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Screening of Custard apple (*Annona squamosa L.*) genotypes for fruit rot resistance

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AICRP on Arid Zone Fruits (Fig and Custard apple) Jadhavwadi, Dist. Pune (M.S.)-412 301

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ABSTRACT

Custard apple (*Annona squamosa* L.) is a hardy dryland horticultural crop with high export potential and significant economic benefits. However, fruit rot, caused by *Colletotrichum gloeosporioides* Penz, is one of the major diseases that can lead to substantial losses in yield. The study revealed that all 30 genotypes were infected by the disease. The Attached Fruit Culture (AFC) method showed that a higher number of genotypes exhibited a highly susceptible reaction when inoculated using the Mycelial Bit Inoculation Method (MBIM) compared to the Micro Droplet Inoculation Technique (MDIT). In the MBIM method, the genotypes Vrudheshwar-3, Verul-2, Karkhel, Vrudheshwar-4, Vrudheshwar-2, Telgaon Local, Humnabad Local, and Dhanagarawadi-3 exhibited moderate resistance. In the MDIT method, Vrudheshwar-3, Verul-2, and Karkhel showed resistance to the disease. Additionally, under natural field conditions, Vrudheshwar-3, Verul-2, Karkhel, Humnabad Local, Dhanagarwadi-3, and Vrudheshwar-4 were recorded as resistant to fruit rot disease.

Introduction

Custard apple (*Annona squamosa* L.) is a significant dryland fruit crop in India, originating from the tropical regions of the Americas and belonging to the Annonaceae family. The Portuguese introduced custard apple to India in the 16th century, and it thrived in the Deccan Plateau's subtropical climate. The Southern Tropical Plateau of India has a long history of cultivating custard apples (Hayes, 1953; Venkataratnam and Satyanarayanaswamy, 1958; Venkataratnam, 1959). Despite its adaptability, both biotic and abiotic factors, particularly diseases caused by various pathogens, are major contributors to reduced yields.

Custard apple is affected by several diseases, including fruit rot caused by *Fusarium*, *Phytophthora*, *Phoma*, *Pestalotia*, among others, Diplodia rot caused by *Botryodiplodia theobromae*, black canker caused by *Phomopsis annonacearum*, leaf spot caused by *Alternaria* spp., *Cercospora* leaf spot (*Cercospora annonae*), and anthracnose/fruit rot caused by

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Colletotrichum gloeosporioides (Mukarji and Bhasin, 1986). Among these, the most serious disease is fruit rot caused by *Colletotrichum gloeosporioides*, which poses a significant threat to custard apple production.

The use of resistant cultivars is one of the most economical and effective strategies for controlling plant diseases. With this objective, custard apple genotypes available at the All India Co-Ordinated Research Project on Arid Zone Fruits (Fig and Custard Apple) in Jadhavwadi were screened in 2022 using various methods to identify sources of resistance and determine the most effective screening technique for fruit rot disease.

Material and Methods

Thirty custard apple genotypes from the All India Co-Ordinated Research Project on Arid Zone Fruits (Fig and Custard Apple) at Jadhavwadi, Pune, were evaluated during 2022 to screen the genotypes against resistance of fruit rot disease. The screening was conducted using the attached fruit culture (AFC) method under controlled field conditions, as well as under natural field conditions.

a. Screening by Attached Fruit Culture (AFC)

