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Comparison of artificial screening methods for evaluation of resistance to *Fusarium* wilt disease of castor (*Ricinus communis* L.)

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Abstract
This study was carried out to compare two artificial screening methods *viz*. Soil Infestation method (SIM) and root dip inoculation technique (RDIT), under glasshouse conditions for the screening of resistant to *Fusarium* wilt. Both the artificial screening methods; SIM and RDIT were statistically similar in respect of wilt incidence. However, the reaction exhibited by the castor genotypes was varied with artificial screening methods. Mean wilt incidence obtained through SIM (53.9%) was higher as compared to RDIT (44.8%). All the genotypes exhibited comparatively higher wilt incidence when screened through SIM as compared to RDIT. Genotype DCS 9 exhibited resistant reaction (15.8 %) when screened through RDIT but was moderately resistant (32.5 %) when screened through SIM. It was concluded that SIM could also be used for screening of castor genotypes for the resistance to *Fusarium* wilt disease. However, lower level of resistance could be evaluated efficiently with saving 8-10 days through RDIT as compared to soil infestation method.

Keywords: Castor, *Fusarium* wilt, Root dip inoculation technique, Screening methods, Soil infestation method.

INTRODUCTION

Castor (*Ricinus communis* L.) is the most important non-edible oilseed crop with immense industrial and commercial values which is widely used as a lubricant in high speed engines and aeroplanes; an important ingredient in manufacture of soaps, printing inks, varnishes, transparent paper, linoleum and plasticizers (Caupin, 1997) and it also a medicinally important oil seed crop (Ganeshkumari *et al.,* 2008; Marwat *et al*., 2017). Castor belonging to family Euphorbiaceae is found across all tropical and sub-tropical regions of the world (Weiss, 2000). Castor has the ability to grow under low rainfall and low fertility conditions and is most suitable for dry land farming. It grows as an indeterminate annual or perennial crop depending on climate and soil types in tropical, sub-tropical and warm temperate regions in the world. It can be grown productively on underutilized marginal uplands. India is the world leader with regards to area, production and productivity. In India, Gujarat is leading castor growing state, contributing around 82 % of total production in the country and has established a virtual monopolistic grip on the international market. The wilt disease caused by *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad is an important seed and soil-borne disease of castor which appears at all crop growth stages and it is more prominent during flowering and spike formation stage. Extent of seed yield loss ranges from 39 to 77 % depending upon the stage of crop (Raoof and Rao, 1999). The disease incidence up to 80% was recorded in Russia (Moshkin, 1986). The losses in yield were realized in all cultivated castor hybrids in Gujarat (Dange *et al*., 1997) and as high as 85 % wilt incidence has been reported under North Gujarat condition (Dange, 2003). Cultivation of resistant castor hybrids and varieties is cheapest and best way to manage *Fusarium* wilt disease and several wilt resistant hybrids and varieties were developed and released. Genetic resistance has one problem that is limited durability of the effectiveness due to genetic adaptation by the pathogen (Niks *et al*., 1993). Screening for disease resistance of available genotypes provides source of disease resistance which is prerequisite to breeding for disease resistance. Important screening techniques to identify *Fusarium* wilt resistance in castor are

wilt sick plot methods for field screening and root dip inoculation technique for artificial screening under glass house conditions (Kumar *et al*., 2015). Present study was carried out to compare two artificial screening methods *i.e.* root dip inoculation technique (RDIT) and soil infestation method (SIM) for evaluation of resistance to *Fusarium* wilt disease of castor.

MATERIALS AND METHODS

The experiment was conducted at Castor and Mustard Research Station, S.D. Agricultural University, Sardarkrushinagar (Gujarat) during 2015-16.

Root dip inoculation technique (RDIT): The technique standardized by Raoof and Rao (1996) and Desai and Dange (2003) was followed with modifications. The pathogen was mass-multiplied on sorghum grain. Sorghum grain was soaked overnight in 2 % sucrose solution and boiled till become soft. After draining excess sucrose solution, boil sorghum grains were filled in conical flask. These flasks were plugged with non-observant cotton and wrapped with butter paper which was followed by sterilization through autoclaving. Inoculation of these flasks was done with 5 mm bits cut from of actively growing colony, under aseptic conditions and incubated in BOD incubator at 28 ± 2 ^{0}C temperature for 12-15 days. Conidia was harvested in sterilised distilled water, concentration of conidia was quantified using haemetocytometer and then its concentration was adjusted at 10^6 conidia/ml suspension by diluting with sterilised distilled water. Castor seedlings of test genotypes were raised on coco pith and coarse sand (1:1 v/v). Seeds of test genotypes were surface sterilized with 2.5 per cent sodium hypochlorite solution for five minutes and then single seed was sown in each well of nursery trays. 10-12 days old seedlings were uprooted, their roots were clipped from distal 1/3rd end. Clipped roots were dipped in conidia suspension for 60 seconds and 10 seedlings were transplanted with maintaining equidistance in the pots filled with sterilized soil and irrigated immediately after transplanting. Two replications of each treatment were maintained. Tap water is used for irrigation in nursery trays and pots as and when needed. After 30 days of transplanting, mean wilt incidence was recorded.

