Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 07, pp.54393-54398, July, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MORPHOLOGICAL AND GENETIC VARIABILITY OF ALTERNARIA ALTERNATA ISOLATES OF SUNFLOWER

***Naresh, N., Santha Lakshmi Prasad, M. and Prasad, R.D.**

Crop Protection, ICAR –Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500030, Telangana, India

ARTICLE INFO

Article History:
Received 18th April, 2017
Received in revised form
05th May, 2017
Accepted 27th June, 2017
Published online 31st July, 2017

Key words:

Sunflower, *A.alternata*,
Isolates,
Morphological,
Genetic Variability,
ISSR Markers.

ABSTRACT

Sunflower (Helianthus annuus L.) is one of the important oilseed crops in the world and it ranks third in the area of cultivation after groundnut, mustard and rapeseed. Thirty isolates of Alternaria alternata causing leaf blight disease of sunflower during rabi season were collected from different conventional sunflower growing areas of India. All the isolates produced light brown obpyriform to ovate to obclavate conidia, which varied in the size (length, width and septa).Based on the variation in pigmentation, color of aerial mycelium the isolates were categorized into five types. Thirty isolates were studied for length, width of conidia and clustering was performed using the Unweighted Pair Group Mean Average which showed 3 major groups. Group I consisted of 16 isolates; Group II includes 12 isolates and Group III contains 2 isolates. Genetic variability and phylogenetic relationships among the collected isolates were studied by employing the Inter Simple Sequence Repeats (ISSR) markers. Thirty two ISSR primers were used to study the genetic relationship among the 30 isolates of A. alternata, out of these twelve primers were selected as polymorphic based on their banding pattern. The genomic DNA isolated from A. alternata isolates a total of 185 bands were amplified with 12 ISSR primers among which 142 bands were showing polymorphism (76%). The maximum genetic similarity observed with ISSR was 0.98 % between A a1 and A a16, while the lowest genetic similarity of 0.22 % was observed between Aa1 and Aa5. Based on the literature cited, this was the first report on morphological and genetic analysis (ISSR analysis) of A. alternata isolates from different sunflower cultivating areas of India.

Citation: Naresh, N., Santha Lakshmi Prasad,M. and Prasad, R.D, 2017. "Morphological and Genetic Variability of Alternaria alternata isolates of Sunflower", *International Journal of Current Research*, 9, (07), 54393-54398.

INTRODUCTION

Nine *Alternaria* species have been reported on sunflower, including *A. helianthi* (Hansford) Tubaki and Nishihara, *A. alternata*, *A. zinniae* Ellis, *A. tenuissima* (Fries) Wiltshire, *A. leucanthemi* Nelen (syn. *A. chrysanthemi* Simmons and Grosier), *A. helianthicola* Rao and Rajagopalan, *A. longissima* Deighton and MacGarvey, *A. helianthinficiens* Simmons, and *A. protenta* Simmons (Kolte, 1985; Prathuangwong, et al., 1991; Simmons, E. G. 1986.) *Alternaria* belongs to division of the fungi called Deuteromycota. The word *Alternaria* means 'neuter fungi' as it has no sexual stage (meiospores). However *Alternaria* still produces spores. The spores in *Alternaria* are multicellular and pigmented and they are produced in chains or branched chains. The spores have a distinctive appearance that makes them easy to recognize. They are broad near the base and taper gradually to an elongate beak. *A. helianthi* has been recognized as the most prevalent and damaging species worldwide (Kolte, 1985). *A. alternata* causes leaf spot with brown spot with concentric rings appearing on the leaves before or during flowering in rabi sown sunflower crop in India. Although this fungus *A. alternata* is usually reported as secondary pathogen after *Alternariaster helianthi* under high humidity conditions (Kintzios et al., 1996). This disease spreads epidemically causing considerable yield losses during rabi season. Normally leaf blight caused by *Alternariaster helianthi* is the major disease in sunflower growing areas of southern and middle parts of India particularly during Kharif season however, leaf blight caused by *A. alternata* appears in rabi sown crop only in northern and eastern parts of India. Leaf blight caused by *A. alternata* is the major disease on sunflower in Greece, Argentina (Lagopodi and Thanassoulopoulos, 1998) Naresh *et al*. (2012) investigated intra; inter specific polymorphisms among the isolates of *A. carthami*, by employing 20 RAPD and 15 ISSR markers. The per cent of polymorphosim was observed as 65 % and 50.6 % with RAPD and ISSR primers respectively. Genetic similarity co-efficient differentiated all isolates from each other and

****Corresponding author:*** **Naresh, N.,**
Crop Protection, ICAR –Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500030, Telangana, India.

revealed considerable variation between the isolates. The present investigation is proposed to conduct a study on sunflower-*Alternaria* system with an aim to detect variability among the isolates selected.

