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Alternaria Disease Screening in Safflower (*Carthamus tinctorius* L.)

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ABSTRACT

A collection of 150 safflower germplasm accessions was evaluated to study *Alternaria* disease reaction under both natural and laboratory conditions. Natural disease screening was carried out during *rabi* 2011-12 at Agricultural Research Station, Annigeri, UAS, Dharwad. Based on intensity of disease scoring GMU 3621, GMU 3636 and GMU 3748 were shown susceptible reaction with the disease intensity of 20-50 *per cent* and remaining all germplasms along with checks were classified under highly susceptible group with more than 50 per cent disease intensity. Leaf detached laboratory assay was done to compare seed yield with disease reaction, and these results revealed that no immune reaction for the *Alternaria* disease among the accessions.

Key words *Alternaria Disease Screening in Safflower (Carthamus tinctorius L.)*

Safflower (*Carthamus tinctorius* L.) is one of the oldest oilseed crops. India is the largest producer of safflower with 54 % area and 40 % production in the world. In India 98 % of the area comes from three states *viz.*, Maharashtra, Karnataka and Andhra Pradesh. In India, It is cultivated in an area of 1.5 lakh ha with a production of 1.13 lakh tons giving a productivity of 726 kg/ha. Maharashtra and Karnataka are the first and second with reference to area and production, respectively whereas, productivity is highest in West Bengal (1000 kg/ha) followed by Bihar (805 kg/ha) and Karnataka (719 kg/ha) (Indiastat, 2014).

The major constraints for its production are abiotic stress. Among abiotic stress this crop mainly affected by disease and pest. The disease survey indicates a major occurrence of leaf spot diseases caused by *Alternaria* or *Cercospora* (Deokar *et al*., 1999). The disease ranged from 45 to 80 % affected leaf area (Mirza *et al.,* 1985) and the losses in yield were estimated to be 10 to 25% due to *Alternaria* leaf spot (Indi *et al*., 1986). Although a number of chemical measures have been developed for the control of diseases, the cultivation of resistant varieties would always be safer and cheaper than chemical control (Ray and Basuchoudhary, 1984). Therefore, Efforts were made to evaluate germplasm accessions generated at the centre for their reaction to aphids and leaf spot diseases.

Breeding safflower for disease resistance is the most economical and convenient method for controlling major diseases. Mundel and Huang, 2003 described in detail how to control major diseases of safflower by breeding and using cultural practices. Though germplasm lines or cultivars showing partial or full resistance to some of the major diseases have been identified, the availability of genetic resistance is rare.

MATERIAL AND METHODS

The experiment was carried out at Agricultural Research Station, Annigeri, University of Agricultural Sciences, Dharwad during *rabi* 2011-12. The research station is situated in the northern dry zone of Karnataka between 15°8'N latitude, 75°3'E longitude and at an altitude of 624.80 meters above the mean sea level. The experimental material for the present study comprised of 150 safflower germplasm accessions obtained from the Germplasm Unit of the Directorate of Oilseeds Research, Hyderabad. Disease screening for *Alternaria* was done under field as well as laboratory technique (Accessions listed in Table 1). Methodology as followed as below.

Scale	Description	Category
0	No symptoms on the leaf	Immune
1	Small, round brown spots covering 1% or less of the leaf Area	Resistant
3	Brown sunken spots covering 1-10% of the leaf area	Partially resistant
5	Brown spots enlarging to form circular spots covering 10-20% of the leaf area	Partially susceptible
7	Circular, brown, sunken spots covering 21-50% of the leaf area	Susceptible
9	Circular to irregular, brown sunken spots covering 51% or more of the leaf area	Highly susceptible

Table 1. List of 35 safflower accessions including checks used for laboratory screening against *Alternaria* disease

