Original Research Article

Screening of China aster [*Callistephus chinensis* (L.)] genotypes and F_1 hybrids against Alternaria leaf spot disease

ABSTRACT

Leaf spot disease caused by *Alternaria alternata* (Fr.) Keissler is a threat of China aster cultivation and is capable of causing yield losses in all production regions. The experiment was undertaken to screen genotypes and F_1 hybrids which would be helpful in developing Alternaria leaf spot resistance varieties in later years. AAC-1 was found to be resistant against *Alternaria* leaf spot in both field and control conditions. In natural disease pressure, Arka Kamini, Arka Shashank and Arka Poornima recorded to be moderately susceptible, susceptible and highly susceptible to *Alternaria* leaf spot, respectively. However, in artificially inoculated condition, Arka Kamini showed susceptible reaction. Among F_1 hybrids, AAC-1 x Arka Kamini and Arka Kamini x AAC-1 showed moderately resistant reaction; AAC-1 x Arka Poornima, AAC-1 x Arka Shashank, Arka Poornima x AAC-1, Arka Shashank x AAC-1 showed moderately susceptible disease reaction for *Alternaria* leaf spot.

Keywords: *Alternaria leaf spot, China aster, Screening, Resistance, Percent Disease Intensity*

1. INTRODUCTION

China aster [*Callistephus chinensis* (L.) Nees.] is an annual, semi hardy crop; cultivated all around the world for its free blooming flower, native of Northern China, a diploid plant (2n=18) and a member of family Asteraceae (Navalinskien *et al*., 2005). In India, it is grown by small and marginal farmers in Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra and West Bengal. The increasing popularity of this crop in major cities of India has led to its cultivation on a commercial scale. It is cultivated as a cut and loose flower both in field and under protection and is also for garden landscaping and used as a potted plant (Hay *et al*., 1976). It is grown successfully in open condition in *kharif*, *rabi* and summer season for year-round production and for a steady supply of flowers to the market. Unfortunately, heavy losses due to diseases, Alternaria leaf spot, Fusarium wilt and Aster yellow diseases wiped out many aster flower production businesses.

Leaf spot disease caused by *Alternaria alternata* (Fr.) Keissler is one of the major potential threats of China aster cultivation which is more prevalent in field and is capable of causing yield losses in all production regions. The main symptoms of the disease are dark to light brown, roundish oval to irregular spots of 1 to 2 mm in diameter with concentric rings in initial stage on foliage. The incidence of the disease on plant was more prominent during humid weather and symptoms were most pronounced on nutrient-deficient leaves (Nagrale *et al*., 2012). *A. alternata* has a high reproduction rate and widespread dispersal of; is more resistant to fungicide treatment and proliferates at higher temperatures (Stammler *et al*., 2014).

The disease can be controlled by spraying the plants with fungicide. The continuous use of fungicides proved to be hazardous, polluting the environment and leading to residual toxicity

(Riaz *et al*., 2008), creating resistance in pathogens and reducing soil fertility (Nazir and Riazuddin, 2008). The manipulation of inherent potential of plants in the form of resistant varieties is a cheap, viable, environment friendly and alternative to reduce losses from biotic stress. The development of *Alternaria* resistant is necessary to alleviate future threats to China aster crop in Indian subcontinent to stabilize the yield potential of China aster varieties. Thus, the present investigation was undertaken to screen genotypes and F_1 hybrids which would be helpful in developing Alternaria leaf spot resistance varieties in later years.

2. MATERIAL AND METHODS

The experiment was conducted in Department of Floriculture and Landscape Architecture, Kittur Rani Channama College of Horticulture, Arabhavi, UHS, Bagalkot campus during 2020-21. The experimental materials consisted of four genotypes (AAC-1, Arka Poornima, Arka Kamini, and Arka Shashank) and six F_1 hybrids of crosses *viz.*, AAC-1 x Arka Poornima, AAC-1 x Arka Kamini, AAC-1 x Arka Shashank and their reciprocal crosses.