MATERIALS AND METHODS

Pathogen isolates

Survey was conducted on Alternaria leaf blight severity in different sunflower growing areas of India during 2009-10 and 2010-11. In 2010-11, In West Bengal disease severity range was 41-50% in South 24 Parganas, while in Haryana disease severity range was 31-40% in Hisar. In Pujab disease severity range was 1-10% in areas of Fategarh sahib, while it was 41-50% in Ludhiana and low disease of 1-10%was recorded in Udam sing nagar of Uttaranchal. Samples collected from Karnataka, Maharashtra, Andhra Pradesh, Tamilnadu, Bihar, West Bengal yielded *A. helianthi*, where as samples collected from Uttaranchal, Punjab and Haryana gave rise to *A. alternata*. In Punjab and Uttaranchal, disease severity of 1-30% was recorded. Thirty isolates of *A. alternata* from sunflower growing areas of Haryana, Punjab, Maharashtra and Uttaranchal during 2009-10, 2010-11and 2011-12. Morphological and cultural characters like length, width, septa of fifty conidia, radial growth of culture, pigmentation and culture margin in petriplate were studied.

Genetic variability

The genetic variability among thirty isolates of *A. alternata* was assessed using ISSR primers, a more potential DNA fingerprinting technique (Sexton and Howlett, 2004).

Statistical Analysis

Completely Randomized Design (CRD) was used for analyzing the data obtained on colony diameter and morphological data. Dendrogram was generated using NTSYSpc software. Binary data was scored from the gel pictures '1' for the presence of the band and '0' for the absence of the band. Binary data was filled in excel sheet and converted to NTEDIT data using NTSYSpc NTEDIT. Similarity was generated among the isolates using the SIMQUAL function from the similarity tab. using the similarity data Dendrogram was generated using SHAN function using UPGMA analysis from clustering tab from NTSYSpc software.

RESULTS AND DISCUSSION

Cultural characterization

The results of colony growth pattern, colony diameter, pigmentation, color of aerial mycelium and sporulation are presented in (Table 1),

Table 1. Cultural characters of 30 isolates of *Altenaria alternata*

	Colony characters		Pigmentation	Color of aerial mycelium	Sporoulation	
Isolate	Growth pattern	Diameter (mm)			x10^4 spore/ml	Grade
*A a*1	Circular	53	Grey	Light grey	8.8	++++
*A a*2	Circular	47	Light grey	Grey to green	9	++++
*A a*3	Circular	53	Grey	Grey	7.2	+++
*A a*4	Circular	51	Light green	Light grey	7.6	+++
*A a*5	Circular	49	Green	Grey to green	6.8	+++
*A a*6	Irregular	48	Dark green	Light green	6.5	+++
*A a*7	Irregular	47	Dark green	Light green	6.6	+++
*A a*8	Irregular	54	Grey	Light grey	6	++
*A a*9	Irregular	50	Grey	Light grey	5.8	++
*A a*10	Circular	51	Grey	Yellow to grey	7.4	+++
*A a*11	Circular	50	Yellow grey	Yellow	10	++++
*A a*12	Circular	53	Yellow grey	Yellow	10.2	++++
*A a*13	Irregular	53	Dark grey	Yellow	7	+++
*A a*14	Circular	54	Green	Light green	6.8	+++
*A a*15	Irregular	49	Green	Light green	6.2	+++
*A a*16	Circular	57	Dark green	Grey to green	7.9	+++
*A a*17	Irregular	59	Dark green	Light green	8	+++
*A a*18	Irregular	48	Yellow to grey	Yellow	7.7	+++
*A a*19	Circular	50	Grey to green	Dark yellow	6.6	+++
*A a*20	Circular	52	Grey	Light grey	6	++
*A a*21	Circular	54	Grey	Light grey	3.8	+
*A a*22	Circular	54	Grey	Light grey	3.9	+
*A a*23	Circular	59	Grey	Yellow to grey	5.6	++
*A a*24	Irregular	56	Dark green	Light green	8	+++
*A a*25	Circular	56	Dark green	Green	7.2	+++
*A a*26	Irregular	57	Grey	Light grey	7.6	+++
*A a*27	Circular	60	Grey	Light grey	6.4	+++
*A a*28	Circular	59	Light grey	Light grey	4	++
*A a*29	Irregular	58	Grey	Yellow	4.8	++
*A a*30	Irregular	57	Light green	Yellow	5.8	++
SED	-	1.56	-	-	2.20	-
CD(P ≥ 0.05)	-	3.19	-	-	1.82	-
CV%	-	3.43	-	-	1.56	-

