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# Development of Fusarium wilt-resistant genotypes in safflower (*Carthamus tinctorius*)

R. Kalpana Sastry and C. Chattopadhyay*
*Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500030, Andhra Pradesh, India;*
**Author for correspondence (Plant Pathology Section, National Research Center on Rapeseed-Mustard, Sewar, Bharatpur, Rajasthan 321303, India; Phone: +5644 260379; Fax: +5644 260419; E-mail: chirantan_cha@hotmail.com)*

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## Abstract

Screening of 51 promising safflower germplasm lines in Fusarium wilt-infested plots resulted in identification of highly wilt-resistant selections viz., 86-93-36A, 237550, VI-92-4-2 and II-13-2A, with some moderate resistance in HUS-305. Progenies from crosses made using these resistant lines were tested for their reaction to wilt. $F_1$ progenies from 86-93-36A × 237550 and 86-93-36A × II-13-2A recorded zero wilt incidence, while 237550 × 86-93-36A was highly resistant to the Rajendranagar geographical isolate. The reaction for the three progenies showed stability for wilt resistance with no segregation until the $F_7$ generation. Geographical isolates of *Fusarium oxysporum* f. sp. *carthami* (*Foc*) were collected from different safflower growing regions and tested for their pathogenic variability on six host differentials under glasshouse conditions. Based on the reaction of the differentials, the *Foc* isolates were grouped into four biotypes. The three resistant progenies were tested for their reaction to the four biotypes. The progeny of cross 86-93-36A × 237550 showed an immune reaction to all the biotypes, except for a highly resistant reaction to biotype 3. The progenies of the two other crosses (86-93-36A × II-13-2A and 237550 × 86-93-36A) exhibited immune reactions to biotypes 2, 3 and 1, 3, respectively, and were highly to moderately resistant to biotypes 1, 4 and 2, 4, respectively.

## Introduction

Safflower (*Carthamus tinctorius*) is one of the major post-rainy season oilseed crops cultivated in the Deccan Plateau region of India. Wilt of safflower caused by *Fusarium oxysporum* Schlecht. f. sp. *carthami* Klisiewicz and Houston (*Foc*) has assumed increased economic importance in recent years with high incidence of the disease being reported in India than the other areas under the crop in the world (Kalpana Sastry, 1996), Egypt (Zayed et al., 1980) and the USA (Klisiewicz and Houston, 1962). The disease is endemic in the Indian Deccan Plateau (17°–20°E and 70°–74°N) with reports of up to 80% incidence resulting in heavy yield losses (Kalpana Sastry, 1996). Use of wilt-resistant cultivars is the most efficient strategy for sustainable safflower cultivation (Kalpana Sastry and Ramachandram, 1992). However, all the safflower cultivars recommended for commercial production in the country lack resistance to safflower wilt. To fulfill this need, efforts were made to develop wilt-resistant safflower genotypes. This paper also reports on the pathogenic variability of the *Foc* population from different safflower growing areas of India and the reaction of the developed genotypes to the different biotypes of the pathogen under glasshouse conditions.

## Materials and methods

For testing the reaction of germplasm lines to the disease, a Fusarium wilt-infested plot measuring

40 m × 25 m was developed at the experimental farm of the Directorate of Oilseeds Research, Hyderabad. The plot had sandy loam soil, moderate organic carbon content and a pH of 8.0. Chopped pieces of diseased plants and adequate quantities of inoculum of *Foc*, multiplied on autoclaved sorghum seed meal (Kalpana Sastry and Chattopadhyay, 1999), were incorporated to infest the plot with wilt.

Soil samples collected randomly from different parts of the plot were air-dried, homogenized and evenly sprinkled on each Petri plate containing modified Czapek Dox agar medium (Kalpana Sastry et al., 1994) for enumeration of the *Foc* population in soil (Kalpana Sastry and Chattopadhyay, 1999). Counts of colonies of *Foc* were taken on the fourth day and the Poisson distribution of the pathogen across the plot was estimated.

