Technical Bulletin

GRAY MOLD OF CASTOR

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Cover Page : A. *Botryotinia ricini* infected spike, B. *B. ricini* sporulation on enriched OMA Medium, C. Gray mold infected Castor Leaf, D. Conidiophore branching and conidia of *B. ricini*.

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FOREWORD

Castor, a non-edible oil seed crop has immense industrial and medicinal value. India is the world's largest producer of castor seed and meets most of the global demand for castor oil. The primary use of castor oil is as a basic ingredient in the production of jet engine lubricants, heavy duty automotive greases, lubricants, many other chemical, pharmaceutical and cosmetic derivatives. India dominates international castor oil trade that earns foreign exchange to the tune of Rs. 3000 crores every year. In India, castor crop is grown in an area of 10.35 lakh ha with production of 12.30 lakh tonnes. The major castor growing states in India are Gujarat, Rajasthan and Telangana.

Gray mold is one of the most destructive diseases of castor In India. In the states of Telangana, Andhra Pradesh, Tamilnadu and Karnataka, the area under castor cultivation is continuously decreasing due to gray mold caused by the fungal pathogen *Botryotinia ricini.* The disease was first reported in Karnataka during the year 1921. The disease appeared in epidemic form during the years 1985 and 1987 causing extensive damage to the crop in erstwhile Andhra Pradesh and Tamil Nadu states. Since then the disease started appearing year after year and attaining serious proportions limiting castor production in southern states of India.

ICAR-Indian Institute of Oilseeds Research is making serious efforts under a 12th Plan Flagship project on "Multipronged/multidisciplinary approaches to ameliorate yield losses caused by gray mold (*Botryotinia ricini*) in castor (*Ricinus communis* L.)" to develop practicable solutions to manage the disease. The bulletin provides an overall view of the disease, research findings pertaining to the etiology of the pathogen, host resistance assessment and management options supported with relevant pictures and illustrations. This compilation is an endeavor to transfer the knowledge regarding gray mold disease of castor among the researchers and developmental and extension officials of public and private sectors. I compliment the team of scientists involved in bringing out this bulletin.

New Delhi
May 1, 2016

Dr. B.B. Singh

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CONTENTS

1. Introduction

Castor is a member of the *Euphorbiaceae* family that is found across all the tropical and semi-tropical regions of the world (Weiss, 2000). After the world war I, the crop lost its importance in developed countries (Godfrey, 1923), but in arid and semi-arid regions of India and Brazil it has remained as the most important non-edible oilseed crop (Dange *et al.,* 2005; Santos *et al.,* 2007). In India, castor crop is grown in an area of 10.35 lakh ha with a production of 12.30 lakh tones (Ministry of Agriculture, Govt. of India, 2014-15).

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 (Chowdhury and Gaur, 1998). Castor oil has been used as purgative since ancient times and is still considered to be safe and effective laxative (FDA, 2003). 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 (Hall *et al.,* 2009; César and Batalha, 2010). The high viscosity over a wide range of temperature makes castor a valuable ingredient of lubricants (Severino *et al.,* 2012).

One of the most destructive diseases of castor is gray mold, caused by the fungus *Botryotinia ricini* (Godfrey) Whetzel. In India, gray mold of castor was first reported in Karnataka (Anonymous, 1921). It appeared as an epidemic in the year 1985 and the pathogen was identified as *Botrytis ricini* (Anonymous, 1986). During *Kharif* 1987, gray mold occurred in an epidemic form causing extensive damage to the crop in erstwhile Andhra Pradesh (Moses and Reddy, 1989) and Tamil Nadu (Anonymous, 1995), which led to the decline in castor cultivation (Rao, 1997). In this bulletin, the major aspects of gray mold disease are discussed.

2. Gray mold of castor

A. Historic and economic importance

Castor gray mold was first reported in the USA in 1918 and the causal organism was identified as an unknown *Botrytis* species (Godfrey, 1923). The first occurrence of this disease in USA was directly linked to seeds imported from Bombay (now Mumbai), India, even though until that time, such disease had not been described in that country (Godfrey, 1923). In Brazil, the disease was first reported in the Sao Paulo state in 1932, but this disease caused

severe losses there in 1936 (Goncalves, 1936). Yield losses up to 100% are quite frequent when highly susceptible cultivars are planted (Soares, 2012). Currently the disease is present in almost all Brazilian states as castor flowering period is coinciding with highly favourable conditions for disease development (Moraes *et al.,* 2009). In Korea, gray mold was observed on leaves of castor grown in Wonju and Okcheon during October 2000 (Hong *et al.,* 2001). In southern states of India, the disease is of regular occurrence during monsoon rains resulting in severe yield losses to farmers year after year. 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.

B. Geographic Distribution

In India, the disease first occurred in Karnataka (Anonymous, 1921) and appeared in epidemic form during 1987 in Andhra Pradesh (Moses and Reddy, 1989). 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 (Dange *et al.,* 2005).

C. Etiology

The causal agent of gray mold of castor was originally described by Godfrey (1919) as *Sclerotinia ricini* Godfrey, based on the holomorph. Gray mold fungus belongs to *Sclerotiniaceae* (Helotiales, Ascomycota). Later, Whetzel (1945) transferred the species *S. ricini* to the genus *Botryotinia*, since then it has been known as *Botryotinia ricini* (Godfrey) Whetzel. Subsequently, the anamorphic state of *Botryotinia ricini* was named as *Botrytis ricini* N.F. Buchw. (Buchwald, 1949). In 1973, to avoid the confusion among non-mycologist communities, Hennebert erected the genus *Amphobotrys* to accommodate the anamorphic state of *B. ricini*, based mainly on the distinctive pattern of conidiophore ramification, and since then the anamorphic state became known as *Amphobotrys ricini* (N.F. Buchw.) Hennebert (Hennebert, 1973).

The phylogenetic relationship of *Botryotinia ricini* with other known *Botrytis* spp. was established by amplifying, cloning and sequencing of three

housekeeping genes G3PDH, HSP60 and RPB2. The consensus sequences of all the three housekeeping genes obtained by re-sequencing a minimum of five clones for each and sequence similarity search indicated a unique position for *B. ricini* in the genus *Botrytis* (Durga Bhavani and Dinesh Kumar, 2009).

The anamorph of *B. ricini* was characterized based on morphological & molecular studies. The morphological studies are in accordance with Hennebert (1973). Molecular Studies based on ITS Sequence analysis confirmed that the anamorph of castor gray mold in Hyderabad region of Telangana state is *Amphobotrys ricini* (Yamuna *et al.,* 2015)

Botryotinia ricini is a homothallic species (Beveer and Weeds, 2007) and sexual reproduction takes place through the sexual state and production of ascocarp, apothecia, asci and ascospores (Godfrey, 1923). Its anamorphic phase is characterized by the production of dichotomously branched conidiophores bearing globose conidia which serve as major source of primary infection.