Note:	Grade	Sporulation	Spore load
	+	Scanty	< 4 x 10^4 spore ml
	+ +	Moderate	4.1 - 6 x 10^4 spore ml
	+++	Good	6.1 - 8 x 10^4 spore ml
	++++	Abundant	> 8 x 10^4 spore ml

Table 2. Morphological characters of 30 isolates of *Alternaria alternate*

Isolate	Conidial measurement		Septation (no.) *	
	Length (µm) *	Width (µm) *	Transverse	Longitudinal
*A a*1	90	30	5	2
*A a*2	91	36	6	3
*A a*3	92	32	6	3
*A a*4	87	30	5	2
*A a*5	86	26	6	1
*A a*6	88	25	5	2
*A a*7	84	24	5	2
*A a*8	85	26	8	3
*A a*9	70	27	6	4
*A a*10	68	26	3	1
*A a*11	88	28	5	2
*A a*12	90	32	5	2
*A a*13	91	30	6	3
*A a*14	87	34	5	3
*A a*15	85	32	6	4
*A a*16	84	30	6	3
*A a*17	84	34	5	2
*A a*18	85	36	5	2
*A a*19	78	36	5	3
*A a*20	81	39	6	3
*A a*21	57	27	5	2
*A a*22	60	32	6	3
*A a*23	89	38	6	4
*A a*24	92	29	4	1
*A a*25	90	26	5	4
*A a*26	90	32	5	3
*A a*27	88	35	6	3
*A a*28	87	34	3	2
*A a*29	78	30	5	4
*A a*30	80	30	6	3
SED	2.2	1.72	1.08	1.9
CD(P ≥ 0.05)	4.5	3.52	2.22	3.9

*Average of 50 conidial observations.