Soil infestation method (SIM): The pathogen was mass-multiplied on coarse sand (90 %) and maize meal (10 %) medium. Both ingredients (900 g coarse sand + 100 g maize meal) and 150 ml water were mixed thoroughly and filled in conical flasks. These flasks were plugged with non-observant cotton and wrapped with butter paper which was followed by sterilization through autoclaving. Inoculation of these flasks was done with 5 mm bits cut from of actively growing colony, under aseptic conditions and incubated in BOD incubator at 28 ± 2 ^{0}C temperature for 20-25 days. After incubation, colonised sand maize meal medium taken out of flasks and dried under shed. Fifty gram of this inoculum was mixed with 1000 gram of sterilized soil thoroughly and filled in pots. Seeds of test genotypes were surface sterilized with 2.5 per cent sodium hypochlorite solution for five minutes. Twenty seeds were sown with maintaining equidistance in each pot and after germination, ten seedlings were maintained in each pot. Two replications of each treatment were maintained. Tap water is used for irrigation as and when needed. Wilt incidence was recorded after 30 days of sowing. Statistical analysis of the data obtained from experiment was done using appropriate programme as per the requirement of the experiment. The critical difference (CD) was calculated at 5% level of significance for comparison of difference between the means of different treatments. Disease progress curves were developed by plotting disease incidence (%) against time. Area under disease progress curve (AUDPC) was calculated by using following formula (Shanner and Finney, 1977):

$$AUDPC = \frac{\left(\frac{D_1+D_2}{2}\times T\right)+\left(\frac{D_2+D_3}{2}\times T\right).....\left(\frac{D_{n-1}+D_n}{2}\times T\right)}{n-1}$$

....Eq. 1

Where,

D = Mean wilt incidence (%) at different time intervals (D_1 D_2 D_3.....D_n)

T = Time interval (days) between two observations

n = Total number of observations

RESULTS AND DISCUSSION

The analysis of data obtained from evaluation of resistance to *Fusarium* wilt disease of castor revealed that both the artificial screening methods; SIM and RDIT were statistically similar (Table 1). However, the reaction castor genotypes (Table 2) exhibited with artificial screening methods was varied. Mean wilt incidence obtained through SIM (53.9 %) was higher as compared to RDIT (44.8 %).

Interaction of screening methods and genotypes was also non-significant. However, all the genotypes exhibited comparatively higher wilt incidence when screened through SIM as compared to RDIT it may be due to the mixing of inoculum with soil so pathogen could infect the plant just after the germination at more tender stage. The reactions of castor genotypes were categorized on the basis of scale presented in Table 2. Genotype DCS 9 exhibited resistant reaction (15.8 %) when screened through RDIT but was moderately resistant (32.5 %) when screened through SIM. Similarly, Genotype DCS 107 exhibited moderately resistant (23.5 %) reaction when screened

Table 1. Comparison of two artificial screening methods *viz*. soil infestation method and root dip inoculation technique for the evaluation of resistance to *Fusarium* wilt of castor.

S. N.	Castor Genotypes	Soil Infestation Method (SIM)		Root Dip Inoculation Technique (RDIT)		Mean
		Plant Stand	Wilt incidence (%)	Plant Stand	Wilt incidence (%)	
1.	Kranti	20	42.5 (40.6)	19	39.5 (38.6)	40.8 (39.6)
2.	DCS 9	20	32.5 (34.7)	19	15.8 (18.6)	23.8 (26.7)
3.	48-1	20	20.0 (26.5)	19	0.0 (4.1)	10.0 (15.3)
4.	JI 35	20	100.0 (85.9)	20	90.0 (74.7)	95.0 (80.3)
5.	DCS 107	18	41.5 (40.0)	17	24.5 (29.6)	33.0 (34.8)
6.	VP 1	19	86.5 (69.5)	20	100.0 (85.9)	93.3 (77.7)
Mean		-	53.8 (49.6)	-	44.8 (41.9)	49.3 (45.7)
CD at 5 %			Methods (A)			ns
			Genotypes (B)			13.7
			A × B			Ns

***** Mean of two replications; Figures in parentheses are angular transformed values.

Table 2. Categorization of castor genotype reaction to *Fusarium* wilt of castor (Mayee and Datar, 1986).