The fungus also survives in seeds especially in the caruncle and beneath the seed coat (Godfrey, 1923). Latent infection was recorded from apparently healthy seeds to an extent of 13% (Anonymous, 2002). *B. ricini* is seed borne to certain extent (Srinivasulu et al., 1994). Although *B. ricini* is a seed-borne fungus, the initial inoculum source of the disease is not likely to be the seed because there is a large time gap between sowing and flowering, so the inoculum will not be available to infect the flowers (Soares, 2012).

D. Host penetration and colonization

The mycelium of the fungus first degrades the cuticle and later penetrates the host tissues (Orellana and Thomas, 1962). Probably *B. ricini* uses both mechanical and chemical processes to penetrate the undamaged host tissue; its penetration will depend on factors such as inoculum type, free water, nutrient availability, cuticle features, castor capsule type (spiny, non- spiny, smooth or warty surface etc. [Fig. 1]), presence of exudates on floral organs and other glands, besides the abundance of natural openings. Waxy coat/ bloom present on the capsules helps in speedy germination of conidia and growth of the pathogen mycelium and predisposes castor to gray mold infections (Prasad, R.D. unpublished). Genotypes without bloom / waxy coat on capsules and other parts of the castor plant are generally less susceptible to gray mold pathogen compared to genotypes with

bloom (Fig. 3). Smooth spines/ hairs present on capsules retain water droplets for longer periods on their tips and helps in spore germination, gaining entry into capsules through spine base compared to non-spiny / non-hairy types (Fig. 1).

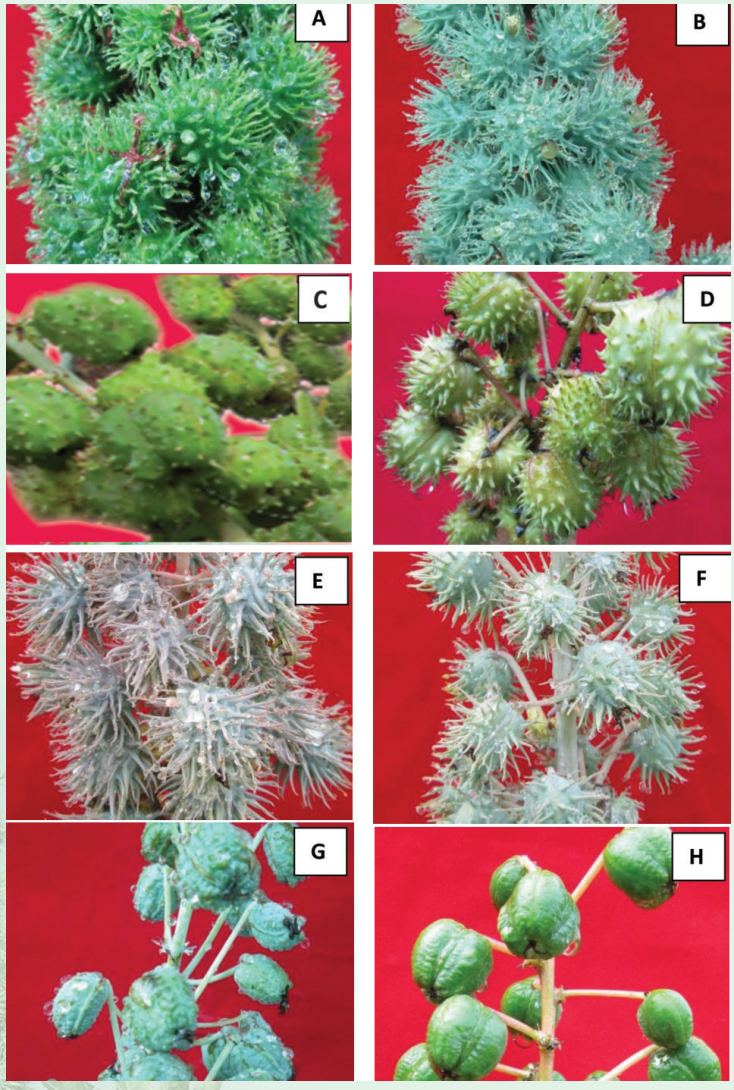

Fig. 1. Intensity of deposit of water droplets on different types of castor capsule, **A & B.** Short spines, **C & D.** Short and Rudimentary spines, **E & F.** Long spines, **G & H.** Non spiny capsules.

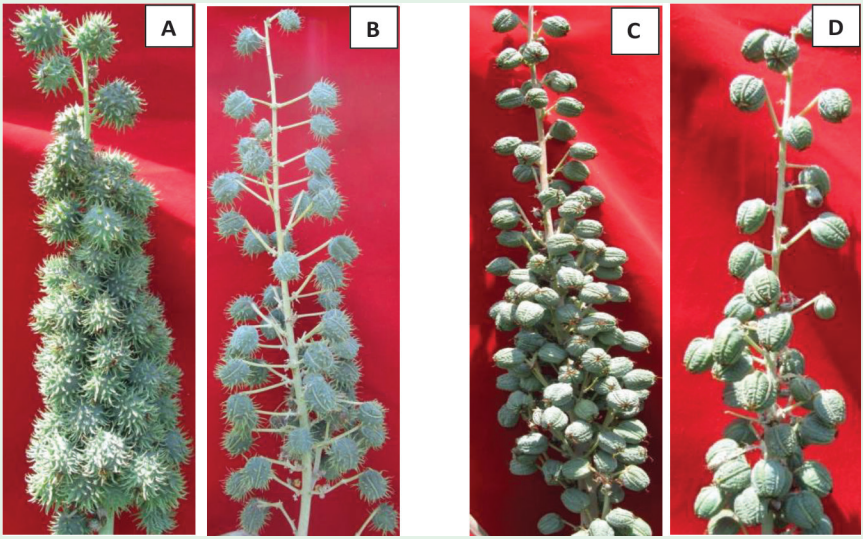

Fig. 2. Different types of spikes. **A.** Compact spiny spike, **B.** Loose spiny spike, **C.** Compact spike with non spiny capsule, **D.** Loose spike with non spiny capsule.

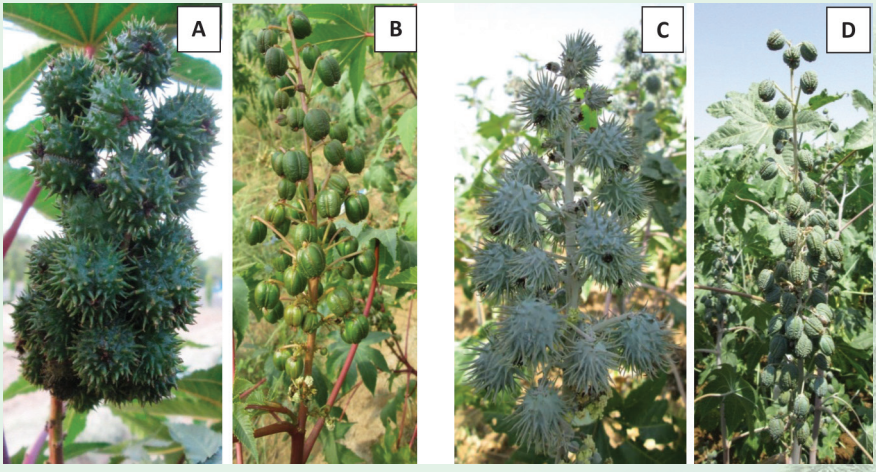

Fig. 3. Castor capsules with bloom and without bloom. **A.** (Spiny), **B.** (Non spiny) capsules without bloom, **C.** (Spiny), **D.** (Non spiny) Capsules with bloom.