2.1 Collection, isolation and maintenance of pathogen

Leaf samples showing typical leaf spot disease symptoms were collected from China aster plants grown in experimental plots. Approximately four 5 mm^2 segments of the leaf per lesion were cut with a disinfected scalpel blade. Each leaf piece included both healthy and infected tissues. The excised leaf pieces were disinfected with 0.5% sodium hypochlorite (NaOCl) solution for 2 minutes followed by washing with sterile distilled water for 2-3 times. The cut samples were dried on sterile blotting paper, placed on Potato Dextrose Agar (PDA) medium amended with streptomycin. The petri-plates were incubated at 27 ± 2º C for 7 days. *Alternaria* cultures were purified from the master plate and plated on PDA media containing the same antibiotics as above. The growth of the fungal mycelia observed after five days of inoculation was sub-cultured on PDA media. The pure fungal pathogen thus obtained was maintained on PDA slants in refrigerator at 4° C.

2.2 Pot culture experiment

Pot culture experiment was conducted for artificial inoculation of *Alternaria* pathogen to China aster parents and F_1 hybrids. For this experiment, China aster seedlings were planted in earthen pots filled with sterilized media. The 45 days old plants were inoculated with 1 x 10^5 spores ml^{-1} conidial spore suspension prepared from 2-week-old *Alternaria* isolates. The spore suspensions were prepared by adding 5 ml of sterile distilled water to each petri-plates and dislodged the conidia with a sterile hockey stick. Each suspension was poured in a beaker and amended with 0.05 microliter Tween 20. The solution was passed through a cheesecloth to remove mycelial fragments from the spore suspension. The spore concentration was determined by using haemocytometer. The conidial suspension was stirred to prevent clumping of conidia and inoculated immediately after preparation by spraying the leaves until run-off with an aerosol sprayer. The plants were then covered with polythene bags to maintain high humidity within the surrounding area of the plants and incubated in a completely random block design in the polyhouse. All treatments consisted of six replicates, with one pot per replicate. The genotypes and F_1 hybrids were scored for their disease reaction at 15 and 30 days after inoculation (DAI).

2.3 Field experiment

The experiment was laid out in randomized block design with three replications. Thirty plants per replication were planted at a distance of 30 cm x 30 cm following ridge-furrow planting system. During the study, the package and practices of crop cultivation were followed but plant protection measures against fungal diseases were not taken. Screening of leaf spot disease was done at 15, 30, 45, 60 and 75 days after transplanting (DAT). The scoring of disease incidence was carried out from 5 randomly selected plants in each replication.

The plant disease severity was assessed on a 0-5 scale based on the percentage of leaf area symptomatic (Xu *et al*., 2011) (Table 1).

Table 1. Disease score scale followed in screening for *Alternaria* leaf spot in China aster

Sl. No.	Disease score	Leaf infected area
1	0	no visible disease symptoms
2	1	≥ 5% leaf area affected
3	2	6% ≥ leaf area affected < 20%
4	3	21% ≥ leaf area affected < 40%
5	4	41% ≥ leaf area affected < 60%
6	5	61% ≤ leaf area affected

The percent disease intensity (PDI) was calculated based on the percentage of infected area on leaves based on the scale of 0-5 grade using the formula given by James (1974):

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of leaves examined} \times \text{Maximum grade}} \times 100$$

The mean PDI of each genotype thus calculated were used to categories host plant response adopting the scale of Xu *et al*. (2011) (Table 2).

Table 2. Grading scale for *Alternaria* leaf spot in China aster

Sl. No.	Category	Percent Disease intensity
1	0	Immune (I)
2	1-15	Resistant (R)
3	16-25	Moderately resistant (MR)
4	26-50	Moderately susceptible (MS)

5	51-75	Susceptible (S)
6	>76	Highly susceptible (HS)

3. RESULTS

The promising four China aster parents and six F_1 hybrids were screened against leaf spot disease to determine the resistance sources. The parents and F_1 hybrids were evaluated using 0-5 scale. Further, these entries were grouped into six categories based on their reaction to the disease Table 3 and 4.