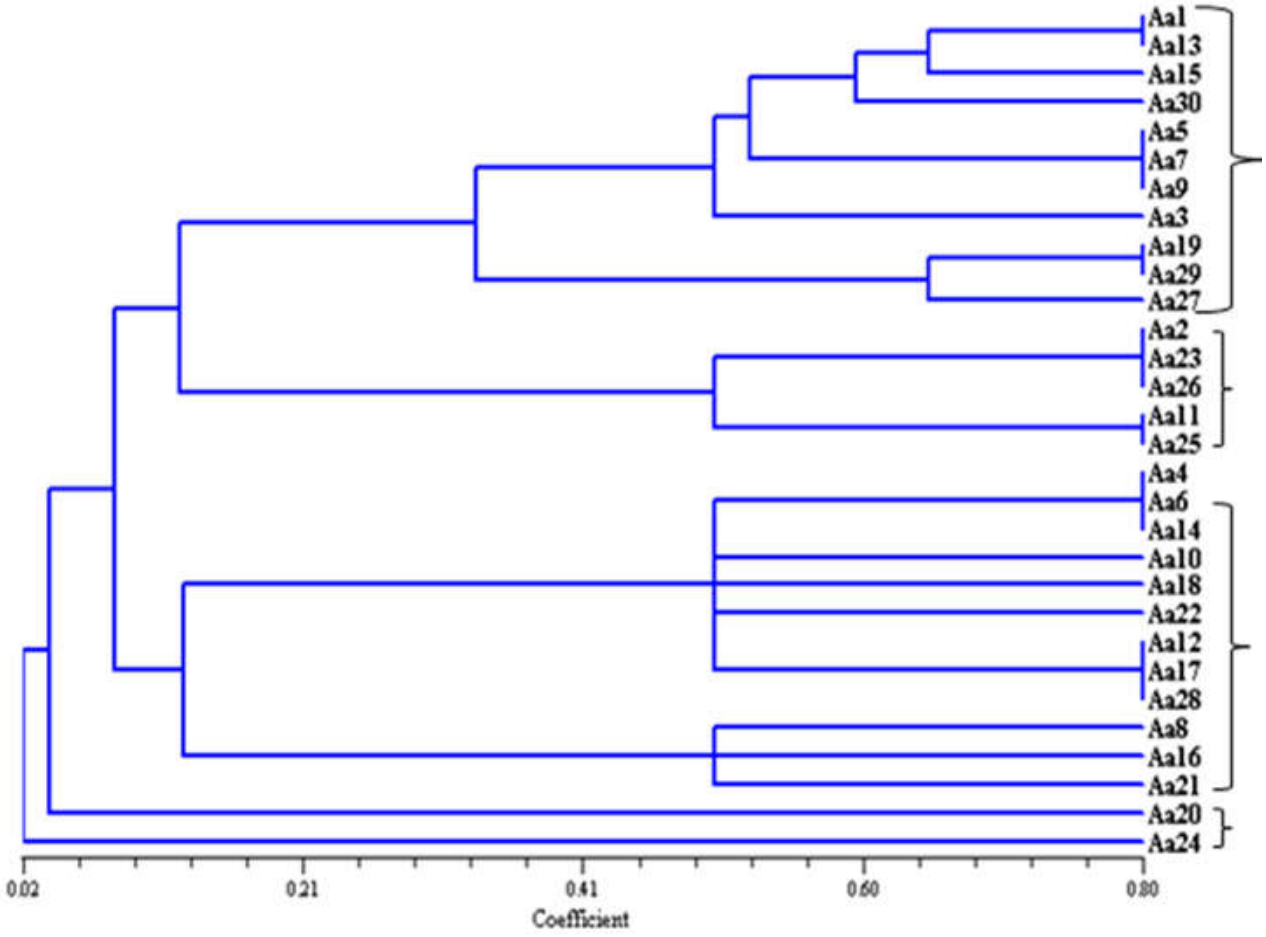

Figure 1. Dendrogram based on conidial characters of isolates of *A. alternata*

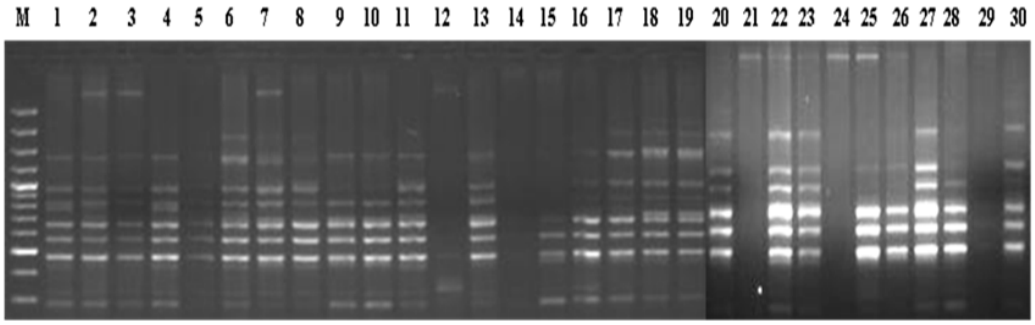

Figure 2. ISSR bands amplified by primer UBC-845. M = marker, Lanes 1-30 *A. alternata* isolates