Conidiospores of *B. ricini* germinates on the capsule surface within 24 h. After germination, the tips of the germ tube come in contact with the

host surface which showed swellings and formation of thick appresoria-like structures. Penetration and extension of hyphae within the host tissue especially in the sub-epidermal region has been noticed (Yasmeen *et al.,* 2003).

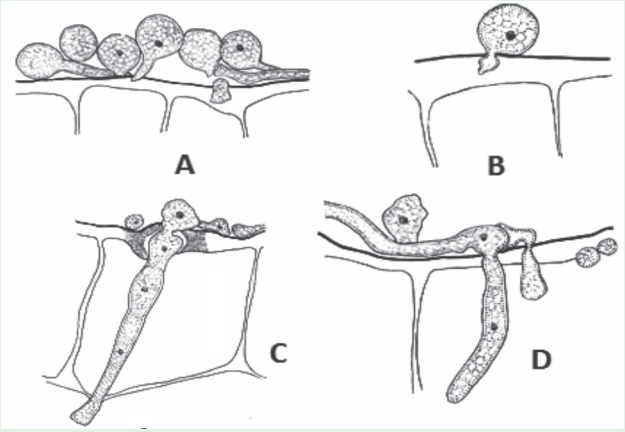

Fig. 4. Host penetration of *Botryotinia ricini* through appresoria formation.

A. Germination of conidiospores on the host surface, **B.** Swellings (appresoria) at the tip of germ tube, **C & D.** Penetration of germ tube within host tissues. [Photos reprinted from Godfrey's publication in Journal of Agricultural Research, Vol XXIII, March, 1923].

E. Symptoms

The primary target of the fungus are the inflorescence and the capsules in any development stage (Araújo *et al.,* 2007; Dange *et al.,* 2005; Lima *et al.,* 2001; and Goncalves, 1936). The fungus has preference for female flowers mainly due to their succulence and retention of water droplets for longer periods which helps in spore germination. Leaves, petioles and stem can also be infected, mainly due to the deposition or fall of infected material from the inflorescence or racemes. 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 into circular or elliptic, sunken, dark coloured spots that can result in rupture of the capsule (Araújo *et al.,* 2007). Bluish spots on capsules from which yellow liquid oozes out could be seen and strands of fungal hyphae emerge out from these spots within a short period (Yasmeen *et al.,* 2003). The capsules in spikes are soon covered by luxuriant, external, grayish fungal growth if high humidity prevails for longer periods. The symptoms as described above are of common appearance during monsoon

and cyclonic rains during the months of July to October in Telangana State. The other uncommon symptom is breaking off of rachis at the point of stem attachment during post rainy season grown castor (January and February months) though low humidity and warm temperature prevails but low intense rain coupled with morning dew triggers gray mold infection. Morning dew trickles down to rachis base and retained there for longer periods encouraging spore germination. This is evident in fields with dense canopy and congenial microclimate created by excessive irrigation and nitrogen fertilizer application.

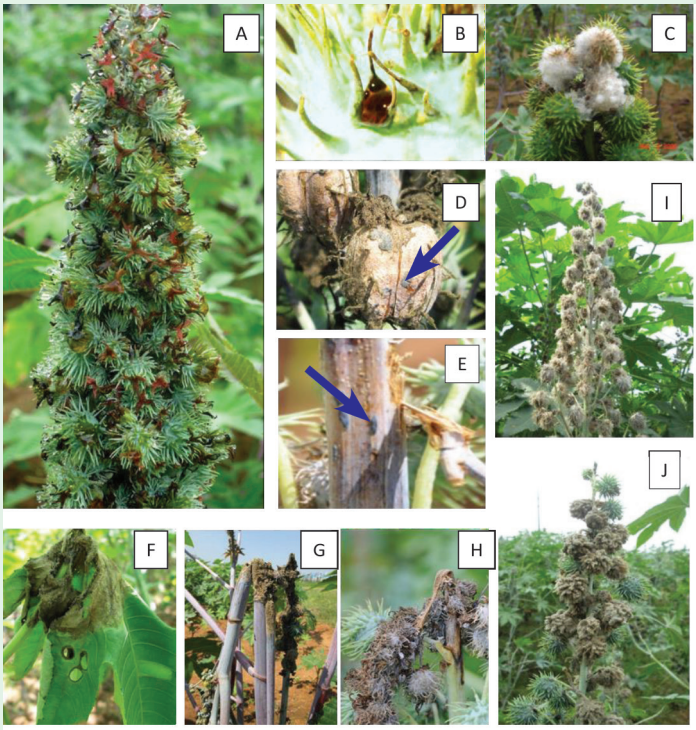

Fig. 5. Symptoms of gray mold on castor plant, **A & B.** Yellow liquid ooze from infected spike, **C.** Whitish mycelial growth on capsules, **D.** Sclerotia formed on dried castor pericarp, **E.** Sclerotia formed on rachis, **F.** Infected castor leaf, **G.** Broken stem after severe infection, **H.** Broken rachis and rotten capsules after infection, **I & J.** Totally infected spike with gray mold growth.

F. Epidemiology

Temperatures around 25^{0}C and high relative humidity are highly favourable to the disease development (Godfrey, 1923). There is a high correlation between the temperature and duration of leaf wetness with the disease incidence and

severity (Sussel *et al.,* 2011). The disease was more intense with a temperature of 28ºC and 72 h of leaf wetness. The fungus survives between the growth seasons as sclerotia, on secondary host plants, and on volunteer castor plants. Growth of the pathogen is maximum and faster at slightly cool temperatures (20-25ºC) and the optimum temperature is 20ºC. Minimum growth is observed at 35ºC and the growth is very slow with very poor sporulation. The detached castor spikes kept above 80% humidity develop symptoms within a week. However, those kept at lower relative humidity do not show any symptoms (Yasmeen *et al.,* 2003).