Wilt incidence (%)	Category
0.0	Highly resistance
0.01 - 20.0	Resistant
20.1 - 40.0	Moderately resistant
40.1 - 60.0	Moderately susceptible
60.1 - 80.0	Susceptible
> 80.0	Highly susceptible

through RDIT, but was moderately susceptible (41.7 %) when screened through SIM. Genotype Kranti exhibited moderately resistant (39.5 %) reaction when screened through RDIT but was moderately susceptible (42.5 %) when screened through SIM. Genotype 48-1 exhibited highly resistant (0.0 %) reaction when screened through RDIT, but was resistant (20.0 %) when screened through SIM. Though, both methods were statistically similar but reaction exhibited by the castor genotypes was varied. All the castor genotypes exhibited higher wilt incidence when screened through SIM as compared to RDIT, except VP 1 where lower wilt incidence (86.5 %) was recorded with SIM as compared to RDIT (100.0 %). Wilt disease progress curve prepared for both methods and leg phase between 15 days to 20 days

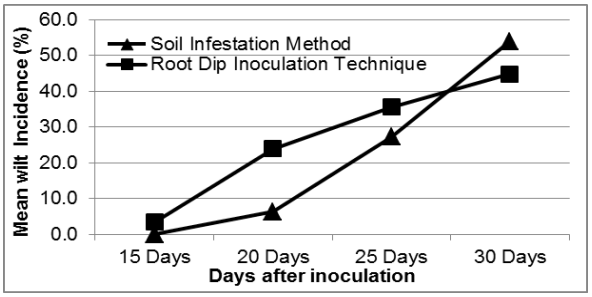

Fig. 1. *Effect of screening methods viz. soil infestation method and root dip inoculation technique on Progress of Fusarium wilt disease on castor.*

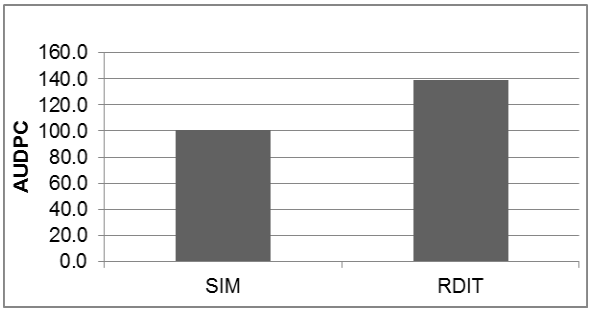

Fig. 2. *Effect of screening methods viz. soil infestation method and root dip inoculation technique on Area Under Disease Progress Curve (AUDPC) of Fusarium wilt disease on castor.*

Table 3. Difference between soil infestation method (SIM) and root dip inoculation technique (RDIT) for the evaluation of resistance to *Fusarium* wilt of castor.

S. N.	Characteristics	Soil infestation Method (SIM)	Root dip inoculation technique (RDIT)
1.	Medium for mass multiplication	Sand + maize meal (9:1 w/w)	Sorghum grains amended with 2 % sucrose solution
2.	Days of mass multiplication	20-25 days	12-15 days
3.	Medium for raising seedling	-	Coco pith and coarse sand (1:1 v/v)
4.	Days taken to raise seedling	-	10-15 days
5.	Dominant Inoculum type	Chlamydospores	Micro and macro conidia
6.	Inoculum load	Not well defined (50 g / kg soil)	Well defined (10^6 conidia /ml)
7.	Time of Inoculation (For each seedling)	Not uniform	Uniform
8.	Days of disease appearance	After 18 days of sowing	After 10 days of inoculation
9.	Progress of disease	Very fast	fast
10.	Days of final observation	30 DAS	30 DAT
11.	Total days required for evaluation	50-55 days	42-45 days

after inoculation was noticed with SIM which was followed by log phase (20 days to 30 days after inoculation) whereas with RDIT, a extended log phase (15 days to 30 days after inoculation was observed (Figure 1). Area under disease progress curve (AUDPC) calculate with SIM (100.8 units) was lower (Figure 2) as compared to RDIT (139.4 units).

Differences between both methods *viz*. soil infestation method (SIM) and root dip inoculation technique (RDIT) are presented in Table 3.

It was noted during mass multiplication of wilt pathogen that on sorghum grains, mostly micro and macro conidia were produced, whereas on sand maize meal medium mostly chlamydospores were produced. This fact could explain the late appearance of *Fusarium* wilt disease with SIM as compared to RDIT. Concentration of inoculum was well defined (10^6 conidia /ml) and uniform on each seedling in RDIT. In RDIT healthy seedling of each genotype was raised in advance and then inoculation of 10-15 days old seedlings was performed by root dip inoculation technique which was followed by transplanting, whereas in SIM there is chance of infection of each seedling just after germination at more tender stage because inoculum is mixed directly into the soil. This could be explain the higher incidence and very fast progress (Figure 1) of *Fusarium* wilt disease recorded with SIM. Raoof and Rao (1996) concluded that by RDIT, lowest form of resistance can be expressed which may be exploited in the breeding program for incorporating this into a high yielding variety. Advanced breeding lines screened at field level in wilt sick plot are simultaneously screened using RDIT for confirmation for wilt resistance for effective screening of castor genotypes. This study is in accordance with finding of Kumar *et al*., (2015) in which they concluded that root dip inoculation technique is also useful in the study of pathogenic variability among *F. oxysporum* f. sp. *ricini* isolates.

Conclusion

It may be concluded that SIM could also be used as alternative of RDIT for screening of castor genotypes for the resistance to *Fusarium* wilt disease. However, the lower level of resistance could be evaluated efficiently with saving 8-10 days through RDIT as compared to soil infestation method.

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