PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(3): 68–74
https://doi.org/10.14719/pst.2057

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Screening of sunflower genotypes for reaction to *Alternaria* leaf blight disease across multi-environments using pooled analysis

Asish K. Binodh[1], Sugitha Thankappan[2*] & M. Paramasivam[3]

[1]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641 003, India
[2]School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore -641 114, India
[3]Department of Plant Pathology, Agricultural College and Research Institute Killikulam, Vallanad Post, Tuticorin Dt -628 252, India

*Email: kumarisugitha@karunya.edu

OPEN ACCESS

ARTICLE HISTORY

Received: 13 August 2022
Accepted: 04 March 2023

Available online
Version 1.0 : 14 April 2023
Version 1.1: 15 April 2023
Version 2.0: 01 July 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Binodh A K, Thankappan S, Paramasivam M. Screening of sunflower genotypes for reaction to *Alternaria* leaf blight disease across multi-environments using pooled analysis. Plant Science Today. 2023;10(3): 68–74. https://doi.org/10.14719/pst.2057

Abstract

Sunflower (*Helianthus annuus* L.) is an oilseed crop with potential health benefits as a source of oil and dietary fiber. The productivity of sunflower is hampered by both biotic and abiotic stresses caused due to climate changes. So far, the available gene pool is having moderately resistant lines. In the present study, we evaluated two screening methods by artificially challenging the plants with the causative agent *A. helianthi*. Significant changes in the disease severity was across 160 pre-breeding lines of sunflower, including the susceptible check, Morden in three different seasons. Among the160 pre-breeding lines derived from a mutant population of sunflower cv. Morden, 6.25 and 5.63 % of lines showed less leaf blight disease incidence under *in vivo* and *in vitro* environments respectively. Moreover, 44.38 % of the sunflower pre - breeding lines demonstrated field tolerance with disease severity in the scale of 5. The promising accessions evaluated in our studies by whole plant assay method and detached leaf technique includes KSFI 19, KSFI 24, KSFI 147, KSFI 56, KSFI 120, KSFI 88, KSFI 152, KSFI 51 and KSFI 115. Therefore, these sunflower lines, could be employed for introgression of resistance genes against leaf blight disease in sunflower cultivars through marker assisted breeding strategies.

Keywords

Alternaria helianthi, Alternaria leaf blight disease, Detach leaf technique, Sunflower genotypes

Introduction

Sunflower (*Helianthus annuus* L.) is a major oilseed crop in India, accounting for approximately 0.24 million ha, producing 0.22 million tonnes with an average yield of 864 kg ha^{-1} in 2019-20 (Salient Statistics on Agriculture, 2021). Sunflower seed oil is an important source of vegetable oil for both food and renewable primary products for bio refineries (1). It is a short duration crop of 80-115 days, and highly suitable to rainfed conditions. Other remarkable features of sunflower like photo-insensitivity, wider adaptability to various cropping patterns, high energy, hull content, drought tolerance and excellent oil quality attracts sunflower cultivation among farmers (2). Sunflower seeds are rich in vitamins (E, B1 and B6) and minerals (Fe, Mn, Cu, K, Zn and Se). Consumption of sunflower seeds in the diet reduces the risk of heart disease and high blood pressure. In India, Karnataka is the leading producer of sunflower, which are mostly grown as arable crops with little irrigation support, followed by Andhra Pradesh, Maharashtra, Bihar, Odisha

and Tamil Nadu (3). However, sunflower farming has been hampered due to various biotic stresses especially diseases at critical stages of development. Leaf blight caused by the genus *Alternaria* has been reported from all sunflower growing regions across the world, which is most prevalent in the tropics and subtropics (4). Among the biotic stresses, leaf blight disease caused by *Alternaria helianthi* reduce the yield by 11.5 % to 73 %. More precisely, Alternari*a* blight in sunflower considerably reduces the seed and oil yield by 27 to 80 and also 17 to 33 % respectively (5). Therefore, the disease highly impacts the number of seeds per head, seed filling, kernel weight and oil content which is greater challenge affecting sunflower productivity.

Cultural, chemical, and biological methods using antagonistic agents such as *Bacillus cereus*, *Pseudomonas fluorescens* and other phylloplane microbes are widely used to manage *Alternaria* blight in sunflower as reported earlier (19). However host plant resistance is the most effective management option. Plant breeding efforts to develop cultivars with tolerance or resistance to the major diseases, are hampered due to limited genetic base. Though, developing resistant cultivars is the most economic management option available, Alternaria leaf blight resistance is not expressed in most of the commercially available hybrids (6). Most disease resistance breeding studies were limited by screening of germplasm under natural conditions and therefore these sources could not be converted into agronomically acceptable cultivars. Screening trials on cultivated sunflower germplasm revealed a lack of genetic diversity for Alternaria leaf blight resistance (2). Despite the fact that the germplasm source with absolute resistance for Alternaria blight is yet to be identified, there is significant potential for using tolerant genotypes in resistant breeding programmes. So far 11 phylogentic groups of Alternaria spp. causing blight in sunflower has been identified (2). Several attempts were made to optimize pathogen challenge technique for screening sunflower germplasm. Reports are on the gametophytic selection by applying pathogen culture filtrate to the stigma and style an hr. before pollination (7). The resistant germplasm were scored based on the % disease index at flowering (15 DAF), physiological maturity and quantifying the economic yield gain (7). Further, Prasad and his coworkers successfully challenged *Alternaria conidia* at a concentration of 10^2 to 10^6 ml^{-1} and observed that the disease intensity is more on 25 days old plants, whereas older plants failed to exhibit visible symptoms (8). Thus, a reliant and consistent disease screening strategy for screening Alternaria disease resistance in sunflower is lacking to develop a competent germplasm pool. Most of the screening methods could express disease severity only in younger plants. However, *Alternaria* spores persists in the soil and cause infection throughout the life cycle. So far, there are no genetic resources that are resistant or tolerant to Alternaria blight. To obtain a reliable resistant genetic source, screening for Alternaria disease resistance should be performed under high disease pressure both in the field and the glass -house environment. Hence, the present study attempts to screen sunflower inbreds under both *in vivo* and *invitro* conditions against the challenged pathogen *Alternaria helianthi* even in older plants. The inbreds identified will be used to develop Alternaria leaf blight resistant cultivars or hybrids.