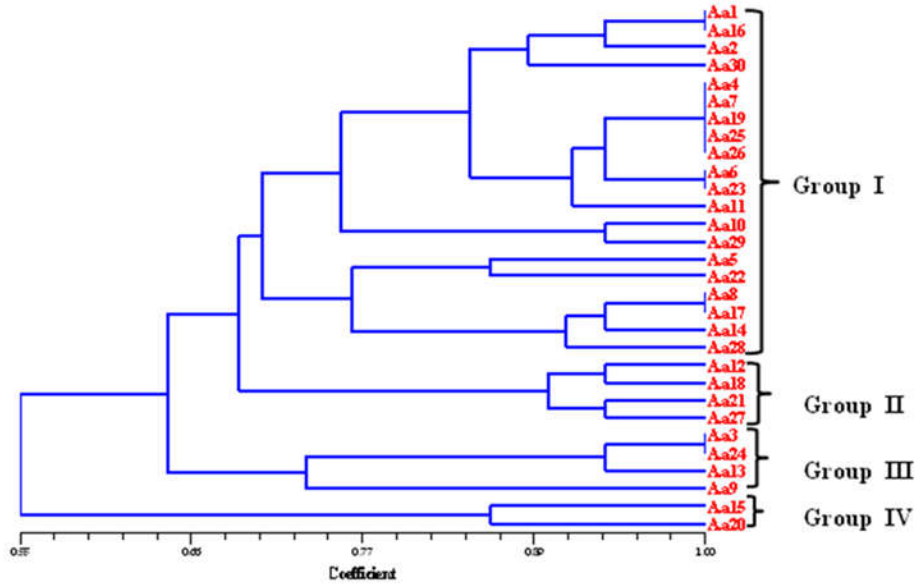

Figure 3. Dendogram representing molecular variation among 30 isolates of *Alternaria alternata*

the colony growth pattern was either circular with the mycelium showing a uniform growth pattern or irregular with mycelium giving irregular growth pattern. Among the 30 isolates, only 12 isolates (*A a*6, *A a*7, *A a*8, *A a*9, *A a*13, *A a*15, *A a*17, *A a*18, *A a*24, *A a*26 *A a*29, *A a*30) showed irregular growth and the remaining isolates (*A a*1, *A a*2, *A a*3, *A a*4, *A a*5, *A.a*-10, *A a*11, *A a*12, *A a*14, *A a*16, *A a*19, *A a*20, *A a*21, *A a*22, *A a*23, *A a*25, *A a*27, *A a*28) recorded circular growth pattern. Based on variation in pigmentation color of aerial mycelium of 30 isolates were categorized into five types viz; Light green to light grey pigmentation with light grey to yellowish aerial mycelium, Dark grey to dark green pigmentation with light green to light grey aerial mycelium, Grey to light green pigmentation with light grey to yellowish aerial mycelium and yellowish to light grey pigmentation with yellowish aerial mycelium. Among the 30 isolates of *A. alternata* studied, isolates (*A a*1, *A a*2, *A a*4, *A a*13 and *A a*19) produced light green to light grey pigmentation with light grey to yellowish aerial mycelium, while the isolates, (*A a*5, *A a*6, *A a*7, *A a*14, *A a*15, *A a*16, *A a*17, *A a*24 and *A a*25) produced dark grey to dark green pigmentation with light green to light grey aerial mycelium, similarly isolates(*A a*8, *A a*9, *A a*10, *A a*20, *A a*21, *A a*23,*A a*26 and *A a*28) produced grey to light green pigmentation with light grey to yellowish aerial mycelium, while the isolates (*A a*11, *A a*12, *A a*18, *A a*29 and *A* a30) showed yellowish to light grey pigmentation with yellowish aerial mycelium and the isolates (*A a*22 and *A a*27) showed light green pigmentation with yellowish aerial mycelium.

Morphological characterization

Thirty isolates of *A. alternata* have shown considerable variation with respect to conidial length, width, and septation. Across the isolates, the range of average conidial length varied from 57 to 92 µm. The isolate *A a*3 (92 µm) had maximum average conidial length followed by *A a*2 and *A a*14 (91 µm). The isolate *A a*1, *A a*13, *A a*25, *A a*26 (90µm) while the isolate *A a*21 (57µm) has recorded a minimum average conidial length followed by *A a*23 (60µm). The average conidial width also ranged from 24 to 39 µm across the isolates. The isolate *A a*20 showed maximum conidial width (39 µm) followed by *A a*23 (38µm) and *A a*2 (36µm). While that of minimum was observed in *A a*7 (24µm) followed by *A a*6 (25µm) and *A a*5 (26µm) (table 2). The average of transverse conidial septation was ranged between 3 to 6. Maximum number of septa were observed in isolate *A a*2 (6) and *A a*3 (6) followed by *A a*1 (5), *A a*4 (5) and *A a*24 (4). The conidial morphological characters of *A. alternata* isolates are in accordance with those described by Tubaki and Nishihara (1969). However, Kual and Saxena (1989) opined that the spore dimensions were not useful in distinguishing *A. solani* strains. Morphological and cultural variations are essential characteristics for differentiating the fungal isolates at the preliminary stage of isolate selection for variation studies. Infact, it is very difficult to group them into distinct categories, variation exist between the isolates in one or the other respect as they were obtained from different locations.