G. Life cycle and host range

The disease cycle starts with spore deposition on the host surface, followed by penetration and colonization of the host tissues. Soon after colonization, the fungus, under favourable conditions, sporulates profusely on the dead tissues, and then the conidia become the primary inoculum source for new infection sites. By infecting the first inflorescence under favourable conditions, the fungus produces abundant sporulation, thus allowing multiple rounds of re-infection, since this pathogen is easily spread by wind, rain splash and, probably by insects (Dange *et al.,* 2005). Godfrey (1923) reported that the fungus survives on soil or crop debris as sclerotia which under favourable condition give rise to apothecia serving primary infection source. However, there is no report of sexual reproduction under natural conditions, other than the original reports of Godfrey (1923), so the role of ascospores as the initial inoculum source remains unclear. Several reports of natural infection of *B. ricini* on members of *Euphorbiaceae* have been made, including both weeds and ornamentals: such as *Caperonia palustris* (Whitney and Taber,1986), *Euphorbia supine* (Holcomb *et al.,* 1989), *E. pulcherrima* (Holcomb & Brown, 1990), *E. heterophylla* (Barreto & Evans, 1998), *E. inarticulate* (Alwadie and Baka, 2003), *Acalypha hispida, Jatropha podagrica* (Lima *et al.,* 2008) and *Acalypha australis* (Zhang *et al.,* 2012). At IIOR, Hyderabad it was observed that *Botryotinia ricini* is able to infect weed species of *Euphorbia pulcherrima*, *E. hirta, E. prostrata, E. geniculata, Jatropha curcus, J. intergerrima, J. multifida, J. podagrica, J. gossypifolia, Hibiscus rosa-sinensis, Tagetes erecta, Cicer aerientinum, Delonix regia, Bougainvillea spectabilis* and *Lantana camara* (Yasmeen, 2004). Though several weed hosts reported to harbor gray mold infections, during rainy season none of the reported weeds developed gray mold before castor produce racemes. Hence, possibility of the infected weeds providing conidia for primary infection is rare (Prasad, R.D Unpublished). The fungus survives on infected castor crop debris for six and nine months under

field and laboratory conditions, respectively. It may also survive in quiescent state in seeds or in other host parts or form dormant resting structures like sclerotia, which may help in disease initiation and development under favourable conditions (Yasmeen, 2004). *B. ricini* produces good sporulation and profuse mycelium (Fig. 6 A & B) on Oat meal agar medium enriched with L-aspargine, gallic acid and castor pericarp extract (Prasad and Bhuvaneswari, 2014).

The diversity in cultural characters of *Amphobotrys ricini* was studied on six different growth media. Radial growth was higher on oat meal agar medium and enhanced sporulation was observed on oat meal enriched medium (Yamuna *et al.,* 2015)

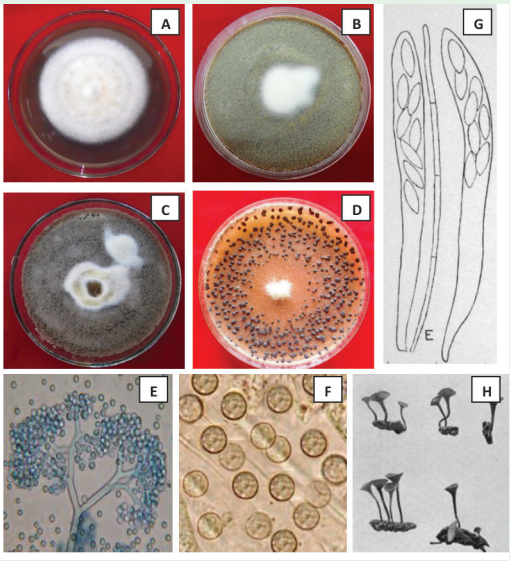

Fig. 6. Morphological characters of *Botryotinia ricini*

A. Pluffy mycelial growth at 5 DAI, **B.** Profuse sporulation on enriched OMA medium, **C & D.** Sclerotia formed under unfavorable condition, **E.** Dichotomously branched conidiophore bearing clumps of conidia, **F.** Conidia, **G.** Ascus bearing eight ascospores **H.** Sclerotia germinated as apothecia.

H. Host resistance

Desired level of resistance is not reported in castor germplasm lines anywhere in the world (Batista *et al.,* 1998; Costa et al., 2004; Lima and Soares, 1990; Anjani *et al.,* 2004; Milani *et al.,* 2005) Plants of ornamental type with capsules in different shades of red or reddish green are more resistant (Godfrey, 1923). Genotypes with less compact spikes, non-spiny capsules,

capsules without waxy coating, long pedicels are less prone to gray mold. Castor capsules with low soluble sugars and water soluble pectin showed more resistance (Thomas and Orellana, 1964).

Host resistance has been on top priority to manage castor gray mold. However, to identify resistant sources reliable screening techniques are a prerequisite. At the Indian Institute of Oilseeds Research, various screening methods *viz.,* 'detached spike technique', 'detached capsule technique', 'detached leaf technique', 'field fogging technique', 'poly house screening' and a 'biochemical technique' are developed for screening castor germplasm accessions and breeding lines for gray mold reaction.

i. Screening techniques for resistance assessment

a. Detached spike/ raceme technique

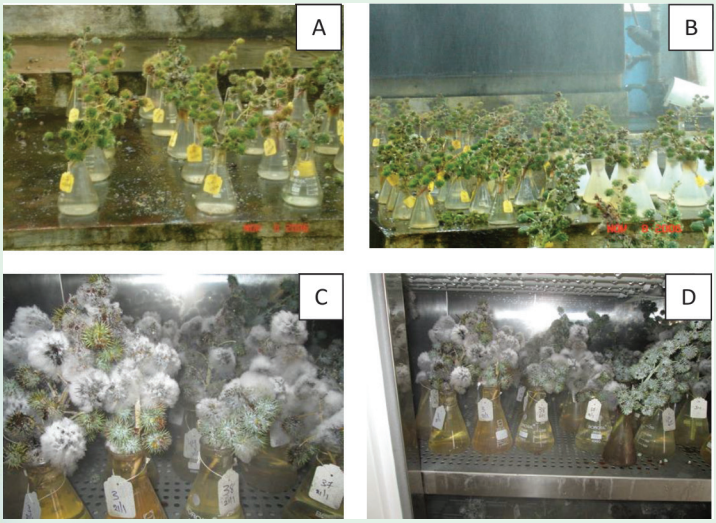

Fig. 7. Screening and selection of resistant sources by detached spike/ raceme technique. **A & B.** In glass house, **C & D.** In growth chamber.

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 (10^6 conidia/ml) of *Botryotinia ricini*. The spikes thus prepared are kept in glasshouse where a humidity of 80%, temperature around 27^0C and continuous capsule wetness are maintained by fogging. In growth chamber, temperature around 20^0C and 90% relative humidity are maintained but capsule wetness is provided by spraying water

thrice a day at 8 h interval as water droplets are retained by spikes only for 7 to 8 h. Initial symptoms of gray mold infection appear 5 days after inoculation. By 7th day, all the capsules will be covered with cottony gray mycelium of the fungus. Screening of large number of castor germplasm/ breeding lines against gray mold can be done using this method.

b. Detached Capsule technique

Capsules of 15-20 days old are detached from castor spikes, surface sterilized and dipped in a spore suspension (10^6 conidia/ ml) of *Botryotinia ricini*. Inoculated capsules are maintained at 20^0C temperature and 90% relative humidity. Wetness on capsules is maintained by spraying water at 8 h interval. Symptoms will appear on capsules at 3-4 DAI. By 6th day capsules are fully covered with mycelium and severity is recorded for assessing the resistance.