Seed weight/plant (g)		Seed yield/ net plot (g)		Checks	Field scored susceptible accessions
High	Low	High	Low		
GMU 3770	GMU 3733	GMU 3663	GMU 3720	A-1	GMU 3648
GMU 3678	GMU 3664	GMU 3667	GMU 3647	A-2	GMU 3705
GMU 3672	GMU 3759			Manjira	GMU 3754
GMU 3662	GMU 3766			JSF-1	GMU 3783
GMU 3638	GMU 3684			Bhima	GMU 3797
GMU 3769	GMU 3741			NARI-6	
GMU 3726	GMU 3791			HUS-305	
GMU 3673	GMU 3764				
GMU 3705	GMU 3757				
GMU 3772					

Alternaria leaf spot scoring under field conditions

Five plants in each genotype were randomly selected and using 0-9 scale, plants were grouped based on per cent leaf area affected for *Alternaria* leaf spot.

Detached leaf bio-assay

The typical technique of screening for resistant germplasm under field conditions depends on favourable environmental conditions. To bypass these inherent difficulties in conducting field tests for resistance, Yates *et al.* (1996) developed a detached leaf method for assessing cultivar resistance to pecan scab. This method has the advantage of a standardized test environment in which the inoculum is being conducted, facilitating reproducibility between tests. This protocol was modified to evaluate fungal potential for screening germplasm.

In this experiment, thirty seven genotypes which included high and low yield accessions already evaluated under field conditions, including checks, were sown under glass house conditions. From 45 days old plants, leaves were detached and tested under laboratory conditions. Scoring was done based on presence or absence of symptoms on leaves. The following procedure was followed.

Collection of the diseased leaf material and isolation of the pathogen: Sample showing clear symptoms of the disease was used for isolation of the pathogen and paper bags were used for collection, storage and transportation to avoid sporophytic growth on the diseased samples. The tissue was disinfected or surface sterilized in 10 per cent clorex (0.5%) solution for two minutes. Thereafter, it was washed thoroughly using sterilized distilled water. Then fresh leaf spot together with healthy tissue were cut in to small sections with a sterilized knife and kept on sterile paper towels to remove the excess water. These sections were transferred in sterilized culture plates (9 mm diameter) of nutrient medium under aseptic condition under laminar flow and incubated at 25-30^{0}C for 7 days. The pathogens grew out of the host tissue and sub culturing was done. Again this culture was incubated for 7 days to sporulate the fungus. This fungus culture was tested under microscope for the confirmation of sporulation and serial dilution was made under laminar flow (10^{-1} to 10^{-6}). Optimum sporulated dilution was used for the bio-assay test.

Procedure for leaf detached bio-assay: From 45 day's old seedlings, leaves were detached with petiole and leaf surface was disinfected with 0.01 % sodium hypochloride solution. Two leaves per accession were taken with two replications and were placed on petri plates having moistened paper. Then, fungal spore suspension of about 10µl was placed on the each leaf as a single drop. To ensure favourable environment for the pathogen development moist cotton swab dipped in 0.2 % carbendazim solution was also kept besides the leaves. These samples were kept under room temperature and observed for the symptom development on the leaves after three and five day's interval from the date of inoculation. Then these

Table 2. Disease reaction of 150 safflower accessions with 7 checks to *Alternaria* leaf spot disease at ARS, Annigeri during *Rabi* 2011-12.