Table 3. Genetic Distance of 30 isolates of *Alternaria alternata* collected from different sunflower growing areas of India

A.a 1	*A.a* 2	*A.a* 3	*A.a* 4	*A.a* 5	*A.a* 6	*A.a* 7	*A.a* 8	*A.a* 9	*A.a* 10	*A.a* 11	*A.a* 12	*A.a* 13	*A.a* 14	*A.a* 15	*A.a* 16	*A.a* 17	*A.a* 18	*A.a* 19	*A.a* 20	*A.a* 21	*A.a* 22	*A.a* 23	*A.a* 24	*A.a* 25	*A.a* 26	*A.a* 27	*A.a* 28	*A.a* 29	*A.a* 30
1.00																													
0.93	1.00																												
0.67	0.77	1.00																											
0.86	0.77	0.67	1.00																										
0.22	0.40	0.55	0.55	1.00																									
0.93	0.86	0.77	0.93	0.40	1.00																								
0.86	0.77	0.67	0.96	0.55	0.93	1.00																							
0.67	0.55	0.67	0.86	0.77	0.77	0.86	1.00																						
0.77	0.86	0.77	0.55	0.67	0.67	0.55	0.55	1.00																					
0.77	0.67	0.55	0.77	0.67	0.86	0.77	0.77	0.67	1.00																				
0.77	0.67	0.55	0.93	0.67	0.86	0.93	0.77	0.67	0.86	1.00																			
0.55	0.55	0.55	0.77	0.86	0.67	0.77	0.77	0.40	0.67	0.67	1.00																		
0.55	0.67	0.93	0.77	0.67	0.67	0.77	0.77	0.67	0.40	0.67	0.67	1.00																	
0.55	0.40	0.55	0.77	0.86	0.67	0.77	0.93	0.67	0.86	0.86	0.67	0.67	1.00																
0.67	0.55	0.40	0.67	0.55	0.55	0.67	0.67	0.22	0.55	0.55	0.77	0.55	0.55	1.00															
0.98	0.93	0.67	0.86	0.22	0.93	0.86	0.67	0.77	0.77	0.77	0.55	0.55	0.55	0.67	1.00														
0.67	0.55	0.67	0.68	0.86	0.77	0.77	0.86	0.90	0.55	0.77	0.77	0.93	0.93	0.67	0.67	1.00													
0.67	0.77	0.40	0.67	0.77	0.55	0.67	0.67	0.55	0.55	0.55	0.93	0.55	0.55	0.86	0.67	0.67	1.00												
0.86	0.77	0.67	0.92	0.55	0.93	1.00	0.86	0.55	0.77	0.93	0.77	0.77	0.77	0.67	0.86	0.86	0.67	1.00											
0.67	0.55	0.40	0.40	0.55	0.55	0.40	0.40	0.55	0.77	0.55	0.55	0.22	0.55	0.86	0.67	0.40	0.67	0.40	1.00										
0.67	0.77	0.67	0.86	0.77	0.77	0.86	0.67	0.55	0.55	0.77	0.93	0.77	0.55	0.67	0.67	0.67	0.86	0.86	0.40	1.00									
0.55	0.67	0.55	0.77	0.86	0.67	0.77	0.77	0.67	0.86	0.86	0.86	0.67	0.86	0.55	0.55	0.77	0.77	0.77	0.55	0.77	1.00								
0.93	0.86	0.77	0.93	0.40	0.85	0.77	0.77	0.67	0.86	0.86	0.67	0.67	0.67	0.55	0.93	0.77	0.55	0.93	0.55	0.77	0.67	1.00							
0.67	0.77	1.00	0.67	0.55	0.77	0.67	0.67	0.55	0.55	0.55	0.93	0.55	0.40	0.67	0.67	0.40	0.67	0.40	0.67	0.55	0.77	0.40	1.00						
0.86	0.77	0.67	0.91	0.55	0.93	0.88	0.86	0.55	0.77	0.93	0.77	0.77	0.77	0.67	0.86	0.86	0.67	0.88	0.40	0.86	0.77	0.93	0.67	1.00					
0.86	0.77	0.67	0.88	0.55	0.93	0.86	0.86	0.55	0.77	0.93	0.77	0.77	0.77	0.67	0.86	0.86	0.67	0.86	0.40	0.86	0.77	0.93	0.67	1.00	1.00				
0.77	0.86	0.55	0.77	0.67	0.67	0.77	0.55	0.67	0.40	0.67	0.86	0.67	0.40	0.77	0.77	0.55	0.93	0.77	0.55	0.93	0.67	0.67	0.55	0.77	0.77	1.00			
0.77	0.67	0.77	0.93	0.67	0.86	0.93	0.93	0.67	0.67	0.86	0.67	0.86	0.86	0.55	0.77	0.93	0.55	0.93	0.22	0.77	0.67	0.86	0.77	0.93	0.93	0.67	1.00		
0.86	0.77	0.40	0.67	0.55	0.77	0.67	0.67	0.77	0.93	0.77	0.55	0.22	0.77	0.67	0.86	0.67	0.67	0.67	0.40	0.77	0.77	0.40	0.67	0.67	0.55	0.55	0.55	1.00	
0.86	0.93	0.86	0.86	0.55	0.93	0.86	0.67	0.77	0.77	0.77	0.77	0.77	0.55	0.40	0.86	0.67	0.67	0.86	0.40	0.86	0.77	0.93	0.86	0.86	0.86	0.77	0.77	0.67	1.00