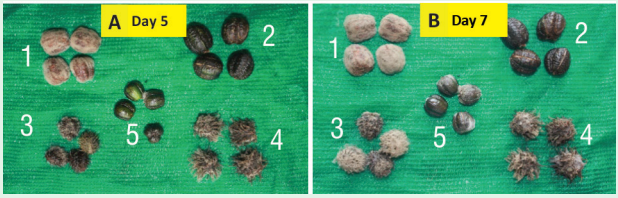

Fig. 8. Screening of castor germplasm by detached capsule technique. **A.** Growth of *Botryotinia ricini* on capsules at 5 DAI **B.** Growth of pathogen at 7 DAI. (1. Pink local, 2. F_6 of JC12 X 48-1, 3. RG 3216, 4. DCH-519, 5. CI-1).

c. Detached leaf technique

A detached leaf technique has been developed at IIOR, Hyderabad to screen castor lines for their reaction to gray mold (Prasad, R. D., Unpublished). In this method, a 5 mm agar disc from a 7-day-old culture plate of *B. ricini* is used for inoculation. Castor leaf facing lower side up is placed in a Petri dish lined with moist blotting paper. The petiole of the leaf is inserted in moist cotton swab to maintain turgidity. The inoculum disc is placed on the detached cut leaf of castor to be screened for disease reaction (Fig. 9). The Petri plates containing inoculated leaves are incubated at 25^oC and 90% RH in a growth chamber with periodic wetting of blotters kept in the plates. The disease severity is recorded 3 days after incubation.

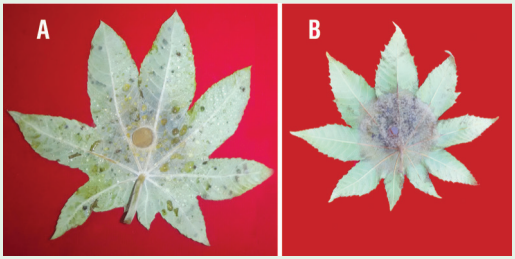

Fig. 9. Detached leaf technique for screening of gray mold disease **A.** Water soaked lesions after 48 h **B.** *Botryotinia ricini* sporulation on leaf at 96 h.

d. Field fogging technique

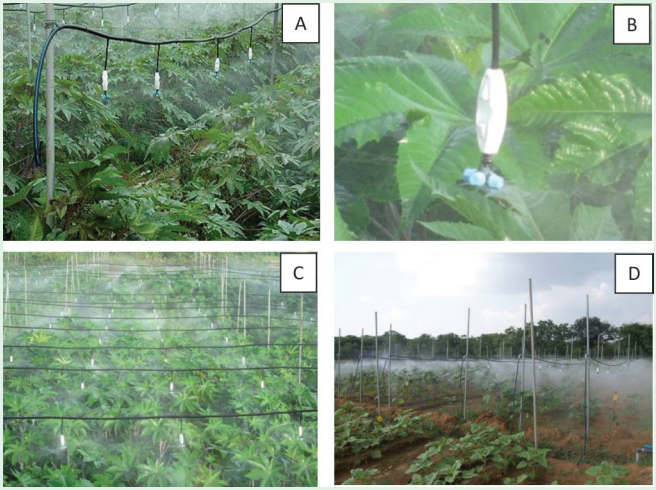

Fig. 10. Screening of castor germplasm by fogging under field conditions. **A.** One line of PVC lateral pipe drawn between two castor rows at 6 ft height, **B.** Close up view of four way fogger, **C & D.** Fog created by the foggers.

To screen castor germplasm and breeding lines for selection of resistance sources under artificial epiphytotic conditions, a field fogging technique has been standardized at IIOR, Hyderabad. Castor lines are sown at a spacing of 90 x 60 cm during second fortnight of June in order to ensure that the flowering and capsule development coincides with the period of maximum rainfall and high humidity which occurs during the months of August and September. For maintaining capsule wetness and humidity, four way foggers are fixed on PVC lateral pipes drawn at a height of 6 ft one line each between two castor rows from underground irrigation pipelines for which pre-filtered (30 m^3 screen filter) irrigation water is supplied at a pressure of 4kg/ cm^2 from water source

(Fig. 10). When castor plants are at an age of 70 days (2-3 spikes/ racemes with 15-20 days old capsules) fogging system is operated every 1h for 10 min during day time so that capsule remain wet at least for 6-8 h/ day. *B. ricini* spore suspension (10^6conidia/ ml) prepared from 10-day-old culture of the fungus is sprayed on spikes/ racemes. Gray mold infection can be seen on the spikes between 5 and 7 days after inoculation on highly susceptible castor lines.

e. Polyhouse screening Technique

Castor crop is grown inside the polyhouse for screening against gray mold disease by maintaining favourable conditions (Fig.11). Humidity & temperature inside the polyhouse are maintained by cooling pad and fan system. Wetness on castor spikes are maintained by foggers fixed on PVC lateral pipes drawn above crop canopy. When castor plants are in 2-3 spikes stage, fogger system is operated for 10 min, every one hour during day time. *B. ricini* spore suspension prepared from 10-day-old culture is sprayed on spikes/ racemes. Graymold infection can be seen on the spikes 3-5 days after inoculation.

Fig. 11. Screening of Castor gray mold disease under poly house condition
A. Castor crop inside the polyhouse **B.** Poly house

f. Biochemical test for resistance assessment

Botryotinia ricini is a necrotrophic fungal pathogen and the pathogenesis process involves production of hydrolytic enzymes like pectinases and cellulases by the pathogen resulting in maceration of pericarp of castor capsules, browning and necrosis. Tests based on these macerating enzymes would help in selection of resistance sources. Reaction of mature castor capsules to these hydrolytic enzymes is tested by modification of earlier described method by Thomas and Orellana, 1963 (Praduman, Y., Unpublished). 25% solution of pectinase (Macerase pectinase from *Rhizopus* sp., Merck) prepared in tris buffer (pH 7)

by adding glycerol as wetting agent and 12% solution of cellulase Onozuka-R-10 (Merck) is prepared in phosphate buffer (pH 4.7) by adding glycerol as wetting agent. Fully developed green capsules of test genotypes of castor are dipped into the solution and incubated at a relative humidity of 100% for 16 h at 33^{0}C. Based on capsule colour, firmness and tissue structure, genotypes can be graded and disease reaction can be correlated with pathological studies. (Fig. 13). Such tests can be indicative in resistance screenings and results should be verified with laboratory/ field screening data.