3.1 Assessment of Alternaria leaf spot disease under artificial inoculation condition

The screening of Alternaria disease development was done at 15 and 30 DAI. The data recorded were computed and presented in Table 3. All the inoculated plants showed disease symptoms on the leaves irrespective of genotypes. At 15 DAI, AAC-1 (9.63%) recorded the least PDI followed by Arka Kamini (30.47%) and Arka Shashank (35.78%) while, Arka Poornima (42.63%) recorded the highest PDI. Among F_1 generation, the minimum PDI was exhibited by Arka Kamini x AAC-1 (14.01%), followed by AAC-1 x Arka Kamini (14.21%) and AAC-1 x Arka Shashank (17.15%) whereas, the maximum was revealed by AAC-1 x Arka Poornima (20.70%). At 30 DAI, AAC-1 (14.62%) has shown resistant reaction; Arka Kamini (57.09%) and Arka Shashank (67.24%) recorded susceptible reaction whereas, Arka Poornima (80.78%) showed highly susceptible reaction. Among the F_1 generation, Arka Kamini x AAC-1 (23.95%), AAC-1 x Arka Kamini (24.52%), Arka Shashank x AAC-1 (35.73%), Arka Poornima x AAC-1 (36.27%) and AAC-1 x Arka Shashank (36.85%) exhibited moderately susceptible disease reaction whereas, AAC-1 x Arka Poornima (49.57%) showed highly susceptible disease reaction when screened under artificial inoculation.

3.2 Assessment of Alternaria leaf spot disease under natural disease pressure condition

Under natural disease pressure condition, it was observed that all plants under study were not completely free from disease. The PDI was recorded at 15, 30, 45, 60 and 75 DAT for parents and F_1 hybrids (Table 4). At 15 DAT, among four parents, AAC-1 recorded lowest PDI (0.18%) and Arka Poornima showed highest PDI (5.44%). In F_1 generation, the cross AAC-1 x Arka Kamini (1.66%) recorded the lowest and the highest was noticed in Arka Shashank x AAC-1 (3.28) and is on par with AAC-1 x Arka Poornima (3.08%). One month after transplanting, the PDI was recorded with a minimum score by AAC-1 (1.41%) while the highest was scored by Arka Poornima (10.02%). Among F_1 population, Arka Poornima x AAC-1 (3.39%) and AAC-1 x Arka Poornima (6.58%) scored the lowest and highest PDI, respectively. Among the parental screened at 60 DAT, the lowest PDI was scored by AAC-1 (12.23%) and the highest was observed in Arka Poornima (45.04%). Among F_1s, Arka Poornima x AAC-1 (16.04%) scored the lowest and AAC-1 x Arka Poornima (35.41) scored the highest PDI.

At 75 DAT, AAC-1 (14.65%) observed resistant reaction, Arka Kamini (47.13%) showed moderately susceptible reaction, Arka Shashank (61.13%) showed susceptible disease reaction and Arka Poornima (76.56%) exhibited highly susceptible disease reaction. Among F_1 generation, AAC-1 x Arka Kamini (20.21%) and Arka Kamini x AAC-1 (22.93%) exhibited resistant disease reaction; Arka Shashank x AAC-1 (26.76), Arka Poornima x AAC-1 (27.22), AAC-1 x Arka Shashank (28.49) and AAC-1 x Arka Poornima (45.16) showed moderately susceptible disease reaction when screened under natural disease pressure condition.

Table 3. Screening of China aster genotypes and F_1 hybrids against *Alternaria alternata* (leaf spot) under artificial inoculation conditions

Treatments	Percent disease intensity		Reaction
	15 DAI	30 DAI	
AAC-1	9.63	14.62	R
Arka Poornima	42.63	80.78	HS
Arka Shashank	35.78	67.24	S
Arka Kamini	30.47	57.09	S
AAC-1 x Arka Poornima	20.70	49.57	MS
AAC-1 x Arka Shashank	17.15	36.85	MS
AAC-1 x Arka Kamini	14.21	24.52	MR
Arka Poornima x AAC-1	17.80	36.27	MS
Arka Shashank x AAC-1	17.19	35.73	MS
Arka Kamini x AAC-1	14.01	23.95	MR
S.Em. ±	0.49	0.56	
CD (5%)	1.39	1.59	
CV (%)	5.44	3.21	