Materials and Methods

The investigation was carried out at the experimental farm of Plant breeding and Genetics, Agricultural College and Research Institute, Killikulam, Vallanad Tuticorin Dt, Tamil Nadu (8 ° 46 'N latitude and 77 ° 42' E longitude). The elevation of the site is 40 m above the mean sea level with relative humidity of 60 to 80%, sub-tropical monsoon climate and red lateritic soil (pH : 6.8 ; EC : 0.05 dS m^{-1}).

Plant materials

Inbred population consisting of 160 individuals derived from mutant population of sunflower cv. Morden was evaluated for Alternaria leaf blight resistance and susceptibility using detached leaf technique and whole-plant assay in 3 consecutive seasons, Rabi- summer 2017-18, Kharif 2018 and Rabi -summer 2018-19.

Inoculation methods

Detached leaf technique

In detached leaf technique leaves from 30-day-old plants (third leaves preferably) were detached and placed in humidity chambers. Each sunflower genotype was replicated thrice on Petriplates, with each replication containing three leaves. The spore suspension (10^6 ml^{-1}) was sprayed uniformly to the leaves using an atomizer. The inoculated leaves were put in humid chambers for 3 consecutive days. The plates were incubated at 25±2 °C with continuous fluorescent illumination, and the leaves were examined for chlorotic/necrotic signs until 72 hr after inoculation at a frequency of 24 hr (8). The % of infected leaf area was calculated by visual inspection wherein each leaf was observed 3 days after inoculation and graded it according to the standard scale of 0 to 9.

Whole Plant Assay

In whole plant assay method, 30 day-old sunflower prebreeding plants grown in pots were challenged with *A. helianthi* spore suspension (10^6spores ml $^{-1}$). Each genotype was repeated 3 times, with 5 plants in each replicate. The plants were covered with a transparent plastic cover for 1-2 days to maintain 100% humidity following inoculation. Following inoculation, plants were individually examined for disease symptoms for 7 days using a standard scale of 0 to 9. The susceptible cv. Morden served as positive controls in both of the experiments.

Symptom assessment and statistical analysis

Leaf blight disease symptoms were observed spontaneously on 35-40 days after sowing. The disease severity was assessed on randomly selected plants in each genotype and scored as per standard scale of 0-9 (9).

Scale	Symptom	Disease severity (%)	Reaction
0	No visible symptoms on leaf	0	Immune
1	Small, circular, scattered, brown spots on leaves covering 1% or less of the leaf area	< 1	Highly resistant
3	Spots enlarging, dark brown in colour, covering 1-10% of the leaf area	1-5	Resistant/ tolerant
5	Spots enlarging, dark brown in colour, target like appearance covering 11-25% of leaf area	5-25	Moderately resistant
7	Spots dark brown, coalescing with target like appearance covering 26-50% of leaf area	25-50	Susceptible
9	Spots uniformly dark brown covering 51% or more of leaf area	>50	Highly susceptible

Screening of genotypes

The disease score was carried out 20 days after inoculation (DAI) and expressed as % Disease Index (PDI) (10).

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all disease ratings x 100}}{\text{Total no: of plants scored x Maximum disease scale}}$$

The disease severity of 160 inbred lines screened with *A. helianthi* was calculated. Pooled analysis for the data obtained from detached leaf and whole plant assay methods was performed using STAR 2.0.1 and TNAUSTAT software package.

Results

After 3 days of incubation (DAI), the plants were evaluated with regard to disease symptoms using the interaction phenotype. The Alternaria blight disease symptoms clearly visible on the inoculated plants indicated that the experimental conditions were conducive for infection by the target pathogen *Alternaria helianthi*. The leaf blight symptoms were distinct on infected plants on 3 DAI, whereas no symptoms noticed in un-inoculated plants. The responses to the leaf blight disease differed both under *in vivo* and *in vitro* conditions. A pooled analysis of variance reveals a significant seasonal difference (Table 1). Significant changes in disease severity have been observed across 160 prebreeding lines of sunflower, including the susceptible check. Based on the disease severity (%) at field conditions, all the genotypes were grouped into 4 categories *i.e.,* resistant (1-5%), moderately resistant (5.1-25%), susceptible (25.1-50%) and highly susceptible (>50%) (Table 2).