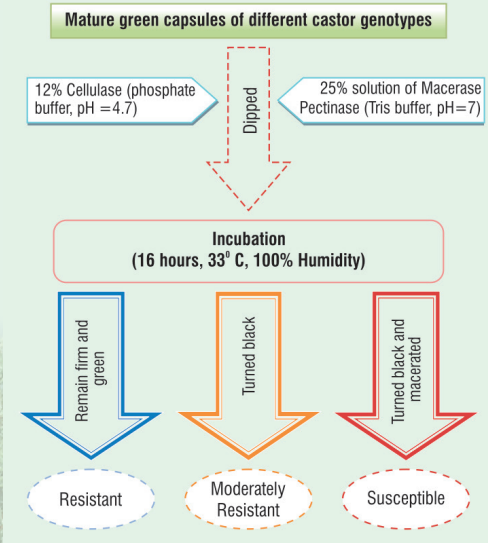

Fig. 12. Biochemical test procedure for resistance assessment

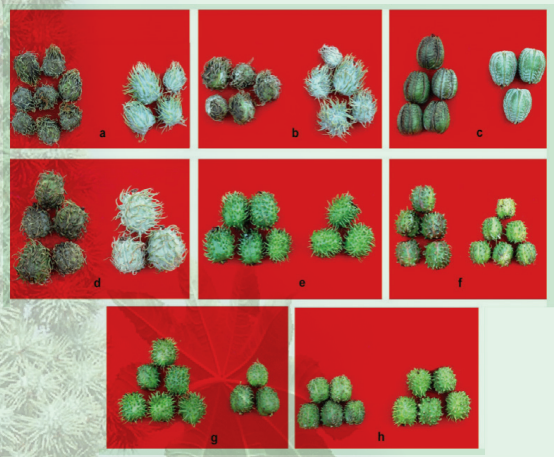

Fig. 13. a. DCH- 519, **b.** DCS-9, **c.** 48-1, **d.** RG 2787, **e.** RG 2810, **f.** RG 3216 (Red), **g.** DPC-9, **h.** RG 3216 (Green). Left-different castor genotypes treated with hydrolytic enzymes, Right-untreated capsules serve as control.

At IIOR, large numbers of germplasm accessions and breeding lines were screened by detached spike/ raceme technique in glasshouse/ growth chamber and by field fogging technique. Few germplasm sources like RG 558, RG 1139, RG 2719 and RG 1963 (Fig. 14) having moderate levels of resistance were identified. Using moderately resistant sources viz, RG1139, 48-1 & DPC9 two breeading lines Viz., CI-1 and CI-2 (Fig. 14) showing better resistance than the available sources were developed (Senthilvel S., unpublished)

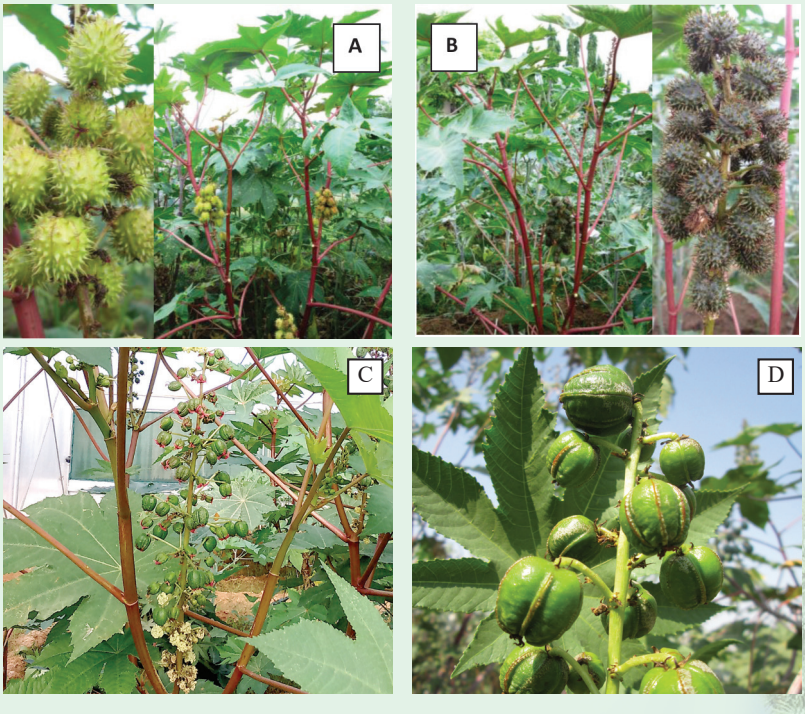

Fig. 14. Moderately resistant germplasm identified by different screening techniques, **A.** RG 2719 **B.** RG 1963 **C.** CI-1, **D.** CI-2

ii. Host resistance assessment

A diagrammatic scale (Fig. 15) to assess gray mold severity in castor has been developed (Sussel *et al.,* 2009). Based on the infection of *Botryotinia ricini* (Fig. 16) on primary, secondary and tertiary racemes (sequence of recemes/ spikes appear on growing plant) a 0-9 scale developed at Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad (Anonymous, 2009)

Disease grade	intensity of infection (%)	Reaction
0	No infection	Immune
1	1 to 10% raceme area infected	Resistant
3	11 to 20% raceme area infected	Moderately resistant
5	21 to 30% raceme area infected	Moderately susceptible
7	31 to 50% raceme area infected	Susceptible
9	>51% raceme area infected	Highly susceptible

The per cent disease index (PDI) can be calculated by using McKinney (1923) infection index.

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Total number of spikes observed X Maximum disease grade}} \times 100$$

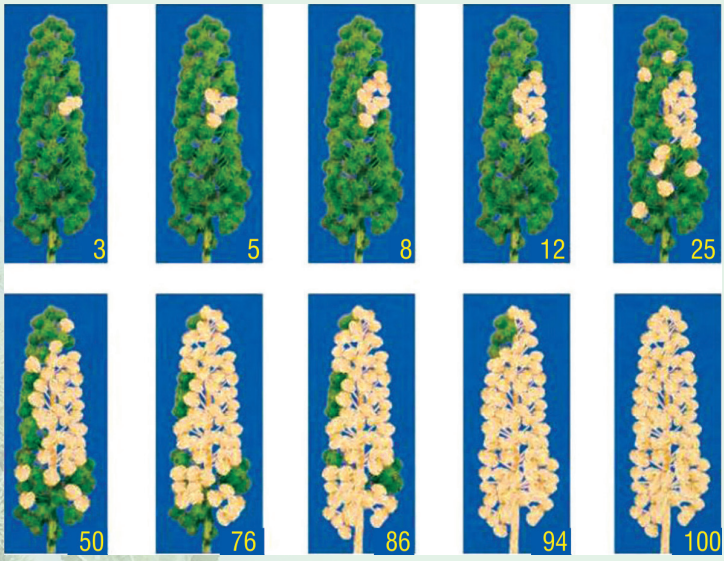

Fig. 15. Diagrammatic scale to assess the gray mold severity on castor (Reprinted from: Sussel *et al.,* Tropical Plant Pathology, Vol. 34, No.3, pp.186- 191, 2009 with permission). Numbers represent the per cent area affected by the disease.

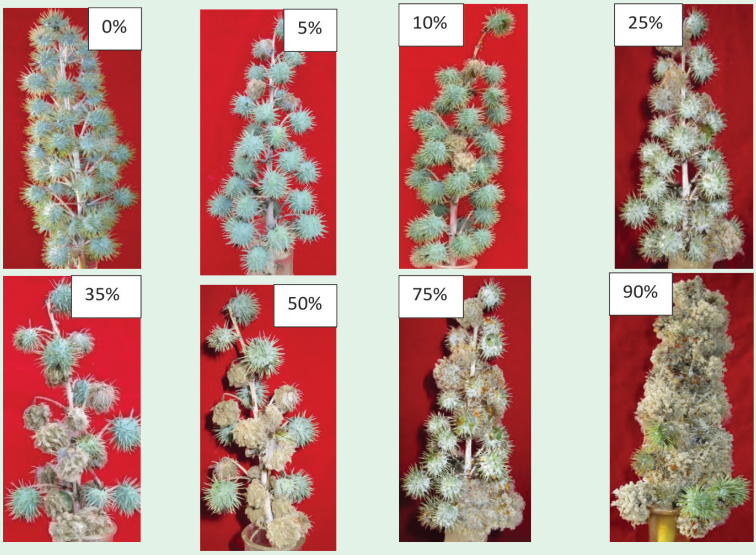

Fig. 16. Pictorial key used for gray mold disease severity assessment at Indian Institute of Oilseeds Research.