DAI – Days after inoculation

Table 4. Screening of China aster genotypes and F_1 hybrids against Alternaria leaf spot under natural disease pressure conditions

Treatments	Per cent disease intensity					Reaction
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	
AAC-1	0.18	1.41	8.37	12.23	14.65	R
Arka Poornima	5.44	10.02	22.60	45.04	76.56	HS
Arka Shashank	3.69	7.66	18.91	36.90	61.13	S
Arka Kamini	2.81	7.46	16.43	32.38	47.13	MS
AAC-1 x Arka Poornima	3.08	6.58	13.61	35.41	45.16	MS
AAC-1 x Arka Shashank	1.75	3.64	9.98	16.30	28.49	MS
AAC-1 x Arka Kamini	1.66	4.06	11.31	16.53	20.21	MR
Arka Poornima x AAC-1	1.73	3.39	9.78	16.04	27.22	MS
Arka Shashank x AAC-1	3.28	7.46	14.22	17.57	26.76	MS
Arka Kamini x AAC-1	1.92	4.91	12.16	16.85	22.93	MR
S.Em. ±	0.33	0.42	0.49	1.01	1.25	
CD (5%)	0.97	1.24	1.47	2.99	3.71	
CV (%)	22.2	12.79	6.22	7.12	5.84	

DAT – Days after transplanting

4. DISCUSSION

Screening of genotypes and F_1 is the most promising method for management of leaf spot disease and development of disease resistant cultivars. Selected parents and their hybrids showed varied levels of resistance and susceptibility but none of them were immune. AAC-1 was found to be resistant against *Alternaria* leaf spot in both field and control conditions. In natural disease pressure, Arka Kamini, Arka Shashank and Arka Poornima recorded to be moderately susceptible, susceptible and highly susceptible to *Alternaria* leaf spot, respectively. However, in artificially inoculated condition, Arka Kamini showed susceptible reaction. Among F_1 hybrids, AAC-1 x Arka Kamini and Arka Kamini x AAC-1 showed moderately resistant reaction; AAC-1 x Arka Poornima, AAC-1 x Arka Shashank, Arka Poornima x AAC-1, Arka Shashank x AAC-1 showed moderately susceptible disease reaction for *Alternaria* leaf spot. These findings were in line with reports of Sujatha *et al*. (2008) who proposed that sunflower genotypes when screened for *Alternaria* leaf blight, none of them showed immune to the disease. Similar results were reported by Vanitha *et al*. (2009), Singh *et al*. (2011), Xu *et al*. (2011), Yadav *et al*. (2014) and Kumari *et al*. (2016) in tomato cultivars for bacterial wilt disease, in tomato for early blight disease, Compositae family plants for *Alternaria* leaf spot, rapeseed mustard for *Alternaria* blight and in sorghum for charcoal rot caused by *Macrophomina phaseolina*, respectively.

The genotypes and F_1 hybrids showed varied reaction against Alternaria leaf spot disease. It is evident that there exists a great scope for developing tolerant/resistant cultivars. The F_1 hybrid plants which exhibited moderately resistant disease reaction can be advanced to next generation. The identification of large number of China aster leaf spot resistance sources and testing them against divergent Alternaria pathogen isolates is essential as the outbreak of new strains of Alternaria pathogens can occur at any time in later years. This can act as a long-term sustainable solution to leaf spot problem in China aster.

5. CONCLUSION

The genotypes and F_1 hybrids showed varied reaction against Alternaria leaf spot disease. It is evident that there exists a great scope for developing tolerant/resistant cultivars. The F_1 hybrid plants which exhibited moderately resistant disease reaction can be advanced to next generation. The identification of large number of China aster leaf spot resistance sources and testing them against divergent *Alternaria* pathogen isolates is essential as the outbreak of new strains of *Alternaria* pathogens can occur at any time in later years. This can act as a long-term sustainable solution to leaf spot problem in China aster.

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