The test material, comprising 51 germplasm lines including five check varieties (Bhima, HUS-305, Tara, A-I and APRR-3), were sown in 5 m row lengths in a randomized block design with two replications in the infested plot (40 m × 25 m) with a spacing of 45 cm × 20 cm. Two susceptible checks, Tara and Manjira, were sown after every four rows of test lines within each replication. The crop was raised according to recommended cultural practices under irrigation (DOR, 1995). Percent disease incidence (PDI) was recorded in each test line wherein the disease was confirmed by isolation of the causal pathogen from randomly selected diseased plants. In accordance with the scale proposed by Mayee and Datar (1986), based on mortality to wilt, the lines were grouped into five categories: immune (PDI: 0–1), highly resistant (PDI: 2–10), moderately resistant (PDI: 11–20), moderately susceptible (PDI: 21–50) and highly susceptible (PDI: ≥51).

The immune/resistant lines 86-93-36A, 237550, VI-92-4-2, II-13-2A and HUS-305 were taken up for the crossing programme with the objective of developing wilt-resistant genotypes. Each of the lines was crossed with one another using them both as female and male and the successful crosses are detailed in the first column of the Table 2.

Fifteen isolates of *Foc*, apart from the one from Rajendranagar, were collected from different safflower growing areas of India (Table 3). The isolates of *Foc* were individually multiplied on autoclaved sorghum seed meal medium and mixed at 3.2/100 g autoclaved soil filled in 30 cm diameter earthen pots inside a glasshouse. The set of host differentials were sown 3 days after mixing the inoculum with the soil and filling of the pots. Reaction of the host differentials was recorded as resistant or susceptible.

Since the new safflower genotypes need to be successful for their wilt-resistant characteristic wherever they are used for safflower cultivation, the resistant progenies were tested for their reaction to the four biotypes. The data were analysed using ANOVA.

## Results and discussion

High PDI in the susceptible checks Tara and Manjira in all parts of the plot and Poisson distribution of the pathogen inoculum therein confirmed the uniform infestation of the plot and its effectiveness for screening the germplasm lines for resistance to the wilt disease. The pathogen population in soil in the infested plot was $35 \times 10^3$ colony forming units of *Foc*/g soil. Line 86-93-36A revealed an immune reaction in both the years with 237550, VI-92-4-2 and II-13-2A also highly resistant (Table 1). Lines 86-93-16A, 86-93-20A, HUS-3128 and HUS-305 were

*Table 1*. Performance of safflower germplasm lines in wilt-induced plot at Rajendranagar

| Germplasm line | Percent wilt incidence* | |
|---|---|---|
| | 1990–91 | 1991–92 |
| 86-93-36A | 4.0 (0.0) | 4.0 (0.0) |
| VI-92-4-2 | 12.9 (5.0) | 4.0 (0.0) |
| 237550 | 12.2 (4.5) | 9.5 (2.7) |
| II-13-2A | 11.8 (4.2) | 14.5 (6.3) |
| 86-93-16A | 22.4 (14.5) | 20.9 (12.7) |
| 86-93-20A | 21.7 (13.7) | 24.9 (17.8) |
| HUS-3128 | 23.9 (16.4) | 23.9 (16.4) |
| HUS-305 | 26.5 (19.9) | 27.6 (21.4) |
| HUS-3182 | 24.1 (16.7) | 34.6 (32.3) |
| A-1 | 40.8 (42.7) | 27.6 (21.4) |
| Bhima | 40.8 (42.7) | 36.9 (36.0) |
| HUS-3159 | 40.7 (42.6) | 29.2 (23.8) |
| HUS-3179 | 40.0 (41.3) | 39.8 (40.9) |
| 398-9 | 33.0 (29.7) | 34.9 (32.7) |
| Manjira | 36.1 (34.7) | 34.9 (32.7) |
| II-14-1A | 31.3 (27.0) | 36.3 (35.0) |
| HUS-3165 | 52.1 (62.2) | 49.0 (56.9) |
| APRR-3 | 50.1 (58.9) | 34.3 (31.7) |
| Tara | 58.4 (72.5) | 72.8 (91.3) |
| L.S.D. ($P < 0.05$) | 9.2 | 6.1 |