The attached fruit culture on different custard apple genotypes was carried out at AICRP on AZF (Fig and custard apple), Jadhavwadi, Pune and disease reactions were recorded. Four fruits of each variety were tagged and bagged. Two fruits were inoculated by mycelial bits inoculation method (MBIM) and other two fruits were inoculated by micro droplet inoculation method (MDIT). The fruits were wounded at stem end by pin prick method and inoculated by using combination of two methods like application of mycelial bits (MBIM) of uniform size (5 mm diameter) on wounded fruit epidermis (Rocha *et al.* 1998) and micro droplet inoculation technique (MDIT) given by Munaut *et.al.* (1997) with spore suspension having 105 conidia per ml. The purpose of using combination of two methods was to have maximum infection of disease. The mycelial discs were placed in inverted position i.e. side of fungal growth on wounded portion and covered with small wet cotton swab to provide moisture for conidial germination and infection. After inoculation fruits were bagged with polythene bags containing cotton swab, saturated with sterile water at the bottom of the bag. During infection and disease development period the relative humidity ranged from 69 to 98 percent and temperature in bag 22 to 30°C. The mycelial bit and cotton swab kept on wounded portion were removed two days after inoculation. Required control set of un-inoculated fruits was provided. The observations regarding latent period were noted beginning at 48 hours from inoculation till appearance of visible symptoms of disease at 24 hours interval. The observations on symptom development were recorded every day after inoculation of fruits.

b. Screening under natural field conditions

The fruit rot disease was studied in field under natural conditions. Custard apple genotypes were observed critically for fruit rot symptoms and using (0 to 7) (Gaikwad, 2002) grade score scale the disease intensity was calculated. The fruit rot severity was recorded by using (0-7) grade scale. The Percent disease intensity (PDI) was calculated by using the formula given by McKinney (1923).

$$PDI = \frac{\Sigma \text{ numerical rating}}{\text{Total no. of fruits observed x maximum grade (}i.e.\text{ 7)}} \times 100$$

Results and Discussion

Attached Fruit Technique (in vivo)

Thirty custard apple genotypes were screened under artificial epiphytotics by attached fruit technique by inoculating each genotype by two inoculation methods; Mycelial bit inoculation method (MBIM) and Micro droplet inoculation technique (MDIT). The data (Table 1) revealed that the treatment differences and their interactions were statistically significant.

A. Mycelial Bit Inoculation Method (MBIM)

Latent Period (LP)

The data presented in Table 1 revealed that the there is significant difference among the different genotypes and latent period. The minimum of 59.0 hrs to maximum up to 115.0 hrs latent period was observed in different custard apple genotypes. The highest latent period (115.0 hrs) was noticed in the Vrudheshwar-3 genotype. The custard apple genotypes Verul-2 (113.0 hrs) and Karkhel (111.0 hrs) were at par with Vrudheshwar-3. The minimum of 59.0 hrs latent period was observed in NMK-1 (Golden) followed by Hyderabad Selection (61.50 hrs) and were at par with each other. The commercial varieties under cultivation have reported latent period of Phule Purandar (90.0 hrs), Balanagar (75.00 hrs), TP-7 (89.0 hrs) and Purandar local (66.0 hrs). The higher la-

Disease rating scale used for recording fruit rot severity

Disease Score	Fruit area infection (%)	Symptoms under		Reactions
		Field	*In vitro*	
0	-	Nil	Nil	Immune
1	<1	Healthy looking fruits, only minute black, pin point, few spots, sometimes yellowish specks may be observed	Healthy looking fruits, only minute black, pin point , few spots, sometimes yellowish specks may be observed	Highly resistant (HR)
2	1.01 to 10.0	Fruits with small, light brownish-black spots of about 0.5 to 1.0 mm in diameter and 5 to 20 spots	Fruits with small, brown to dark brown discoloured area of about 0.5 to 2.0 mm in diameter with or without yellow halo	Resistant (R)
3	10.01 to 20.0	Fruits with small, slightly sunken reddish brown spots of 1.0 to 2.0 mm in size and 10 to 25 spots	Light violet to black discolouration of about 5 to 10 mm in diameter without any fungal growth	Moderately resistant (MR)
4	20.01 to 30.0	Fruits with medium sized moderately sunken light violet to blackish spots of about 2 to 4 mm diameter and 20 to 50 spots	Light violet-black discolouration of about 10 to 20 mm but < 30% fruit surface infection with or without fungal growth	Moderately susceptible (MS)
5	30.01 to 40.0	Fruits with medium to large sized, sunken violet – black spots of about 3 to 8 mm in diameter and 50 to 100 spots scattered all over the fruit, with or without sub-epidermal acervuli	Fruits with violet – black discoloration of about 20-30 mm in diameter, but <40 per cent area of fruit with scanty to moderate ashy-white fungal growth with or without acervuli	Susceptible (S)
6	40.01 to 50.0	Fruits with large deeply sunken , dark violet- black spots of 5 to 8 mm in size and few to many (up to 190) isolated but adjoining spots with sub-epidermal acervuli	Fruits with dark violet – black discoloration of about 30-40 mm in diameter, but <50 per cent area of fruit surface, with good, pinkish- white fungal growth containing acervuli of pathogen	
7	>50.01	Fruits with several typical anthracnose type spots or dark violet to black spots which coalesce with each other resulting into blackening of fruits (dry fruit rot) with numerous orange to black, pin point sub-epidermal fungal bodies (acervuli)	Fruits with dark violet – black to brownish discoloration covering >50 per cent area of fruit surface, with luxuriant orange – white fungal growth containing numerous acervuli of the pathogen, finally resulting into hard, compact, dry fruit mass	Highly susceptible (HS)