Rabi summer 2017-18

Leaf spot incidence was less in entries KSFI 19 (3.42 %), KSFI 24 (4.14 %), KSFI 56 (4.17 %), KSFI 120 (4.18 %) and KSFI 147 (4.18 %) when compared to other entries under *in vitro* conditions during Rabi summer 2017-18 (Supplementary Table). In rest of the entries leaf spot incidence ranged between 5.24 - 87.47 %. The highest incidence of 87.47 % was recorded in KSFI 114. The susceptible check, Morden recorded 77.48 % disease incidence. All these entries were also screened under *in vivo* conditions by artificially inoculating 40 days old plants. The incidence was on par with *in vitro* screening as the disease pressure was very high under artificial conditions. KSFI 56 recorded least leaf spot incidence of 3.43 % followed by KSFI 120 (3.57 %). KSFI 19, KSFI 152 and KSFI 21 also recorded low leaf spot incidence and ranged between (3.59 % - 3.67 %). Maximum incidence of 87.16 % was observed in KSFI 18. Incidence of disease in check variety Morden was 75.91 %.

Kharif 2018

During Kharif 2018 the severity of the disease ranged from 2.47 (KSFI 19) to 84.70 (KSFI 18) for *in vivo* screening whereas *in vitro* screening registered scores from 2.63 (KSFI 19) to 85.86 (KSFI 82). When compared to other entries, leaf spot incidence was lower in KSFI 19 (2.47 %), KSFI 24 (2.72 %), KSFI 147 (3.02 %), KSFI 56 (3.24 %), KSFI 120 (3.27 %), KSFI 88 (3.60 %), KSFI 152 (4.07 %), KSFI 51 (4.09 %) and KSFI 115 (4.42 %) under field conditions. The incidence of leaf spots in the remaining entries ranged from 9.27 to 84.70 % KSFI 18 had the highest incidence rate of 84.70 %. The susceptible check Morden had a disease incidence of 73.03 % (Supplementary Table 1).

Rabi summer 2018-19

None of the entries registered immune reaction to Alternaria leaf spot incidence. Leaf spot incidence was less in entries KSFI 19 (3.07 %), KSFI 88 (3.84 %) and KSFI 56 (4.09 %) when compared to other entries under field conditions. It was observed in the range of 4.24 - 89.93 % in other entries. In general, the disease severity ranged from 4.05 (KSFI 19) to 93.66 (KSFI 158) and 3.07 (KSFI 19) to 89.93 (KSFI 82) for *in vitro* screening and *in vivo* screening. All

Table 1. Pooled analysis of Variance for prebreeding lines against Alternaria leaf spot

Source	DF	Whole Plant Assay			Detached Leaf Technique		
		SS	MSS	F ratio	SS	MSS	F ratio
Treatment	159	523490.6	3292.393	488.6466	564722.4	3551.713	367.83
Environment	2	4263.07	2131.535	316.3557	3084.083	1542.041	159.7
Environment x Treatment	318	2873.911	9.0375	1.3413	10428.64	32.7945	3.4
Total	1439	543590.5			587504.8		
MEAN			31.99			35.53	
CV(%)			8.11			8.75	
SE for envt x treat			1.4986			1.4439	

SS – sum of squares; MSS – mean sum of square

Table 2. Grouping of sunflower genotypes based on disease severity (%) against Alternaria leaf blight disease under field conditions