The variability of *A. helianthi* was in confirmation with the earlier studies conducted by Santha Lakshmi Prasad *et al*. (2009). Where in the classified 30 isolates of *A. helianthi* into four distinct groups based on cultural and morphological characters. Based on spores produced per unit area on culture medium, the isolates of *A. alternata* were categorized in to 4 types viz; scanty sporulating (<4×10^4 spores ml^{-1}) isolates (*A a*21 and *A a*22), moderate sporulating (4.1-6×10^4 spores ml^{-1}) isolates (*A a*8, *A a*9,*A a*20,*A a*28,*A a*29 and *A a*30), good sporulating (6.1-8×10^4 spores ml^{-1}) isolates (*A a*3, *A a*4, *A a*5, *A a*6, *A a*7, *A a*10, *A a*13, *A.a-14, A a*15, *A a*16, *A a*17, *A a*18, *A* a19, *A a*24, *A a*25, *A a*26 and *A a*27) and abundant sporulating (8.1-10.2×10^4 spores ml^{-1}) isolates (*A a*1, *A a*2, *A a*11 and *A a*12).

Grouping of isolates based on morphological and colony character. Thirty isolates of *A.alternata* were studied for colony growth pattern, colony diameter, pigmentation, color of aerial mycelium and sporulation and Principal component analysis showed 4 broad groups. Group I consisted of 11 isolates; Group II Includes 5 isolates while Group III contained 12 isolates and Group IV consisted of two isolates (Fig. 1)

Molecular variability studies

Hundred ISSR primers were used to study the genetic diversity among thirty isolates of *A. alternata,* out of these twelve primers were selected as polymorphic based on their repeatability of banding pattern.

