile transfer loop. The resulting suspension was filtered through two layers of sterile muslin cloth. The conidia suspension was adjusted to 10<sup>6</sup> conidia/ml with sterile distilled water using haemocytometer. *B. ricini* conidio spore suspension (10<sup>6</sup> spores/ml) prepared from 7-day-old culture grown on OMA medium was used. The fungal suspension (spore and hyphae) was mixed and homogenized using sterile water and 0.03% Tween-20 Spikes or racemes of 20 days old are cut from castor plants and immersed in 2 % sucrose solution in conical flask and Spore suspension of *B. ricini* (10<sup>6</sup> conidia/ml) was sprayed on all castor cultivars including the DCH-519 control which served as pathogen check. Gray mold incidence was scored by determining the percentage of capsules infected on racemes using a 0-9 scale for the gray mold in castor . Based on the infection of *Botryotinia ricini* (on primary, secondary and tertiary racemes (sequence of racemes/ spikes appear on growing plant) a 0-9 scale developed at Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad<sup>1</sup>.

Disease grade	Intensity of infection %	Reaction
0	No infection	Immune
1	1-10	Resistant
3	11-20	Moderately resistant
5	21-30	Moderately susceptible
7	31-50	Susceptible
9	>51	Highly susceptible

Spikes/ racemes of 15-20 days old along with 10cm stalk are cut from castor plants, cut end of stalks immersed in 2% sucrose solution in

conical flasks and sprayed with a spore suspension (10<sup>6</sup> conidia/ml) of *Botryotinia ricini*. The spikes thus prepared are kept in

glasshouse where a humidity of 80%, temperature around 27°C and continuous capsule wetness are maintained by fogging<sup>12</sup>.

To identify suitable cultivars for weather-disease relationship studies, different cultivars of castor were grown in a poly house and artificial inoculation of pathogen was done and humidity and temperature (N average humidity > 80% and temperature around 25°C were maintained by cooling pad and fan system and fogging for 3 min every one hour during day and 1 min per every 1 hr during night<sup>12</sup>. Disease severity recorded on castor racemes with right age marked at the beginning and disease severity recorded every day starting from 3<sup>rd</sup> day till complete spread on a susceptible genotype. Screening techniques developed for identification of resistance to individual mold pathogens by artificial inoculation<sup>16</sup>.

## RESULT AND DISCUSSION

### Symptoms development on Castor spikes and leaves (Detached spike and fogging technique in poly house)

Symptoms of the disease can be seen on leaves, stem, flowers and capsules, being prominent on spikes. Initial symptoms of gray mold infection occurred 3 the day after

inoculation on susceptible cultivars. Infected capsules are covered by characteristic grey or ash coloured growth of the fungus. Subsequently the disease spreads upward infecting all flowers and capsules which are covered by the fungus thereby involving the entire spike. Infection at flowering results in flower rot and affects seed filling. Infected spikes become sterile without capsules. Yellowish drops of liquid exude from these portions which are covered by fluffy grey fungal growth. Infected capsules rot and shed off. Infection spreads to the seed also on which black sclerotia develop. The symptoms were circular spots on leaves at early stage of infection. As symptoms progressed, irregular light brown lesions are covered by the gray colour growth of the pathogen on leaves. Disease severity of the *Botrytis ricini* was recorded on both leaves and spikes. Disease severity was recorded 3<sup>rd</sup> days after inoculation. The first symptoms are visible as bluish spots on the inflorescences, on both female and male (before anthesis) flowers, and on developing fruits. On fruits, the symptoms can evolve to circular or elliptic, sunken, dark coloured spots that can result in rupture of the capsule<sup>3</sup>.

**Table 1: Screening of Germplasm lines against Gray mold disease under glasshouse conditions (IIOR, Hyderabad)**

S.No	Cultivars	Disease severity	
		Poly house	Detached spikes
1	DCS-9	93	95
2	JC-12	84	90
3	RG-1139	0	11
4	RG-1963	No spikes	No spikes
5	RG-3309	0	20
6	RG-3216	35	40
7	CI-1	0	6
8	CI-2	0	9
9	DPC-9xCI-2	No spikes	No spikes
10	DPC-9xCI-1	83	85
11	RHC-247xCI-1	82	75
12	M-574xCI-2	76	80
13	M-574xCI-1	36	45
13	RG-3200	8	30
14	RG-3202	12	20
15	RG-3206	0	35
16	RG-3207	No spikes	No spikes
17	RG-3243	No spikes	No spikes
18	RG-3257	No spikes	No spikes
19	DCH-519	89	95
20	48-1	42	50
21	M-574	76	75
22	RG-3308-1	72	80

**NS: NO Spikes**

Twenty one castor cultivars were screened for their reaction against *Botryotinia* gray mold under artificial epiphytotic conditions in glasshouse and also in poly house by using detached spike technique. Out of twenty germplasm CI-1 and CI-2 showed better resistant to the gray mold disease, only one germplasm source RG-1139 having moderate levels of resistance were identified. Remaining all germplasm sources showed more than >\_20 disease severity. Whereas in susceptible check DCH-519 recorded more than 89% of disease severity. Gray mold incidence was scored by determining the percentage of capsules infected on racemes using a 0-9 scale for the gray mold in castor. Few germplasm lines CI-1, CI-2, RG-1139 were showed least susceptibility to gray mold disease when compared to susceptible check DCH-519(95 %). Out of the 66 accessions, only four guinea sorghum lines as resistant identified four grain guinea sorghum lines as resistant out of 66 accessions. Recently, moderate to high level of mold resistant has been identified in elite white grain sorghum lines<sup>15</sup>.

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