Category	Disease Severity (%)	Genotypes		
		R/S 17-18	Kh 18	R/S 18-19
Resistant	(1-5%)	KSFI 56, KSFI 120, KSFI 19, KSFI 152, KSFI 24, KSFI 147, KSFI 115, KSFI 88, KSFI 51	KSFI 24, KSFI 56, KSFI 147, KSFI 19, KSFI 120, KSFI 88, KSFI 152, KSFI 51, KSFI 115, KSFI 39	KSFI 19, KSFI 88, KSFI 56, KSFI 24, KSFI 120, KSFI 147, KSFI 51, KSFI 115, KSFI 39, KSFI 152
Moderately Resistant	(5.1-25%)	KSFI 39, KSFI 7, KSFI 43, KSFI 66, KSFI 4, KSFI 6, KSFI 22, KSFI 44, KSFI 79, KSFI 62, KSFI 46, KSFI 2, KSFI 5, KSFI 65, KSFI 47, KSFI 9, KSFI 112, KSFI 121, KSFI 58, KSFI 3, KSFI 92, KSFI 141, KSFI 110, KSFI 8, KSFI 11, KSFI 30, KSFI 144, KSFI 90, KSFI 103, KSFI 101, KSFI 70, KSFI 15, KSFI 16, KSFI 12, KSFI 37, KSFI 54, KSFI 34, KSFI 23, KSFI 143, KSFI 36, KSFI 95, KSFI 26, KSFI 10, KSFI 50, KSFI 59, KSFI 60, KSFI 57, KSFI 74, KSFI 14, KSFI 63, KSFI 31, KSFI 77, KSFI 68, KSFI 33, KSFI 72, KSFI 17, KSFI 73, KSFI 25, KSFI 13, KSFI 76, KSFI 71, KSFI 35, KSFI 75, KSFI 27, KSFI 64, KSFI 67, KSFI 32, KSFI 28, KSFI 69, KSFI 52, KSFI 83, KSFI 48, KSFI 29	KSFI 43, KSFI 47, KSFI 7, KSFI 58, KSFI66, KSFI 6, KSFI 4, KSFI 54, KSFI 141, KSFI 92, KSFI 44, KSFI 5, KSFI 112, KSFI 9, KSFI 121, KSFI 110, KSFI 3, KSFI 2, KSFI 46, KSFI 8, KSFI 11, KSFI 144, KSFI 103, KSFI 22, KSFI 50, KSFI 90, KSFI 59, KSFI 70, KSFI 101, KSFI 16, KSFI 15, KSFI 79, KSFI 62, KSFI 30, KSFI 60, KSFI 12, KSFI 65, KSFI 37, KSFI 26, KSFI 57, KSFI 95, KSFI 23, KSFI 34, KSFI 143, KSFI 63, KSFI 36, KSFI 10, KSFI 74, KSFI 77, KSFI 14, KSFI 73, KSFI 72, KSFI 71, KSFI 31, KSFI 17, KSFI 64, KSFI 76, KSFI 68, KSFI 75, KSFI 13, KSFI 33, KSFI 83, KSFI 27, KSFI 28, KSFI 25, KSFI 69, KSFI 32, KSFI 52, KSFI 67, KSFI 35, KSFI 48, KSFI 29, KSFI 15	KSFI 7, KSFI 2, KSFI 62, KSFI 22, KSFI 44, KSFI 46, KSFI 66, KSFI 79, KSFI 6, KSFI 65, KSFI 5, KSFI 4, KSFI 9, KSFI 8, KSFI 37, KSFI 3, KSFI 43, KSFI 73, KSFI 121, KSFI 47, KSFI 92, KSFI 144, KSFI 112, KSFI 70, KSFI 141, KSFI 110, KSFI 16, KSFI 103, KSFI 90, KSFI 101, KSFI 11, KSFI 143, KSFI 74, KSFI 12, KSFI 95, KSFI 30, KSFI 10, KSFI 23, KSFI 50, KSFI 54, KSFI 77, KSFI 15, KSFI 83, KSFI 72, KSFI 58, KSFI 26, KSFI 34, KSFI 36, KSFI 76, KSFI 25, KSFI 31, KSFI 75, KSFI 69, KSFI 52, KSFI 59, KSFI 48, KSFI 67 KSFI 35, KSFI 27, KSFI 68, KSFI 57, KSFI 32, KSFI 60, KSFI 28, KSFI 14, KSFI 17, KSFI 13, KSFI 71
Susceptible	(25.1-50%)	KSFI 157, KSFI 155, KSFI 21, KSFI 154, KSFI 113, KSFI 119, KSFI 149, KSFI 129, KSFI 104, KSFI 133, KSFI 145, KSFI 139, KSFI 125, KSFI 124, KSFI 97, KSFI 132, KSFI 93, KSFI 148, KSFI 86, KSFI 111, KSFI 109, KSFI 108, KSFI 126, KSFI 81, KSFI 137, KSFI 117, KSFI 130, KSFI 131, KSFI 107, KSFI 159, KSFI 98, KSFI 123, KSFI 151, KSFI 122, KSFI 150, KSFI 45, KSFI 99, KSFI 1, KSFI 100, KSFI 140, KSFI 134, KSFI 118, KSFI 91, KSFI 116, KSFI 55, KSFI 42, KSFI 102, KSFI 138, KSFI 135, KSFI 85, KSFI 94, KSFI 105, KSFI 136, KSFI 128, KSFI 89, KSFI 53, KSFI 20, KSFI 156, KSFI 78	KSFI 155, KSFI 21, KSFI 154, KSFI 113, KSFI 119, KSFI 149, KSFI 104, KSFI 129, KSFI 133, KSFI 145, KSFI 97, KSFI 125, KSFI 124, KSFI 139, KSFI 132, KSFI 148, KSFI 159, KSFI 93, KSFI 108, KSFI 86, KSFI 109, KSFI 111, KSFI 126, KSFI 107, KSFI 117, KSFI 137, KSFI 131, KSFI 130, KSFI 81, KSFI 123, KSFI 151, KSFI 122, KSFI 150, KSFI 45, KSFI 98, KSFI 99, KSFI 140, KSFI 134, KSFI 100, KSFI 118, KSFI 1, KSFI 116, KSFI 91, KSFI 102 KSFI 135, KSFI 42, KSFI 105, KSFI 55, KSFI 138, KSFI 94, KSFI 85, KSFI 136, KSFI 128, KSFI 89, KSFI 53, KSFI 20, KSFI 156,	KSFI 63, KSFI 64, KSFI 29, KSFI 33, KSFI 157, KSFI 155, KSFI 21, KSFI 93, KSFI 86, KSFI 154, KSFI 119, KSFI 149, KSFI 81, KSFI 129, KSFI 55, KSFI 139, KSFI 104, KSFI 113, KSFI 85, KSFI 159, KSFI 133, KSFI 145, KSFI 1, KSFI 99, KSFI 132, KSFI 97, KSFI 137, KSFI 124, KSFI 131, KSFI 148, KSFI 122, KSFI 150, KSFI 123, KSFI 151, KSFI 111, KSFI 125, KSFI 91, KSFI 117, KSFI 45, KSFI 109, KSFI 100, KSFI 126, KSFI 108, KSFI 98, KSFI 138, KSFI 130, KSFI 118, KSFI 102, KSFI 42, KSFI 136, KSFI 116, KSFI 94, KSFI 134, KSFI 140, KSFI 128, KSFI 89, KSFI 107, KSFI 53, KSFI 135, KSFI 105, KSFI 78, KSFI 156
Highly susceptible	(>50%)	KSFI 153, KSFI 158, KSFI 80, KSFI 87, KSFI 106, KSFI 40, KSFI 49, KSFI 160, KSFI 61, KSFI 114, KSFI 127, KSFI 142, KSFI 146, KSFI 38, KSFI 84, KSFI 96, KSFI 82, KSFI 41, KSFI 18, Morden	KSFI 78, KSFI 153, KSFI 146, KSFI 158, KSFI 80, KSFI 87, KSFI 106, KSFI 84, KSFI 40, KSFI 49, KSFI 61, KSFI 160, KSFI 114, KSFI 127, KSFI 142, KSFI 38, KSFI 96, KSFI 82, KSFI 41, KSFI 18, Morden	KSFI 20, KSFI 153, KSFI 146, KSFI 127, KSFI 142, KSFI 40, KSFI 49, KSFI 61, KSFI 160, KSFI 106, KSFI 114, KSFI 38, KSFI 158, KSFI 80, KSFI 87, KSFI 84, KSFI 41, KSFI 96, KSFI 18, KSFI 82, Morden