tent period results in to slow disease development. Hence, custard apple genotypes Vrudheshwar-3, Verul-2, Karkhel, Vrudheshwar-4, Vrudheshwar-2, Telgaon Local, Humnabad Local and Dhanagarawadi-3 possess slow disease development characters.

Per cent Disease Intensity (PDI)

The observations (Table 1) showed that none of the variety or genotype was free or resistant to fruit rot disease. The minimum of 7.50 per cent disease intensity was observed in Vrudheshwar-3. It was at par with Verul-2 (8.15 %) and followed by Karkhel, Vrudheshwar-4, Vrudheshwar-2, Telgaon Local, Humnabad Local and Dhanagarawadi-3 genotypes and showed moderately resistant reaction to disease. Remaining of the germplasm showed moderately to highly susceptible reaction. The genotypes NMK-1 (Golden) recorded maximum of 50.40 per disease severity and exhibited highly susceptible reaction to the fruit rot disease.

B. Micro Droplet Inoculation Technique (MDIT)

Latent Period (LP)

The observations (Table 1) divulge that the there is significant difference among the different genotypes in latent period was observed. The minimum of 73.0 hrs to maximum up to 117.50 hrs latent period was observed in different custard apple genotypes. The highest latent period (117.50 hrs) was noticed in the Vrudheshwar-3 which was at par with Verul-2 (117.0 hrs) and Karkhel (117.0 hrs). The next best genotypes were Vrudheshwar-4, Humnabad Local, Vrudheshwar-2, Dhanagarawadi-3, Phule Purandar, Dhanagarawadi-7 and Purandar Local. The minimum latent period was noticed in NMK-1 (Golden) (73.0 hrs).

Per cent Disease Intensity (PDI)

The observations (Table 1) showed that none of the variety or genotype was free or resistant to fruit rot disease. The minimum of 5.90 per cent disease intensity was observed in Vrudheshwar-3 genotype and showed resistant reaction to disease. This genotype was at par with Verul-2 (6.60%) and Karkhel (7.50%) which also showed resistant reaction to fruit rot disease. The next best genotypes were Vrudheshwar-4, Humnabad Local, Vrudheshwar-2, Dhanagarawadi-3, Phule Purandar, Dhanagarawadi-7 and Purandar Local which showed moderately resistant reaction to disease The genotype NMK-1 (Golden) recorded 43.60 per cent disease intensity followed by Hyderabad selection recorded 43.30 per cent disease intensity and were at par with each other and showed highly moderately susceptible reaction to the disease..

Screening of custard apple genotypes under natural field condition against fruit rot disease

Thirty custard apple varieties and genotypes were screened under natural conditions for their resistance to *C. gloeosporioides* at All India Coordinated Research Project on Arid Zone Fruits (Fig & Custard apple), Jadhavwadi, Tal.- Purandar, Distt.- Pune during 2022-23 where the disease pressure remains very high.