A total of 185 bands were obtained with 142 bands (76%) polymorphic from PCR amplification with 12 primers using genomic DNA from 30 *A alternata* isolates (Fig 2). The similarity matrix indicated that most of the isolates exhibited 0. 98% similarity coefficient. The maximum genetic similarity observed with ISSR was 0.98 % between *A a*1 and *A a*16, while the lowest genetic similarity of 0.22 % was observed with *A a*1 and *A a*5 (Table 3). Combined data set of amplified bands obtained for all isolates and primers was analyzed using UPGMA method and Jaccard's coefficient. The resulting dendrogram showed that isolates were divided into five groups in the dendrogram generated with the ISSR markers at genetic similarity of 0.55%. Group I was the largest consisting of 14 isolates and it was subdivided into three subgroups with 82.2 % similarity. The isolates *A a*1, *A a*16, *A a*2 and *A a*30 formed the first subgroup with 88 % genetic similarity, while *A a*4, *A a*7, *A a*19, *A a*25, *A a*26, *A a*6, *A a*23 and *A a*11 formed second subgroup with 92 % genetic similarity, *A a*10 and *A a*29 formed 3 rd sub group respectively. Group II had six isolates (*A a*5, *A a*22, *A a*8, *A a*17, *A a*14 and *A a*28) with 76 % genetic similarity among them. The isolate *A a*12, *A a*18, *A a*21 and *A a*27 formed Group III the genetic similarity was 91%. Group-IV was divided into two sub clusters containing three (*A a*3, *A a*24 and *A a*13) and one isolates (*A a*9) with 71 % genetic similarity. Isolates *A a*15 and *A a*20 having the common host sunflower, but from different locations namely Bilaspur, (Punjab) and Ludhiana, (Punjab) includes Group V the genetic similarity was 87 % (Fig 3). The breeding programmes are based mainly on the introduction and assessment of a large number of lines of different origins. Similarly, in case of *A. brassicicola,* Bock *et al*. (2002) reported that multiple isolates collected from particular locations tended to cluster together implying the potential for population structure and moderate levels of genetic diversity existing within isolates. Naresh *et al*. (2012) investigated intra; inter specific polymorphisms among the isolates of *A. carthami*, by employing 20 RAPD and 15 ISSR markers. The per cent of polymorphosim was observed as 65 % and 50.6 % with RAPD and ISSR primers respectively. Genetic similarity co-efficient differentiated all isolates from each other and revealed considerable variation between the isolates. Based on the results obtained in the present study demonstrates the morphological and genetic variation exists in the *A. alternata* isolates, which were collected from different areas of India.

Acknowledgement: The authors are thankful to the Project Director, India Institute of Oilseeds Research, Hyderabad for constant encouragement and for providing necessary facilities to carry out the research work.

REFERENCES

Bock, C.H., Thrall, P.H., Brubaker, C.L and Burdon, J.J. 2002. Detection of genetic variation in *Alternaria brassicicola* using AFLP fingerprinting. *Mycological Research*. 106: 428-434.

Kaul, A.K and Saksena, H.K. 1989. Conidial morphology of isolates of *Alternaria solani* showing cultural and pathogenic variability. *Plant Disease Research*. 4: 184-186.

Kintzios S, Koliopoulos A, Karyoti E, Drossopoulos J, Holevas CD, Grigoriu A and Panagopoulos CG. 1996. In vitro reaction of sunflower (Helianthus annuus L.) to the toxin (s) produced by Alternaria alternata, the casual agent of brown leaf spot. *Journal of Phytopathology* 144, (9-10): 465–470.

Kolte S J. 1985. Diseases of annual edible oilseed crops. Pages 9-96 in: Sunflower, Safflower and Nigerseed Diseases. Vol. III. CRC Press.

Lagopodi A L and Thanassoulopoulos C C. 1998. Effect of a leaf spot disease caused by *Alternaria alternata* on yield of sunflower in Greece. *Plant Dis*. 82:41-44.

Naresh, N., Santha Lakshmi Prasad, M and Sujatha, K. 2012. Molecular characterization of *Alternaria carthami* of safflower using RAPD and ISSR markers. *Journal of Oilseeds Research*. 29 (*Special issue*): 336-338.

Prathuangwong S, Kao SW, Sommartya T and Sinchaisri P. 1991. Role of four *Alternaria* spp. causing leaf and stem blight of sunflower in Thailand and their chemical controls. Kasetsart J. Natur. Sci. 25:112-124.

Santha Lakshmi Prasad, M., Sujatha, M and Chander Rao, S. 2009. Analysis of cultural and genetic diversity in *Alternaria helianthi* and determination of pathogenic variability using wild *Helianthus* species. *Journal of Phytopathology*. 157: 609-617.

Sexton AC and Howlett BJ 2004. Microsatellite markers reveal genetic differentiation among populations of Sclerotinia sclerotiorum from Australian canola fields. *Curr. Genet*. 46: 357-365

Simmons EG. 1986. *Alternaria* themes and variations (17-21). VII. Some species of *Alternaria* on *Helianthus*. *Mycotaxon* 25: 203- 216.

Tubaki, K and Nishihara, N. 1969. *Alternaria helianthi* (Hansf.) Comb. Nov. *Transactions of British Mycological Society*. 53: 147-149.

****