these entries were screened under greenhouse conditions in pot culture by artificially inoculating with 9 day old culture of *Alternaria helianthi* on 40 day old plants. KSFI 19 has recorded the least leaf spot incidence of 4.05 % followed by KSFI 56 (4.14 %). KSFI 158 recorded the highest incidence of 93.66 % (Supplementary Table).

Discussion

In the present investigation, leaf blight symptoms were distinctly observed in infected plants on 3 DAI, whereas no symptoms noticed in un-inoculated plants. Significant changes in disease severity have been observed across 160 pre-breeding lines of sunflower, including the susceptible check, sunflower cv. Morden. The pre-breeding lines demonstrated a broad range of disease response to leaf blight, from resistance to highly susceptible. In the field, the mutant lines of cultivated sunflower cv. Morden variety showed more tolerance and moderately resistant reactions. Among the 160 pre-breeding lines derived from a mutant population of sunflower cv. Morden, 6.25 % of lines had less leaf blight disease in the field and 5.63 % under *in vitro* condition. Moreover, 44.38 % of the sunflower pre-breeding lines demonstrated field tolerance with disease severity with a scale value of 5 (Fig. 1). The pre-breeding lines developed in the present study showed disproportionately higher number of lines with high to moderate resistant responses to leaf blight disease.

Further, continuous evaluation of pre- breeding lines over 3 seasons, suggests a clear demarcation in the occurrence of leaf blight among the lines examined. The disease severity ranged from 3.42 (KSFI 19) to 87.47 (KSFI 114) and 4.05 (KSFI 19) to 93.66 (KSFI 158) respectively for *in vitro* screening during Rabi Summer 2017-18 and 2018-19 respectively. During Kharif 2018 the severity of the disease ranged from 2.47 (KSFI 19) to 84.70 (KSFI 18) for *in vivo* screening whereas *in vitro* screening registered scores from 2.63 (KSFI 19) to 85.86 (KSFI 82). During Rabi Summer 2017-18 and 2018-19 the severity of the disease ranged from 3.43 (KSFI 19) to 87.16 (KSFI 18) and 3.07 (KSFI 19) to 89.93 (KSFI 82) for *in vivo* screening respectively. The sensitive cv. Morden, exhibited high susceptible reaction. The severity of leaf blight <3 score in the scale was