I. Disease management

i. Cultural

The use of varietal resistance is one of the major strategy for disease management. However, as highlighted previously, there are no genotypes with satisfactory resistance levels to gray mold (Araújo *et al.,* 2007; Cook, 1981; Dange *et al.,* 2005; Kolte, 1995; Milani *et al.,* 2005). Different researchers have recommended the use of healthy seeds, removal of plant debris, proper choice of planting area and growing season, and use of tolerant cultivars (Galli *et al.,* 1968; Massola and Bedendo, 2005; Sussel, 2009). Gray mold incidence was higher in July-sown crop than in August-sown crop regardless of cultivars and hybrids. It is also recommended to use plant spacing adjusted for maximum aeration (Kolte, 1995; Lima *et al.,* 2005). Elimination of alternate and reservoir hosts (Euphorbiaceous hosts), as well as removal and burning of gray mold infected castor spikes/ capsules has been recommended (Anonymous *et al.,* 2002) for reducing the inoculum load of the pathogen. Adjusting sowing time such that the crop maturation occurs during dry season helps in disease

escape (Raoof and Nageshwar Rao, 1999). Wider spacing between the plants and between the rows (Fig. 17A) has also been recommended as one of the important components for castor gray mold management as it provides good aeration in the crop canopy and film of water on capsules dries up fast thus limiting conidia germination.

Fig. 17. Cultural management of Botryotinia gray mold. **A.** Wider spacing; **B.** Castor crop with open canopy.

ii. Chemical

Seed treatment has been the management strategy most frequently recommended (Araújo *et al.,* 2007; Batista *et al.,* 1998; Godfrey, 1923; Gonçalves, 1936; Massola Jr. & Bedendo, 2005; Milani *et al.,* 2005; Sussel, 2009), mainly to avoid the introduction of the pathogen into new areas. Spraying of systemic fungicides soon after the appearance of the first symptoms delay the epidemic and reduces disease progress (Araújo *et al.,* 2007). Preliminary studies under controlled conditions have shown that carbendazim and azoxystrobin are effective against the gray mold pathogen (Bezerra, 2007). Chagas (2009) found azoxystrobin to be ineffective against *B. ricini,* while carbendazim and several other fungicides, including tebuconazole, iprodione and procymidone are highly effective. Under *in vitro* conditions carbendazim + mancozeb completely inhibit the growth of the fungus even at the lowest concentration of 100 ppm and mancozeb, iprodione, captan are effective at a concentration of 250 ppm (Yasmeen *et al.,* 2003).

Fungicides are also effective in managing gray mold disease under field conditions. Depending on cyclone warning centre's forecast, one spray of

carbendazim/ thiophanate methyl 1 g/ lit before onset of cyclonic weather and one more spray after disease appearance were recommended as chemical control for gray mold (Anonymous, 2002). Prophylactic spray with carbendazim (1 g/ l), removal of affected spikes and application of 20kg N/ha and 20kg P_2O_5/ha is effective in reducing the yield loss due to *Botryotinia* gray mold (Anonymous, 2001).

Spray of carbendazim + iprodione (quintal) 0.1% was found to be significantly superior recording the lowest gray mold incidence (28.9%) and maximum seed yield (1620 kg/ ha). Spraying of thiophanate methyl was on par with quintal in disease management. Removal of affected spikes, application of 20kg N/ha after rains followed by spray of carbendazim (1g/ lit) recorded lowest incidence of gray mold and increased yields (Anonymous, 2005). Chemical fungicide propiconazole 0.1% is also very effective in disease control (Anonymous, 2010).

iii. Biological

There are several studies dealing with the use of biological control agents, mainly *Trichoderma* spp. and *Clonostachys rosea* to control diseases caused by *Botrytis* spp. (Elad and Stewart, 2007). Some studies conducted with *Trichoderma* spp. and *Clonostachys rosea* for the control of gray mold of castor gave promising results (Bhattiprolu and Bhattiprolu, 2006; Chagas, 2009; Demant *et al.,* 2006; Raoof et al., 2003; Tirupathi *et al.,* 2006). *T. harzianum* Th4d SC 2ml/l and consortia of *T. harzianum* Th4d + *T. asperellum* Tv5 SC 1ml/l resulted maximum gray mold reduction with a better persistence on racemes up to 2 weeks and resulted in higher yield compared to pathogen check (Anonymous, 2012).

iv. Biotechnological approaches

Castor transgenic plants tolerant to gray mold disease are being developed at IIOR using the multiple genes imparting resistance to fungal Pathogens. With the aim of testing the cumulative effects of multiple genes in imparting resistance to *Botrytis*, two polygene cassettes have been developed using genes that impart partial resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. In the first construct, three genes ERF1 (Ethylene Response Factor

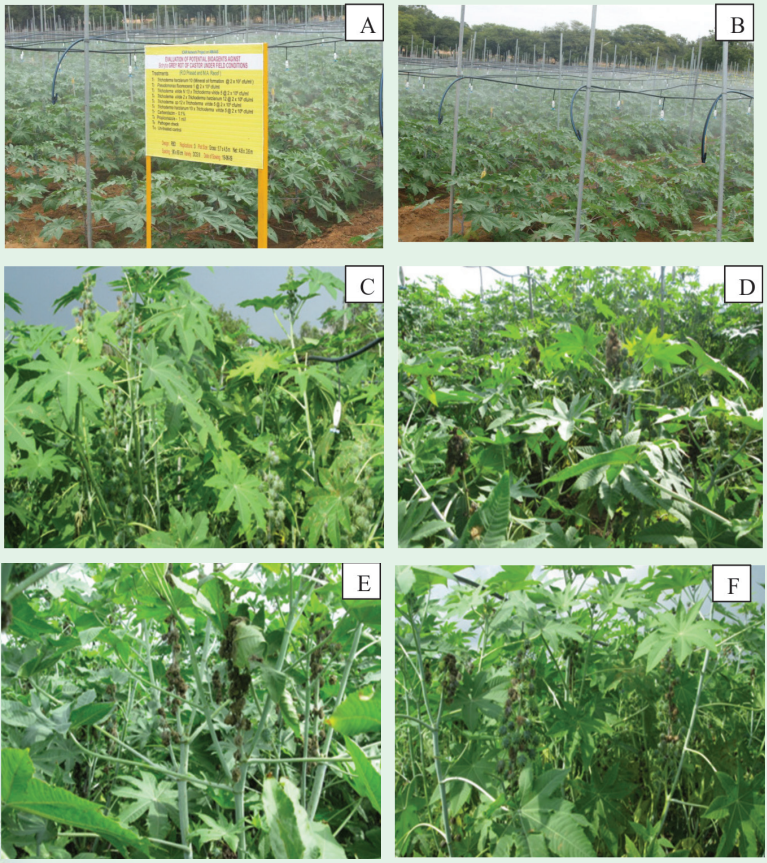

Fig. 18 Evaluation of chemical fungicides and bioagents against Botryotinia gray mold of castor. **A.** Field view, **B.** Fogging system, **C.** Propiconazole 0.1% treated plot, **D.** Carbendazim + iprodione 0.1% treated plot, **E.** Pathogen check, **F.** *Trichoderma harzianum* Th4d SC 2 ml/ l sprayed plot.