Figures in parentheses are actual percent disease incidence and others are arc sin transformed values.
*Mean of two replications.

moderately resistant. Similar reports of resistance to Fusarium wilt in safflower have been made earlier (Klisiewicz, 1980). The rest of the lines showed moderately to highly susceptible reactions. These highly resistant lines, and HUS-305 among the moderately resistant ones, were not significantly different ($P < 0.05$) in seed yield, test weight of seed and oil content from the national checks Bhima and A-1, which are traditionally cultivated and occupy large areas under safflower cultivation (Kalpana Sastry and Chattopadhyay, 1997). So 86-93-36A, 237550, VI-92-4-2, II-13-2A and HUS-305 were used for the crossing programme for developing wilt-resistant genotypes.

$F_1$ progenies from 86-93-36A × 237550 and 86-93-36A × II-13-2A recorded no incidence of wilt while the cross 237550 × 86-93-36A gave a highly resistant reaction to the Rajendranagar isolate (Table 2). Hence, 86-93-36A (immune) when crossed with 237550 and II-13-2A (highly resistant) resulted in the $F_1$ progenies also being immune. The reciprocal cross between 237550 and 86-93-36A also exhibited the wilt-resistant characteristic, but 86-93-36A performed better as a female parent than as the male one. The reciprocal differences observed in the performance of the cross made between parents 86-93-36A and 237550 for the wilt incidence indicated the influence of cytoplasm of the maternal parent in conferring wilt resistance. The other progenies were moderately resistant. Similar results were also observed in flax when a similar method of crossing was followed to breed for Fusarium wilt resistance (Luchina, 1979). However, Bockelman (1974) reported involvement of one major and one minor gene for conferring resistance to Fusarium wilt in safflower. Singh et al. (2001) indicated the role of inhibitory gene action in the expression of wilt resistance in safflower, where entry 237550, which is included in the present study, was used as one of the wilt-resistant parents in the investigation of inheritance of wilt resistance. The reaction of the two immune progenies and the one highly resistant to the Rajendranagar isolate showed stability for wilt resistance when grown in the same wilt-infested plot with no segregation for wilt resistance till $F_7$ generation. That the two immune (86-93-36A × 237550 and 86-93-36A × II-13-2A) and one highly resistant (237550 × 86-93-36A) progenies showed the same respective reactions till the $F_7$ generation in the wilt-infested plot at Rajendranagar was evidence for the resistance being stable and durable.

*Table 2*. Wilt incidence in progenies from crosses of highly resistant safflower lines

| Entry | Percent wilt incidence* |
|---|---|
| 86-93-36A × 237550 | 4.0 (0.0) |
| 86-93-36A × II-13-2A | 4.0 (0.0) |
| 237550 × 86-93-36A | 7.7 (1.8) |
| II-13-2A × 86-93-36A | 19.4 (11.0) |
| VI-92-4-2 × HUS-305 | 20.3 (12.0) |
| HUS-305 × II-13-2A | 22.6 (14.8) |
| II-13-2A × HUS-305 | 24.3 (17.0) |
| II-13-2A | 14.5 (6.3) |
| 86-93-36A | 4.0 (0.0) |
| 237550 | 16.2 (7.8) |
| VI-92-4-2 | 17.0 (8.6) |
| HUS-305 | 26.5 (19.9) |
| A-1 | 33.9 (31.1) |
| Tara | 73.3 (91.7) |
| L.S.D. ($P < 0.05$) | 2.3 |

Figures in parentheses are actual percent disease incidence and others are arc sin transformed values.

*Mean of three replications.

In the studies on host differentials, Tara, Manjira and A-1 were susceptible whilst 86-93-36A was resistant to all the geographical isolates (Table 3). Differentiation of the isolates was made possible through the reactions of II-13-2A and VB-75-4. Accordingly the isolates were grouped into four biotypes (Table 3). This confirmed the variability in the *Foc* population in different parts of the safflower growing areas. Such variability in population of *Foc* has been reported earlier (Chakrabarti, 1979).