The observations (Table 2) showed that none of the variety was immune to fruit rot disease. From the data it is revealed that out of 30 genotypes only six genotypes which found resistant to fruit rot disease, were Vrudheshwar-3, Verul-2, Karkhel, Humnabad Local, Dhanagarwadi-3 and Vrudheshwar-4 where they recorded 4.80, 5.91, 6.42, 8.57, 9.09 and 9.55 per cent disease intensity, respectively. Rest 14 genotypes were found moderately resistant to fruit rot disease.

In present investigation it was observed that the fruit rot intensity under natural field condition was very less. The present investigation the genotypes Vrudheshwar-3, Verul-2, Karkhel, Humnabad Local, Dhanagarwadi-3 and Vrudheshwar-4 recorded resistant reaction to fruit rot disease. Padule and Kaulgud (1993) who studied the reaction of 14 different custard apple varieties to fruit rot caused by *C. gloeosporioides* under natural and field condition and found that four varieties *viz.,* Bullock's heart, Island gem, Pink's mammoth and Annona hybrid No. 2 were free from the disease.

The findings of present investigation are in agreement with the earlier work carried out at All India Coordinated Research Project on Arid Zone Fruits (Fig & Custard apple), Jadhavwadi, Tal.- Purandar, Distt.-Pune during 2020, they observed that out of 72 lines screened against *Colletotrichum* leaf blight of custard apple, none of the line was found free. Atemoya and Jalke Khurd were found moderately resistant while rest of the genotypes were found resistant against the disease (Anonymous, 2020). During 2021, out of 72 lines screened against Colletotrichum leaf blight of custard apple at AICRP-AZF, Jadhavwadi, none of the line was found free. 57 germplasm were found resistant while 15 genotypes were found moderately resistant against the disease at Jadhavwadi (Anonymous, 2022).

Conclusion

The various custard apple genotypes were evaluated under natural field conditions using the attached fruit technique. All 30 genotypes tested were found to be infected by the pathogen *Colletotrichum gloeosporioides*. However, the genotypes Vrudheshwar-3, Verul-2, Karkhel, Vrudheshwar-4, Vrudheshwar-2, Telgaon Local, Humnabad Local, and Dhanagarawadi-3 showed moderate resistance using the attached fruit technique. In this study, it was observed that the intensity of fruit rot under natural field conditions was very low. Of the

Table 1. Reaction of different custard apple genotypes to fruit rot disease caused by *C. gloeosporioides* in Attached Fruit Culture (AFC)