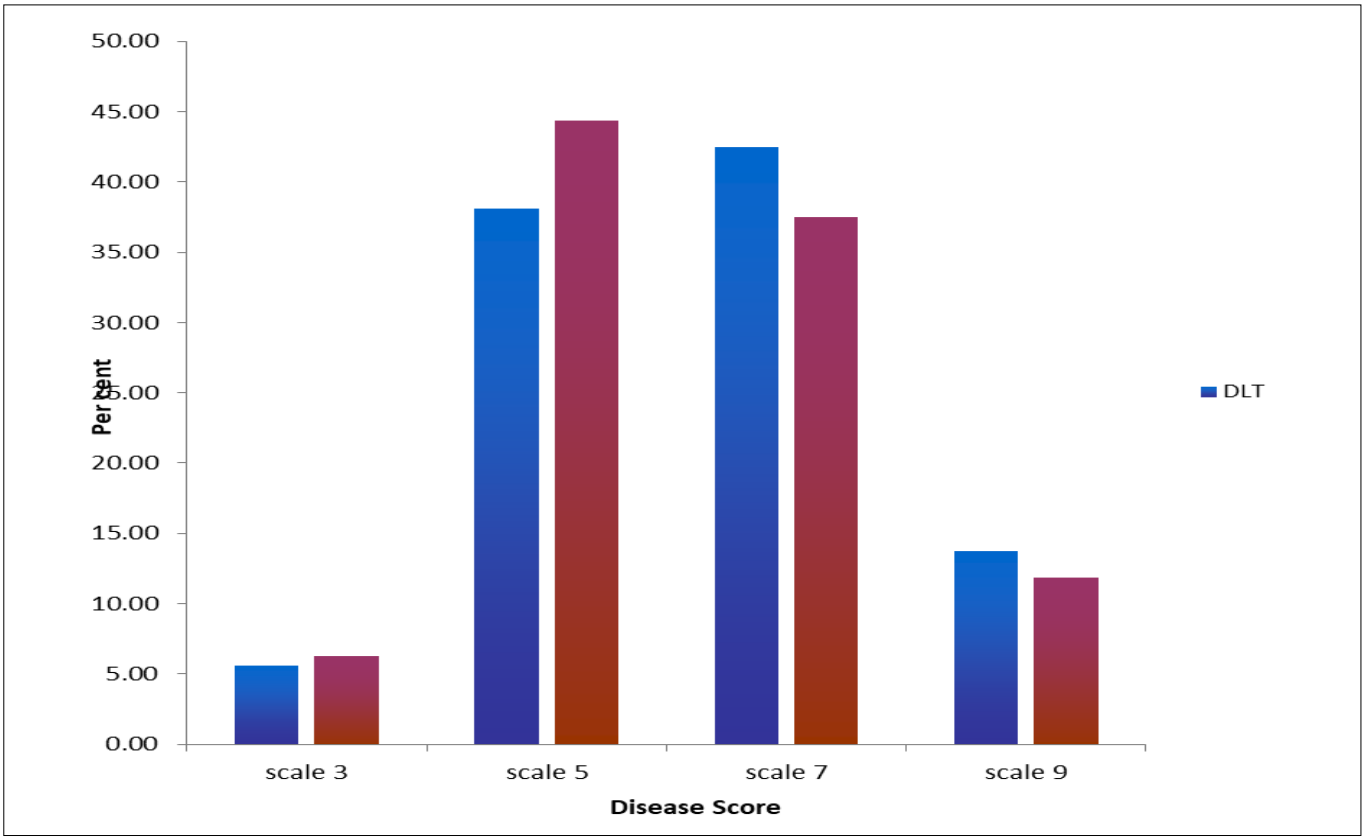

Fig. 1. Disease score on screening the sunflower inbred lines for Alternaria leaf blight resistance/susceptibility.

observed in 10 pre-breeding lines under *in vivo* condition, indicating that environmental factors such as temperature, rainfall and dew period may influence the expression of resistance to leaf blight. However, the disease severity was severe under field conditions in 160 in breds evaluated in Rabi summer 2017-18. Majority of the lines showed moderate resistance to susceptible responses.. However, KSFI 19, KSFI 24, KSFI 147, KSFI 56, KSFI 120, KSFI 88, KSFI 152, KSFI 51 and KSFI 115 were consistently low in leaf blight severity under field conditions. The reason for less leaf blight disease severity during Kharif season might be attributed due to the prevalence of less relative humidity (RH %) during the entire crop period (11). Leaf spots typically appear 35-40 days after planting and as weather conditions are favorable, disease spreads quickly to upper leaves and covers the entire leaf, resulting in blight symptoms. When compared to other entries, leaf spot incidence was lower in KSFI 19 (2.47 %), KSFI 24 (2.72 %), KSFI 147 (3.02 %), KSFI 56 (3.24 %), KSFI 120 (3.27 %), KSFI 88 (3.60 %), KSFI 152 (4.07 %), KSFI 51 (4.09 %) and KSFI 115 (4.42 %) under field conditions during Kharif 2018. The incidence of leaf spots in the remaining entries ranged from 9.27 to 84.70 %. KSFI 18 had the highest incidence rate of 84.70 %. Morden, the susceptible check, had a disease incidence of 73.03 %. KSFI 19 and KSFI 24 showed low leaf blight disease in the promising pre-breeding lines evaluated with *A. helianthi* isolates during Rabi summer 2018-2019. KSFI 19 had the lowest leaf-spot incidence of 3.07 %, followed by KSFI 24 (3.83 %) and KSFI 56 (3.83 %) (4.08 %). The disease incidence rate in check variety Morden was 84.84 %. Among the 160 in breds tested, 68 lines registered disease scale of 5, whereas 21 lines showed susceptible reaction with disease score of more than 50% to leaf blight. No definite trend of association with field score was noticed which is in accordance with previous reports (12).

Pathogen spore load may be low in most crop seasons under natural field conditions, and plants under evaluation may have escaped infection. Since all inbred lines showed different levels of disease resistance to leaf blight under field conditions, they were further tested *in vitro* using the detached leaf technique to avoid disease severity escapes. All of these entries were also screened in a greenhouse setting using artificially inoculated 40-day-old plants. The incidence was higher in all entries, indicating that the disease severity depends on several factors which can be controlled under glass house conditions. When compared to field screening, the disease pressure was extremely higher under artificial or controlled environment. Optimal inoculum concentration will reduce the possibility of overlooking susceptible plants and enables to differentiate between different resistaces level (13). Among the 160 in breds evaluated using the detached leaf technique nine lines recorded score of 3 and 61 lines exhibited tolerant reaction with disease severity of 25%. On the contrary, 22 lines are highly susceptible to leaf blight with a score value of 9 (Fig. 1). The current study strongly supports the previous findings that most of the sunflower genotypes are susceptible to Alternaria leaf blight (14).

The resistance reaction of the 9 pre - breeding lines KSFI 19, KSFI 24, KSFI 147, KSFI 56, KSFI 120, KSFI 88, KSFI 152, KSFI 51 and KSFI 115 under field and laboratory conditions was confirmed by the *in vitro* screening results (Fig. 2). Under both field and laboratory conditions, none of the pre - breeding lines demonstrated either an immune or highly resistant response to the disease. Previous workers also reached similar conclusions while screening different sunflower genotypes for Alternaria resistance (15, 16).

