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Screening germplasm lines for identification of resistant source against gray mold disease (*Amphobotrys ricini* (N.F. Buchw.) Hennebert) of castor

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ABSTRACT

The present study was aimed to screen castor germplasm lines varying in wax content against gray mold disease for the identification of resistance source under *in vitro* conditions using detached spike and detached capsule technique. Out of thirty-three lines screened against gray mold under *in vitro* conditions six lines with low wax content *viz*., RG-1754 (0.24 µg/mg), RG-1875 (0.12 µg/mg), RG-1915 (0.21 µg/mg), RG-1919 (0.24 µg/mg), RG-1972 (0.08 µg/mg) and RG-1926 (0.08 µg/mg) recorded significantly low levels of infection ranging from 10 to 20 per cent 7 days after inoculation. Whereas the susceptible cultivar DCH -519 has recorded disease severity of more than 90 %. The pearman's rank correlation analysis showed a strong positive relation between disease severity and capsule wax content, with $p < 0.01$ and $r = 0.884$.

Introduction

Castor (*Ricinus communis* L.), a predominant non-edible oilseed crop that is native to Ethiopia and North Africa, is a member of the Euphorbiaceae family (Ramanjaneyulu *et al.,* 2017). Castor seed, primarily grown in tropical and subtropical climates contains 50-55 per cent oil and plays a significant role in the Indian oil economy. India produces approximately 73 per cent of the world's castor with an annual production of around 1.19 million tonnes. India earned about 780 million USD from the sales of castor seed, castor oil and its derivatives during 2020-21 (SEA, 2021). Currently, India leads the world in both castor production and area. Castor oil has several industrial applications and is valuable as a source of therapeutic oil. In addition to being used in the production of a number of complex products such as nylon fibres, lubricants for jet engine, hydraulic fluids, polymers, and synthetic leather. Dehydrated castor oil is utilised as an antifreeze for engine fuels and lubricants used in spacecraft and rocket. (1982 by Ogunniyi, 2006). As the oil contains up to 90% of the hydroxy fatty acid ricinoleic acid, castor oil stands out from other vegetable oils. Due to the huge demand for castor oil and its derivatives, India is expected to stay as a major producer of castor seeds worldwide with China and Brazil as its only competitors. However, India has not yet fully utilised the potential of its monopoly because few value-added goods and largely raw oil are exported. (Bhavanidurga, 2013;

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Severino *et al*., 2012; Jeong and Park, 2009).Castor crop inspite of its importance as commercial crop and hardy nature is attacked by numerous bacterial and fungal diseases. At various crop growth stages there are 150 or more identified pathogens of castor plant. Among these, the most destructive disease is gray mold caused by the fungus *Amphobotrys ricini* (N.F. Buchw.) Hennebert (Prasad *et al.,* 2016). The anamorphic phase is mainly responsible for causing disease epiphytotics. Heavy yield losses were observed, as the inflorescence (raceme) and the capsules are the primary targets of the fungal infection. Heavy rains that last for a long time during the spike/capsule formation stage results in significant losses. As the development of pathogen is favored by the prevalence of high relative humidity and extended wet conditions during the flowering stage. This disease causes significant yield losses up to 100 % in India (Soares, 2012). which pose a serious hazard to the crop's commercial cultivation. As a result, there have been only a few advancements in the management of gray mold disease. Although breeding initiatives to create resistant genotypes have been unsuccessful, those with a moderate level of tolerance have been identified (Anjani, 2012). The preliminary studies of Ayesha *et al.* (2022) indicates a positive correlation between wax content and gray mold severity. The present study was conducted to identify resistant sources against gray mold disease by screening different germplasm lines varying in bloom content under *in vitro* conditions.

Material and Methods

Isolation of pathogen

The castor capsules with typical gray mold symptoms obtained from research farm, ICAR-IIOR, Hyderabad were used for isolation of the fungal pathogen. The pathogen was isolated from the pericarp of infected castor capsules The pericarp pieces along with fungal growth were picked, surface sterilized and isolated on the enriched oat meal agar media (OMA). The mycelial growth appeared 48 h after inoculation and the pathogen was further purified by single spore isolation method.

Estimation of wax content of the capsules

Wax content of the capsules was estimated using calorimetric approach. From each germplasm line with 15 day old spike, by using rapid calorimetric method (Ebercon *et al.,* 1977).

Screening against gray mold disease

Castor germplasm lines were screened against gray mold under *in vitro* conditions using detached spike and detached capsule techniques (Prasad *et al.,* 2016).

Preparation of Inoculum of the pathogen

`Pathogen inoculum for disease screening was prepared from seven day-old, highly sporulating culture of *Amphobotrys ricini* grown on oat meal agar medium. (Prasad & Bhuvaneswari, 2014).