S.No.	Custard apple genotypes	MBIM			MDIT		
		LP (hrs)	PDI (%)	Reaction	LP (hrs)	PDI (%)	Reaction
1	Phule Purandar	90.00	25.40	MS	112.00	16.80	MR
2	Purandar Local	66.00	44.40	HS	109.00	19.00	MR
3	Pimple local	87.25	28.30	MS	99.00	23.10	MS
4	Jalkehurd-1	87.00	28.50	MS	100.00	22.50	MS
5	Jalkehurd-2	77.50	33.00	S	95.50	25.75	MS
6	Vrudheshwar-1	84.00	31.75	S	96.00	24.50	MS
7	Vrudheshwar-2	107.00	18.00	MR	114.00	15.40	MR
8	Vrudheshwar-3	115.00	7.50	R	117.50	5.90	R
9	Vrudheshwar-4	107.00	16.40	MR	114.75	12.80	MR
10	Verul-1	86.00	28.50	MS	104.00	22.00	MS
11	Verul-2	113.00	8.15	R	117.00	6.60	R
12	Karkhel	111.00	12.80	MR	117.00	7.50	R
13	Humnabad Local	105.00	19.20	MR	114.00	13.90	MR
14	Patoda-1	83.00	32.80	S	93.25	26.75	MS
15	Patoda-2	87.50	27.60	MS	96.00	24.50	MS
16	Patoda-3	93.00	25.10	MS	107.50	20.40	MS
17	Jahangirmona	77.00	35.30	S	87.00	32.40	S
18	Dhanagarawadi-3	97.00	19.20	MR	112.00	15.50	MR
19	Dhanagarawadi-4	77.00	35.00	S	89.25	32.20	S
20	Dhanagarawadi-6	84.50	31.60	S	97.00	23.70	MS
21	Dhanagarawadi-7	96.50	23.20	MS	111.00	17.30	MR
22	Telgaon Local	107.00	18.60	MR	98.50	23.30	MS
23	NMK-1 (Golden)	59.00	50.40	HS	74.00	43.60	HS
24	Arka sahan	85.00	30.50	S	93.00	28.50	MS
25	Balanagar	75.00	35.50	S	91.00	30.60	S
26	Raidurga	83.50	32.30	S	94.00	26.30	MS
27	APK1	68.00	36.90	S	76.00	33.20	S
28	Sindhan Local	85.00	30.00	MS	97.50	23.50	MS
29	Hyderabad Selection	61.50	47.75	HS	73.00	43.30	HS
30	TP-7	89.00	26.60	MS	96.00	25.20	MS
	SEm±	1.74	1.35		2.54	0.86	
	CD (0.05)	5.06	3.92		7.40	2.50	
	CV (%)	2.79	6.80		3.60	5.31	

MBIM: Mycelial Bit Inoculation Method MDIT: Micro Droplet Inoculation Technique

Table 2. Screening of custard apple genotypes under natural field condition against fruit rot disease

S.No.	Genotypes	PDI (%)	Reaction	S.No.	Genotypes	PDI (%)	Reaction
1	Phule Purandar	10.92 19.26	MR	16	Patoda-3	15.93 23.49	MR
2	Purandar Local	24.86 29.89	MS	17	Jahangirmona	25.26 30.14	MS
3	Pimple local	16.97 24.29	MR	18	Dhanagarawadi-3	9.09 17.54	R
4	Jalkehurd-1	16.97 24.31	MR	19	Dhanagarawadi-4	24.29 29.45	MS
5	Jalkehurd-2	20.59 26.55	MS	20	Dhanagarawadi-6	18.46 25.43	MR
6	Vrudheshwar-1	20.00 19.39	MR	21	Dhanagarawadi-7	12.79 20.94	MR
7	Vrudheshwar-2	11.03 12.63	MR	22	Telgaon Local	18.70 25.61	MR
8	Vrudheshwar-3	4.8 17.95	R	23	NMK-1 (Golden)	40.00 39.22	S
9	Vrudheshwar-4	9.55 24.44	R	24	Arka sahan	27.03 31.30	MS
10	Verul-1	17.14 14.04	MR	25	Balanagar	29.70 33.00	MS
11	Verul-2	5.91 14.67	R	26	Raidurga	20.00 26.53	MR
12	Karkhel	6.42 17.01	R	27	APK1	26.28 30.80	MS
13	Humnabad Local	8.57 30.14	R	28	Sindhan Local	17.33 24.58	MR
14	Patoda-1	20.00 26.52	MR	29	Hyderabad Selection	35.71 36.66	S
15	Patoda-2	18.75 25.63	MR	30	TP-7	24.44 29.56	MS
			SEm± 0.60 CD (0.05) 1.73 CV (%) 5.37				

Note: Figures in bold faces are arc sin values

HR : Highly resistant (>1.00 %)
R : Resistant (1.00 to 10.00 %)
MR : Moderately resistant (10.01 to 20.00 %)
MS : Moderately susceptible (20.01 to 30.00 %)
S : Susceptible (30.01 to 40.00 %)
HS : Highly susceptible (>40.00 %)

30 genotypes, Vrudheshwar-3, Verul-2, Karkhel, Humnabad Local, Dhanagarwadi-3, and Vrudheshwar-4 demonstrated resistance against fruit rot under natural field conditions.

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