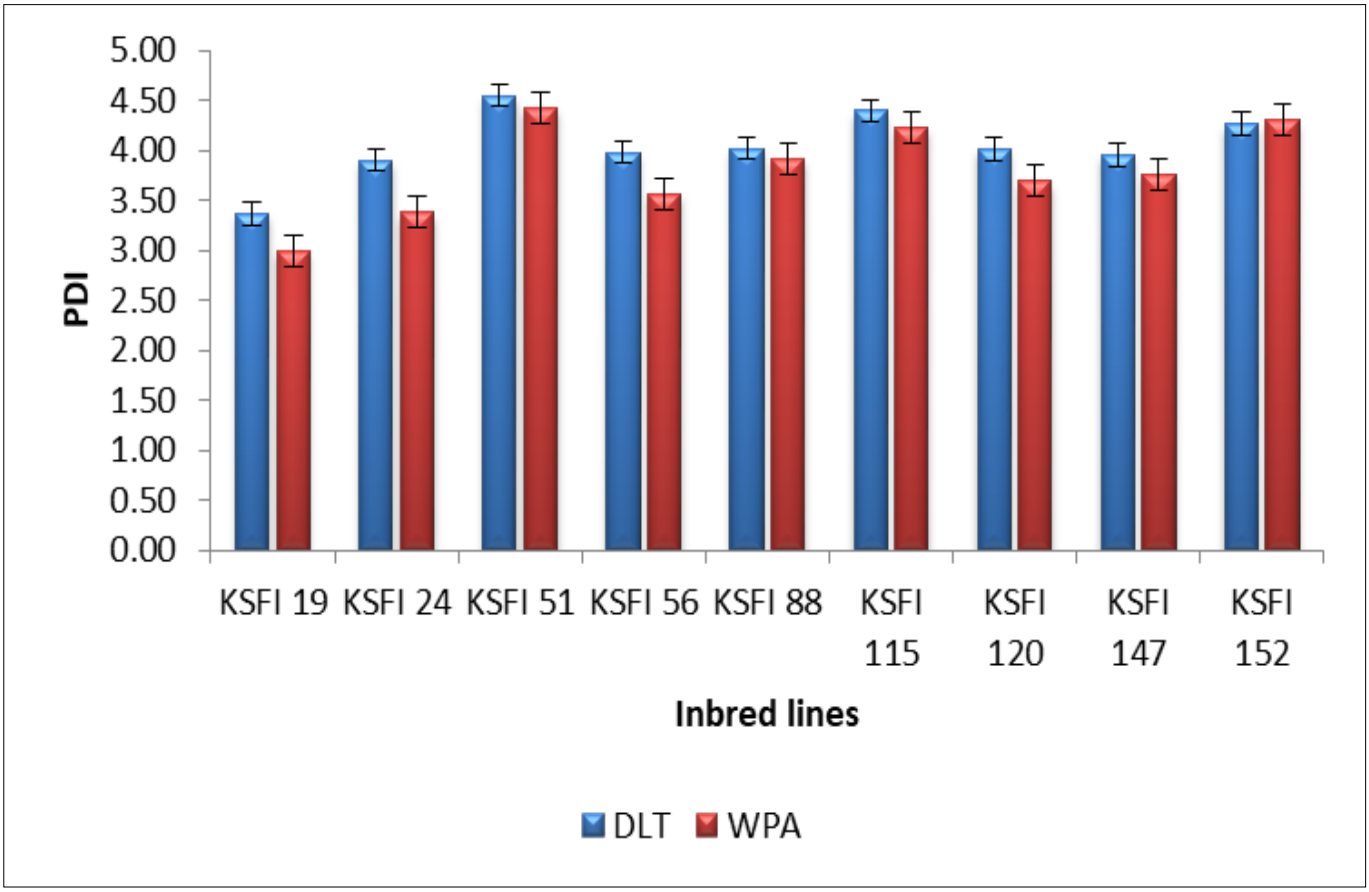

Fig. 2. Percentage disease index for all the inbreds aginst Alternaria leaf blight. Data represented are mean (±standard error) (n=3)

The wide difference in disease reaction between genotypes in the field and in the laboratory could be attributed to differences in environmental conditions and inoculum load availability (17). This shows that genes for resistance to *Alternaria* blight are dispersed differently in genotypes and the favourable alleles get accumulated to give resistant disease reaction. The present findings are in agreement with the earlier investigations (15, 18).

Conclusion

Sunflower breeding is challenged with limited gene pool of moderate resistant lines. The present study evidenced that, few sunflower genotypes showed less disease incidence to Alternaria leaf blight in detached leaf technique, whereas higher blight intensity was observed in the greenhouse assay. Therefore, sunflower pre- breeding lines with less disease incidence could be used in further breeding programme to develop sunflower hybrids with built-in resistance to Alternaria leaf blight. The elite sunflower accessions evaluated in our studies by whole plant assay method and detached leaf technique include KSFI 19, KSFI 24, KSFI 147, KSFI 56, KSFI 120, KSFI 88, KSFI 152, KSFI 51 and KSFI 115, that can be introgressed to develop leaf blight resistant genotypes in sunflower. Therefore, the pooled analysis of multi-seasonal data using whole plant assay method and the detached leaf technique under controlled environment can be regarded as most reliable, accurate and consistent for identifying resistant sunflower genotypes. The selected inbred lines in the investigation would benefit to develop Alternaria leaf blight resistant and high yielding sunflower hybrids to cater the needs of healthy cooking oil and confectioneries.

Acknowledgements

Authors are grateful to the Department of Plant Breeding and Genetics, AC &RI, Killikulam for extending lab and field facilities to carry out the work.