Sl No.	Disease reaction	Disease grade	Number of accessions	Accession list
1	Resistant (0 per cent)	1	00	NONE
2	Moderately resistant (0 – 10 per cent)	3	00	NONE
3	Moderately susceptible (10 – 20 per cent)	5	00	NONE
4	Susceptible (20 – 50 per cent)	7	3	GMU 3621, GMU 3636,GMU 3748
5	Highly susceptible (more than 50 per cent)	9	154	GMU 3623,GMU 3624, GMU 3625, GMU 3626, GMU 3628, GMU 3631, GMU 3632, GMU 3633, GMU 3634, GMU 3635, GMU 3637, GMU 3638, GMU 3639, GMU 3640, GMU 3641, GMU 3642, GMU 3643, GMU 3644, GMU 3645, GMU 3646, GMU 3647, GMU 3649, GMU 3650, GMU 3651, GMU 3652, GMU 3653, GMU 3654, GMU 3656, GMU 3658, GMU 3659, GMU 3660, GMU 3661, GMU 3662, GMU 3663, GMU 3664, GMU 3666, GMU 3667, GMU 3668, GMU 3670, GMU 3671, GMU 3672, GMU 3673, GMU 3674, GMU 3675, GMU 3676, GMU 3678, GMU 3679, GMU 3680, GMU 3681, GMU 3682, GMU 3683, GMU 3684, GMU 3685, GMU 3686, GMU 3687, GMU 3689, GMU 3690, GMU 3691, GMU 3692, GMU 3693, GMU 3694, EC 523367, GMU 3696, GMU 3698, GMU 3699, GMU 3700, GMU 3701, GMU 3702, GMU 3703, GMU 3704, GMU 3705, GMU 3706, GMU 3708, GMU 3709, GMU 3711, GMU 3713, GMU 3715, GMU 3716, GMU 3718, GMU 3719, GMU 3720, GMU 3721, GMU 3722, GMU 3723, GMU 3725, GMU 3726, GMU 3737, GMU 3729, GMU 3730, GMU 3731, GMU 3733, GMU 3734, GMU 3735, GMU 3736, GMU 3737, GMU 3738, GMU3739, GMU 3740, GMU 3741, GMU 3743, GMU 3744, GMU 3745, GMU 3746, GMU 3747, GMU 3748, GMU 3749, GMU 3750, EC 523373, GMU 3752, GMU 3753, GMU 3754, GMU 3756, GMU 3757, GMU 3759, GMU 3760, GMU 3761, GMU 3762, GMU 3764, GMU 3765, GMU 3766, GMU 3768, GMU 3769, GMU 3770, GMU 3771, GMU 3772, GMU 3773, GMU 3774, GMU 3775, GMU 3776, GMU 3777, GMU 3778, GMU 3780, GMU 3780, GMU 3781, GMU 3782, GMU 3783, GMU 3786, GMU 3787, GMU 3789, GMU 3790, GMU 3791, GMU 3792, GMU 3793, GMU 3794, GMU 3796, GMU 3798, HUS-105, A-1, Manjira, JSF-1, Bhima

accessions were categorized as resistant or susceptible based on the appearance of disease symptoms or not, respectively.

RESULTS AND DISCUSSION

The occurrence of natural epiphytotics of *Alternaria* significantly reduce seed yield in safflower. It is one trait which requires attention especially in the current scenario of climatic uncertainty. Literature reveals that resistance to *Alternaria* leaf spot is genetically controlled by monogenic recessive gene and sources of resistance are available only in wild species because of the histological nature of epidermal tissues in those species. However, in the present investigation 150 germplasm accessions were screened for the identification of resistance or even tolerance for the disease.

Screening under field conditions: The incidence of *Alternaria* leaf spot was observed in the field under natural conditions and the results are presented in Table 2. Accessions were grouped based on 0 to 9 disease rating scale. Three accessions showed susceptible reaction with grade 7, remaining accessions showed highly susceptible reaction with 9 grade and there is no resistance or tolerance in any entry. It is already confirmed by earlier studies of Heatonand Lisiewicz, 1980, Desai