1), BIK1(*Botrytis* Induced Kinase1) and AtEBP (*Arabidopsis thaliana* Ethylene responsive Element Binding Protein), involved in signal transduction during plant-pathogen interaction of necrotrophic fungi, have been used. These genes have been cloned under three independent promoters with known inflorescence elevated expression pattern and the resultant cassettes have been cloned in tandem within a single T-DNA of the binary vector so that the transgenic plants realized with this vector will express all the three gene cassettes independently. In the second polygene construct, three genes RsAFP2 (*Raphanus sativus* - Anti Fungal Protein2), Chitinase and AceAMP1 (*Allium cepa* - Anti Microbial

Peptide1) have been cloned under the same promoter (35S, a constitutive promoter) and are separated by 2A signal peptide sequence so that three genes are expressed as a single polycistron and subsequently as a self-cleaving polyprotein.

To validate these gene constructs, tobacco plant is being used as a model crop species. Tobacco transgenic plants carrying individual gene cassettes used in the development of the first polygene cassette have been realized and analysed for the stable expression of the transgene(s) in T1 generation transgenic tobacco plants. These T1 generation plants subjected to *Botrytis* infection showed considerably high resistance to *Botrytis* than control indicating the positive effect of the introduced transgenes (Fig. 19). Transgene cassettes are being stacked into the same plant by following appropriate crossing programme with the transgenic tobacco plants carrying the individual gene cassettes. Transgenic plants with combinations of gene cassettes have been identified in the segregating populations and they are being tested for tolerance to necrotrophic fungi in the laboratory assays. Also, attempts are being made to develop tobacco plants with both the triple gene cassette vectors. Castor is being transformed with the developed constructs using both *in vitro* and *in planta* transformation methods.

3. Proactive mitigation of Gray mold by holistic crop management

As evident from the various reviews on the topic, stable resistance sources to gray mold disease of castor are not available though few morphotypes having certain level of resistance identified. But inheritance pattern of resistance, involvement of major/minor gene action are not elucidated. It is also known that the disease is highly weather dependent, but quantification of the dependency of the disease on weather variables for developing dynamic models and forewarning disease occurrence has not been attempted. It is also observed that morphological characters of racemes/spikes such as less compact spikes with non-spiny/sparsely spiny and hard spines on capsules, capsule with long pedicels, and absence of waxy coating on capsules (ideal plant types) play a significant role in field resistance of castor cultivars. Castor plant architecture especially open canopy type (Fig. 17B) attracts less disease due to aeration and shorter periods of capsule wetness. Excessive foliage growth due to high

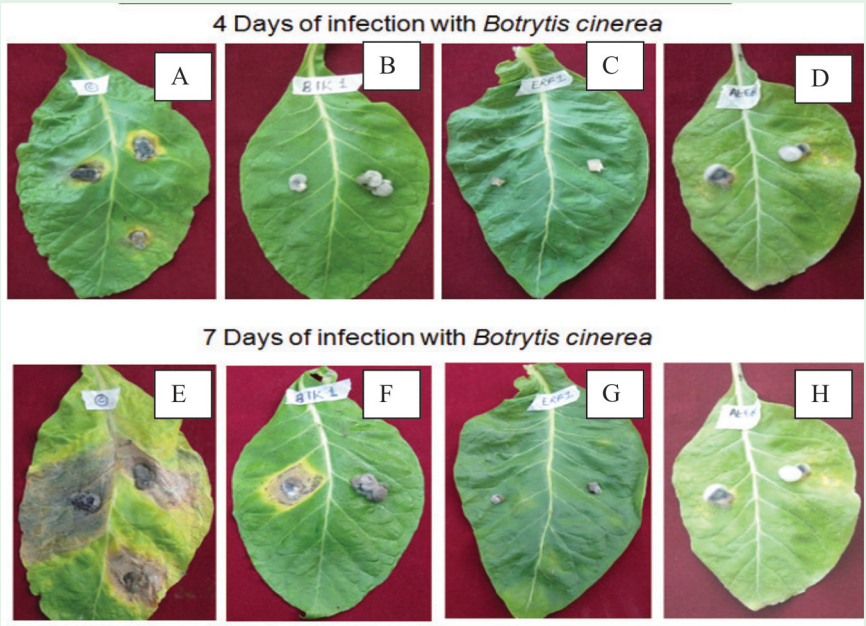

Fig. 19. Excised leaf assay of tobacco control and transgenic plants using *Botrytis cinerea* 4 days and 7 days post infection studies. **A & E**. Tobacco control leaf. **B & F**. BIK1 tobacco leaf. **C & G**. ERF1 tobacco leaf. **D & H**. AtEBP1 tobacco leaf

nitrogenous fertilizer applications and excess irrigation results in congenial microclimate that predispose castor crop to gray mold. It is also strongly believed that sclerotia are the major source of primary inoculum (conidia) initiating the infections on plants during monsoon season. The role of weeds and perennial castor plants as source of primary inoculum is not certain.

In the light of above observations, proactive mitigation of gray mold by holistic crop management by involving/ adopting some of the known approaches listed below is important.

a. Identifying ideal plant types among released varieties/ hybrids having field tolerance and deployment of those cultivars in endemic locations.

b. Appropriate prophylactic measures like fungicidal sprays, scheduling fertilizer application and irrigation based on local weather forecasts should be

taken up till the development of disease forewarning system based on models developed by integrating information on disease cycle and weather variables.

c. Application of fungicide, carbendazim 0.1% or propiconazole 0.1% before onset of congenial conditions.

d. Adoption of various cultural practices that encourage beneficial mycoparasitic organisms that brings down primary source of pathogen inoculum in the soil.

e. Adjusting sowing time in such a way that the crop maturation occurs during dry season and adopt wider spacing to create uncongenial micro climate for gray mold development.

4. Future Challenges

The major challenges in tackling gray mold disease are ;

- Precise information on infection process and disease progression is meagre
- Role of weather variables on disease development and primary source of inoculum initiating first infections during crop season is not known.
- Forecasting time of onset of disease for proactive attempts using available management strategies.
- Finding stable resistance sources with desirable level of resistance for developing resistant cultivars is still elusive.
- Explorations for collecting *Botryotinia* resistant germplasm need to be taken up.
- Though the disease is considered a monsoon dependent occurring during rainy season (*kharif*) grown castor, and it is presumed that growing castor in late *kharif* and *rabi* situation is safe cultural practice to avoid gray mold, the possible effects of climate change, unseasonal precipitation and dew fall in during *rabi* also may attract gray mold disease.

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