Authors contributions

AKB conceptualized the idea and obtained an in-house project from Tamil Nadu Agricultural University; AKB initiated the lab studies, conducted most of the experiments and drafted the manuscript; MP assisted in field screening; ST assisted AKB in data curation, statistical analysis and editing the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

Supplementary data

Supplementary Table 1. Screening of pre-breeding lines over seasons against Alternaria leaf spot under *in vitro* and *in vivo* conditions.

References

1. Zeinalzadeh-Tabrizi H, Hosseinpour A, Ghaffari M, Haliloglu K. Genetic structure and marker-trait associations in parental lines of sunflower (*Helianthus annuus* L.). Iran J Plant Physiol. 2022;12 (1):3955-71.
2. Lakshmi Prasad MS, Naresh N, Sujatha K, Usha D, Sujatha M, Sarada C et al. Population structure of Alternaria species causing leaf blight of sunflower (*Helianthus annuus* L.) in India. Phytoparasitica. 2020;48(3):335-56. https://doi.org/10.1007/s12600-020-00796-6
3. Kulkarni S, Biradar R, Rathod A, Sharanappa P. Impact of frontline demonstration on adoption of improved practices of sunflower (*Helianthus annuus* L.). Intl J of Curr Microbiol and Applied Sci. 2018;7(9):2511-15. https://doi.org/10.20546/ijcmas.2018.709.311
4. Prakash N, Vishunavat K, Khan GT, Prasad P. SA, ABA and Pseudomonas fluorescens elicit defense responses in tomato against *Alternaria* blight. J of Plant Biochem and Biotechnol. 2021;30 (1):13-25. https://doi.org/10.1007/s13562-020-00564-x
5. Kgatle M, Flett B, Truter M, Aveling T. Control of Alternaria leaf blight caused by *Alternaria alternata* on sunflower using fungicides and *Bacillus amyloliquefaciens*. Crop Protection. 2020;132:105146. https://doi.org/10.1016/j.cropro.2020.105146
6. Mesta R, Benagi V, Srikant K, Basavarajappa M. Management of Alternaria blight of sunflower through fungicides. Karnataka J of Agri Sci. 2011;24(2):149-52.
7. Shobha Rani T, Ravikumar R. Sporophytic and gametophytic recurrent selection for improvement of partial resistance to Alternaria leaf blight in sunflower (*Helianthus annuus* L.). Euphytica. 2006;147(3):421-31. https://doi.org/10.1007/s10681-005-9039-6
8. Prasad MSL, Sujatha M, Rao SC, Sarada C, Singh H. A new technique for evaluating sunflower germplasm against Alternaria leaf blight. J Mycol and Plant Pathology. 2008;38:39-46.
9. Mayee C, Datar V. Phytopathometry Tech Bull-1. 1986. Marathwad Agricultural University: Parabhani, India.p:186.
10. Wheeler BEJ. An introduction to plant diseases. An introduction to plant diseases. London:John Wiley & Sons Ltd., 1969. p :134.
11. Brand SI, Heldwein AB, Radons SZ, Maldaner IC, Hinnah FD, Guse FI et al. Effect of Alternaria and Septoria spot on sunflower yield. Int J Biometeorology. 2020;64(12):2153-60. https://doi.org/10.1007/s00484-020-02006-8
12. Shaik S, Ravikumar R. Association between field and *in vitro* reaction to alternaria leaf blight in sunflower genotypes/ correlación entre la reacción del campo y la reacción *in vitro* en alternariosis de la hoja en genotipos de girasol/corrélation entre les réactions à la rouille des feuilles dans les champs et *in vitro* dans le génotype du tournesol. Helia. 2003;26(38):109-14. https://doi.org/10.2298/HEL0338109S
13. Surujdeo-Maharaj S, Umaharan P, Butler D, Sreenivasan T. An optimized screening method for identifying levels of resistance to *Crinipellis perniciosa* in cocoa (*Theobroma cacao*). Plant Pathology. 2003;52(4):464-75. https://doi.org/10.1046/j.1365-3059.2003.00865.x
14. Gopalakrishnan C, Manivannan N, Vindhiyavarman P, Thiyagarajan K. Evaluation and Identification of Alternaria leaf spot resistant sunflower genotypes. Electronic J Plant Breeding. 2010;1(2):177-81.
15. Reddy C, Reddy A, Sinha B, Lakshmi MS. Screening of sunflower genotypes for resistance against Alternaria blight. Asian J Plant Sci. 2006;5(3):511-15. https://doi.org/10.3923/ajps.2006.511.515
16. Sujatha M, Reddy AV, Sivasankar A. Identification of sources of resistance to Alternaria blight in sunflower. Current Biotica. 2008;2(3):249-60.
17. Viriyasuthee W, Saepaisan S, Saksirirat W, Gleason ML, Chen RS, Jogloy S. Effective plant ages for screening for field resistance to Alternaria leaf spot (caused by *Alternaria* spp.) under natural infection in Jerusalem artichoke (*Helianthus tuberosus* L.). Agronomy. 2019;9(11):754. https://doi.org/10.3390/agronomy9110754
18. Vear F. Changes in sunflower breeding over the last fifty years. OCL Oilseeds and fats crops and lipids. 2016;23(2):1-8. https://doi.org/10.1051/ocl/2016006
19. Jeyapradha C, Yesu Raja I. A review of eco-friendly management of *Alternaria* spp. Indian Journal of Hill Farming. 2016; 29(2):106-19.