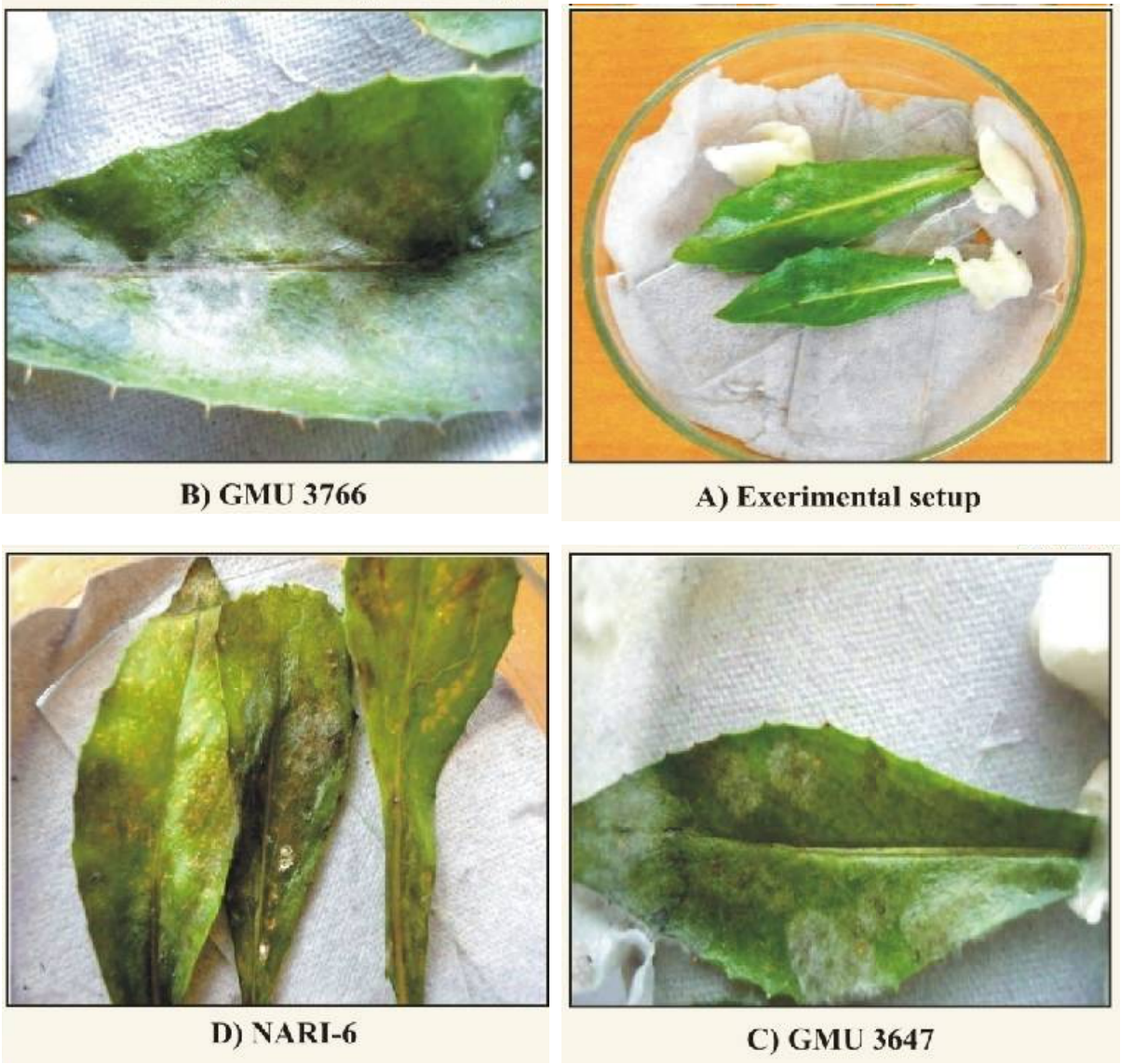

Fig. 1. Leaf detachment technique showing *Alternaria* disease symptoms on accessions

and Radder, 1984, Mirza *et al.,* 1985, Silva *et al.,* 1986, Deokar *et al.,* 1999, Gadekar and Jambhale, 2002, Madhavi *et al.,* 2005, Prasad and Anjani, 2008, Gud *et al.,* 2008, Murumkar *et al.,* 2009, Gerald, 2010 and Xu *et al.,* 2011 that there is no resistance available in the cultivated genotypes of safflower.

Screening under laboratory conditions: Thirty five accessions which were already evaluated under field conditions including checks were evaluated by detached leaf bio-assay. Scoring was done based on the development of symptoms on leaves. All accessions again showed complete susceptibility to *Alternaria* (Plate 1) revealing its pernicious nature. These findings were also reported by Prasad and Anjani, 2008.