Evaluation of castor germplasm lines using detached spike technique against *A. ricini* infection

Thirty three germplasm lines along with resistant (ICS-324) and susceptible (DCH-519) checks were screened against gray mold disease under glass house conditions by following the standard screening protocol given by Prasad *et al* (2016). The Gray mold disease severity was recorded manually based on the visual symptoms starting from 3rd day till the entire spike of the susceptible check (DCH-519) was covered by the disease. Disease severity and host resistance were assessed based on the diagrammatic scale developed by Sussel *et al*. (2009) and the disease reaction was categorized based on the scale developed by Prasad *et al.* (2016). (Table-1)

Table 1: Disease scale for host resistance assessment against gray mold of castor

Disease scale	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

Evaluation of castor germplasm lines using detached capsule technique against *A. ricini* infection

The germplasm lines were screened against gray mold infection under *invitro* conditions by using detached capsule method (Prasad *et al*., 2016). The disease reaction was scored on 1-4 scale as given in table 2.

Table 2: Disease scale for assessment against gray mold of castor in detached capsule technique

Disease scale	Symptoms observed
1	Browning of capsules
2	Little mycelia growth
3	Mycelium development with few sporulation
4	Entire capsule covered with high sporulation

Results and Discussion

Per cent disease severity and wax intensity on capsules

Thirty three germplasm lines varying in wax content were screened under artificial epiphytotic conditions in glass house. The initial symptoms of *A. ricini* infection appeared on third day after inoculation and the disease severity was recorded using the disease severity scale at regular intervals till maximum disease severity occurred on susceptible check cv. DCH-519. All the germplasm lines showed disease severity of >10 per cent. Of 33 germplasm lines, six lines with low wax content *viz*., RG-1754 (0.24 µg/mg), RG-1875 (0.12 µg/mg), RG-1915 (0.21 µg/mg), RG-1919 (0.24 µg/mg), RG-1972 (0.08 µg/mg) and RG-1926 (0.08 µg/mg) recorded significantly low levels of infection ranging from 10 to 20 per cent. The germplasm lines RG-1836 (0.19 µg/mg), RG-1881 (0.27 µg/mg), RG-1839 (0.22 µg/mg), RG-155 (0.35 µg/mg), RG-1905 (0.10 µg/mg) and RG-1906 (0.25 µg/mg) recorded disease severity levels of 20-30 per cent, which were moderately resistant. The remaining lines exhibited disease severity levels greater than 30 per cent showing a susceptible reaction. (Table-3 fig-1)

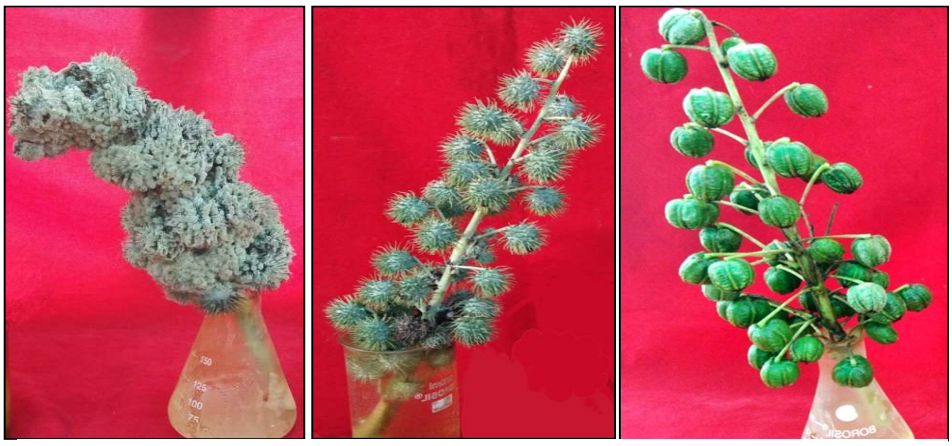
Detached capsule technique (*in vitro* screening)

There was significant difference in disease severity readings among germplasm lines varying in wax content and the results were in accordance with detached spike screening. Six germplasm lines with low wax content (RG-1754, RG-1875, RG-1915, RG-1972, RG-1906, RG-1926) have shown low level of disease infection with browning observed after 7 days of inoculation. The germplasm line RG-1919, RG-1836, RG-155, RG-1881, RG-1851 have shown little mycelial growth on the capsules, which were moderately resistant. The remaining germplasm lines have shown to take up infection at greater rate with 10 lines showing profuse mycelial growth with little sporulation and 12 lines have recorded high disease infection rate shown as entire capsule covered with high sporulation, which was similar to the susceptible check DCH-519 showing a susceptible reaction (Table 3 and figure 2). The Pearman's correlation analysis between per cent intensity of infection from *in vitro* screening with biochemically extracted wax on the capsules revealed that wax content and per cent disease severity were significantly correlated at 0.01 level (2-tailed) with $p < 0.01$ and $r = 0.884$ representing a strong positive correlation. Gray mold is considered as the most destructive disease of castor, the mechanisms underlying the resistance to this fungal pathogen remains unclear. Understanding the interaction between the host and the pathogen and identifying the resistance sources is of great significance in plant breeding programmes. The present study aimed to identify the resistant sources against the gray mold disease from the existing castor germplasm lines. The results of the present study, indicates that among the germplasm lines screened ICS-324 having low amount of capsular wax has shown significantly less amount of disease severity under *invitro* conditions. The results are further supported by Sujatha *et al*. (2016) also screened four castor genotypes using detached capsule technique and reported that genotype DCS-9 was highly susceptible to the disease, while genotype RG-3216 R exhibited least susceptibility. Also, variable degree of tolerance to gray mold pathogen in castor genotypes has been reported (Araújo *et al*., 2007; Anjani, 2012). The study further indicate a significant positive correlation between the gray mold severity and the amount of capsule wax content which is in accordance with earlier studies of Ayesha *et al*. (2022) indicating a possible role of wax governing the pathogenesis. Studies so far have focused on the kind of bloom (no bloom, single, double, and triple bloom) and its