The progeny from 86-93-36A × 237550 showed an immune reaction to all the biotypes except for a highly resistant reaction shown to biotype 3 (Table 4). Two other progenies, 86-93-36A × II-13-2A and 237550 × 86-93-36A showed immune reaction to biotypes 2, 3 and 1, 3, respectively, and were moderately resistant to biotypes 1, 4 and 2, 4, respectively. This test of the reaction of different genotypes to pathogenic biotypes provided essential information about the level of sustainability of each genotype regarding wilt resistance. Depending on the presence and absence of biotypes at a particular location, the appropriate resistant genotypes can be suitably adopted. The use of stable and durable resistance to Fusarium wilt, preferably against more pathogenic biotypes combined with high yields may be a very effective strategy for sustainable

*Table 3*. Reaction of host differentials to geographical isolates of *Fusarium oxysporum* f. sp. *carthami*

| Isolate[1] | Reaction of host differentials[2] | | | | | |
|---|---|---|---|---|---|---|
| | Tara | Manjira | A-1 | II-13-2A | VB-75-4 | 86-93-36A |
| Rajendranagar (Andhra Pradesh) | S | S | S | R | R | R |
| Phaltan (Maharashtra) | S | S | S | R | R | R |
| Hingoli (Maharashtra) | S | S | S | R | S | R |
| Patancheru (Andhra Pradesh) | S | S | S | S | R | R |
| Solapur (Maharashtra) | S | S | S | S | S | R |
| Tandur (Andhra Pradesh) | S | S | S | R | R | R |
| Devalgaon (Maharashtra) | S | S | S | S | R | R |
| Jalna (Maharashtra) | S | S | S | S | R | R |
| Jalgaon (Maharashtra) | S | S | S | S | R | R |
| Annigeri (Karnataka) | S | S | S | S | S | R |
| Indore (Madhya Pradesh) | S | S | S | S | S | R |
| Latur (Maharashtra) | S | S | S | R | R | R |
| Akola (Maharashtra) | S | S | S | R | S | R |
| Buldana (Maharashtra) | S | S | S | R | S | R |
| Parbhani (Maharashtra) | S | S | S | R | R | R |
| Arnej (Gujarat) | S | S | S | S | S | R |

| *Biotype* | *Isolates* |
|---|---|
| 1 | Rajendranagar, Tandur, Phaltan, Latur, Parbhani |
| 2 | Hingoli, Akola, Buldana |
| 3 | Patancheru, Devalgaon, Jalna, Jalgaon |
| 4 | Solapur, Indore, Annigeri, Arnej |

[1]Names of places wherefrom the isolates were collected with names of states within parentheses.
[2]S: susceptible; R: resistant.

*Table 4*. Reaction of promising safflower genotypes to *Fusarium oxysporum* f. sp. *carthami* biotypes

| Genotypes | Percent wilt incidence by biotype* | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 86-93-36A × 237550 | 4.0 (0.0) | 4.0 (0.0) | 14.9 (6.7) | 4.0 (0.0) |
| 86-93-36A × II-13-2A | 26.6 (20.0) | 4.0 (0.0) | 4.0 (0.0) | 26.6 (20.0) |
| 237550 × 86-93-36A | 4.0 (0.0) | 21.4 (13.3) | 4.0 (0.0) | 31.1 (26.7) |
| 86-93-36A | 4.0 (0.0) | 21.4 (13.3) | 4.0 (0.0) | 35.2 (33.3) |
| 237550 | 4.0 (0.0) | 4.0 (0.0) | 35.2 (33.3) | 21.4 (13.3) |
| II-13-2A | 35.2 (33.3) | 26.6 (20.0) | 4.0 (0.0) | 35.2 (33.3) |
| Tara | 90.0 (100.0) | 90.0 (100.0) | 90.0 (100.0) | 90.0 (100.0) |
| L.S.D. ($P < 0.05$) genotype × biotype: 9.9 | | | | |

Figures in parentheses are actual percent wilt incidence and others are arc sin transformed values.
*Mean of four replications.

safflower cultivation. This study provides clues for future research on the pattern of inheritance of wilt resistance in safflower.

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