Safflower is seriously affected by the *Alternaria* leaf spot disease leading to severe yield loss. An attempt was made to screen and evaluate the germplasm accessions both under natural field and laboratory conditions. Screening for *Alternaria* disease revealed no resistance in the accessions studied

LITERATURE CITED

Deokar, C. D., Mehtre, S. P., Deshmukh, D. P. and Akashe, V. B., 1999, *Alternaria* leaf spot resistance in exotic and indigenous safflower germplasm. *Sesame and Safflower Newslett.,* **14** : 114-116.

Desai, S. A. and Radder, G. D., 1984, Relative performance of sunflower and safflower varieties with reference to date of planting and disease incidence. *Plant Pathology Newslettr.*, **2 :** 12

Gadekar, D. A. and Jambhale, N. D., 2002, Inheritance of seedling and adult plant resistance to *Alternaria* leaf spot in safflower [*Carthamus tinctorius* (L.)]. *Indian J. Genet, Pl. Breed*., **62**(3) : 238-239.

Gerald J. S., 2010, Utilization of wild *Helianthus* species in breeding for disease resistance. *Intl. Symp. of Sunflower Breeding on Resistance to Disease.,* pp 36-51.

Gud, M. A. Murumkar, D. R. Shinde S. K. and Kadam, J. R., 2008 Correlation of weather parameters with development of leaf spot of safflower caused by *Alternaria carthami*. 7th *Intl. Safflower Conf.,* Wagga Wagga, Australia**.**

Indi, D.V., Lukade, G.M. and Patil, P.S. 1986. Influence of *Alternaria* leaf spot (*Alternaria carthami* Chowdhary) on growth and yield of safflower. *Curr. Res. Rep.* 2(1): 137-139.

Heaton, T. C. and Lisiewicz M. J., 1980, A disease-resistant safflower alloploid from *Carthamus tinctorius (*L.*)* x *C. Lanatus(* L.*) Can. J. Plant Sci.,* **61** : 219-224.

Madhavi, K. J., Sujatha, M., Reddy, R. D. and Chandra Rao, S., 2005, Culture characteristics and histological changes in leaf tissues of cultivated and wild sunflowers infected with *Alternaria helianthi. Helia*, 28, **43** : 1-12.

Mirza, M. S., Beg, M. A., and Ali, N., 1985, Succeptibility of safflower genotypes to *Alternaria*leaf spot disease. *Pakistan J. Agric. Res.,* **6**(2) : 123-127

Mundel, H. H. and Huang, H. C., 2003, Control of major diseases of safflower by breeding for resistance and using cultural practices. *In Advances In Plant Disease Management.,* **21 :** 562-568.

Murumkar, D. R., Indi, D. V., Akashe, V. B., Patil. and Gud., 2009, Multiple resistance sources against major diseases and pests of safflower. *J. Oilseeds Rec.,* **26**(2) : 175-176.

Prasad, R. D. and Anjani, K., 2008, Sources of resistance to Alternaria leaf spot among *Carthamus* wild species, *7th Intl. Safflower Conf.*, Wagga Wagga Australia.

Ray, S and Basuchoudhary, K. C., 1984, Field evaluation of some improved lines of safflower to leaf blight caused by *A. carthami* at Varanasi. *J.Oilseeds Res.,* **1:** 91-94.

Silva, H. N. and Gordon, I. L., 1986, Susceptibility of safflower lines to head rot and leaf spot disease : New Zealand. *J. Exper. Agril.,* **14** (4) : 469-472.

Xu, G., Liu,Y., Chen, S. and Chen, F., 2011, Potential structural and biochemical mechanisms of compositae wild species resistance to *Alternaria tenussiame*. *Mol.General Genet.,* **253:**427-433.

Yates, I. E., Maxey, D., Sparks, D. and Reilly, C. C., 1996, Developing the pecon scab fungus on susceptible and resistant host and non host leaves. *J. Amer. Soc. Hort. Sci.,* **121**:350-357.

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