influence on the severity of gray mold (Prasad *et al*., 2016), and also pathogenesis of various diseases in the presence or absence of wax in various host plants (Wang *et al.,* 2008; Bourdenx *et al.,* 2011; Hansjakob *et al.,* 2011; Zhu *et al.,* 2017) governing disease. The wax layer acting as protective barrier is playing a role in pathogenesis which is similar to the results observed by Weidenbach *et al.* (2014) where cuticular wax determined the pathogenicity of

Table 3: Evaluation of castor germplasm lines for their reaction to gray mold

Germplasm lines	Wax content (µg/mg)	Disease severity in detached spike method	Disease scale	Disease severity scale in detached capsule method
RG-1754	0.240	18.0 (25.08)*	3	1
RG-1875	0.128	13.3(21.3)	3	1
RG-1915	0.219	11.6(19.9)	3	1
RG-1919	0.242	20.0 (26.5)	3	2
RG-1836	0.194	21.6 (27.7)	5	2
RG-1843	0.387	51.3 (45.7)	9	3
RG-1972	0.083	17.3 (24.5)	3	1
RG-1853	1.030	71.6 (57.8)	9	4
RG-1859	0.520	41.6 (40.1)	7	3
RG-155	0.355	27.0 (31.2)	5	2
RG-1961	0.103	43.3 (41.1)	7	3
RG-1971	0.120	46.6 (43.0)	7	3
RG-1920	0.513	53.3 (46.8)	9	3
RG-1966	1.510	85.0 (67.18)	9	4
RG-1906	0.257	25.0 (29.9)	5	1
RG-1855	0.627	63.3 (52.7)	9	4
RG-1881	0.277	28.6 (32.3)	5	2
RG-1839	0.220	23.0 (28.6)	5	2
RG-1998	0.323	43.3 (41.1)	7	3
RG-1926	0.087	12.3 (20.4)	3	1
RG-1854	0.907	73.3 (58.9)	9	4
RG-1860	1.470	85.0 (67.2)	9	4
RG1905	0.103	29.0 (32.5)	5	2
RG-1851	0.530	48.3 (44.0)	7	3
RG-1872	0.740	51.6 (45.9)	9	3
RG-1921	1.013	42.3 (40.5)	7	3
RG-1952	0.960	75.0 (59.9)	9	4
RG-1791	0.640	58.0 (49.5)	9	3
RG-1917	1.010	70.0 (56.7)	9	4
RG-1999	1.120	76.0 (60.6)	9	4
RG-2058	0.427	53.3 (46.8)	9	4
RG-2012	1.083	71.6 (57.8)	9	4
RG-1777	0.857	66.6 (54.7)	9	4
DCH-519	1.927	93.3 (75.2)	9	4
ICS-324	0.150	7.3 (15.6)	1	1
C.D.@ 5%	0.158	2.822		
SE(m)	0.056	0.998		
SE(d)	0.079	1.412		
C.V.	16.658	3.739		



DCH-519 RG-1919 ICS-324

Figure 1: Reaction of castor germplasm lines to gray mold in detached spike technique

DCH-519 RG-1919 ICS-324

Figure 2: Reaction of castor germplasm lines to gray mold in detached capsule technique

powdery mildew. Similar results were observed by Ayesha (2020) who screened 26 castor genotypes varying in the morphological characters against gray mold infection using detached capsule technique. However, the focus of this investigation was to identify low waxy content germplasm lines and their reaction to gray mold disease which further help in identification of resistant sources. Considering the impact of waxy bloom levels on the severity of the gray mold disease severity helps in eliminate high wax plants at early stages saving time and improving the quality of breeding programmes. Additionally, breeding programmes aimed to develop gray mold resistant cultivars can select germplasm lines with low wax content which are expected to be least affected by fungus.

Conclusion

From the study it can be concluded that there exist a positive correlation between wax content and gray disease severity. The germplasm lines RG-1754, RG-1875, RG-1915, RG-1919, RG-1972 and RG-1926 with low level of wax serve as better source of resistance to gray mold disease of castor and the amount of wax on the castor capsule can be used as a biochemical marker to screen large number of germplasm lines or early generation breeding material with large population size.

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Conflict of interest

The authors declare that they have no conflict of interest.

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