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RESEARCH ARTICLE

Occurrence of Alternaria leaf blight of groundnut in Gujarat and reaction of some genotypes against the disease

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ABSTRACT: Outbreak of a hitherto minor disease, Alternaria leaf blight was observed in farmer's fields during 2009 in summer crop of groundnut in Saurashtra and Kuchchh regions of Gujarat. Though, this disease has been reported to occur two decades ago, this is the first report of its widespread occurrence from any state of India. The characteristic symptoms of the disease are blighting of apical portions of the leaflets, which later curl inward and become brittle giving the leaf a ragged appearance. The pathogen identified was *Alternaria alternata* (Fr.) Keissler on the basis of its morphological and growth characteristics. The pathogenicity test confirmed the etiology. The disease severity in farmers' fields in Junagadh and Rajkot districts of Gujarat ranged from zero to 67 percent across the cultivars and crop growth phases. The cultivars, TG-37A, TPG-41 and JL-42 were found more susceptible than others. Losses of crop were in terms of reduced pod and fodder yields. The outbreak of the disease could be due to changes in cropping patterns, the cultivars and the climatic parameters.

Key words: *Alternaria alternata,* Alternaria leaf blight, groundnut

Diseases are one of the major constraints in achieving high production of groundnut (*Arachis hypogaea* L.) crop. The foliar fungal diseases, early leaf spot (*Cercospora arachidicola* Hori), late leaf spot [*Phaeoisariopsis personata* (Berk. & Curt) V. Arx] and rust (*Puccinia arachidis* Speg.) are widespread and destructive. The magnitude of losses in yield due to these diseases range from 10 to 70% (Ghewande, 1990). In last few years, however, there has been a change in the appearance of diseases and their relative importance in groundnut. The seed and seedling diseases *viz.*, collar rot (*Aspergillus niger* Van. Teighem) and stem rot (*Sclerotium rolfsii* Sacc.) have assumed importance because they cause severe seedling mortality resulting in 'patchy' crop stand and associated loss of yield from 25 to 50% (Chohan, 1974; Ghewande, 1985; Pande and Rao, 2000). Similarly, among the viral diseases, peanut bud necrosis and peanut stem necrosis diseases cause significant losses to the crop. The relative economic importance of these diseases varies in different states due to local cultivation practices, the environment and the production system. The leaf blight disease caused by different species of *Alternaria* has been hitherto a minor disease. The leaf blight disease of groundnut caused by *Alternaria alternata* (Fr.) Keissler, was reported by Balasubramanian (1979), Subrahmanyam *et al.* (1981), Vyas *et al.*(1985) and Narain *et al.*(1987). Patil and Hiremath (1989) and Ghewande *et al.* (1982) had reported *Alternaria tenuissima* (Kunze. Fr) Wiltshire causing leaf blight disease. Two other species of *Alternaria* reported from groundnut are *Alternaria arachidis* Kulk. described by Kulkarni (1974) which causes brown leaf spot symptoms, and *Alternaria longipes* described by Giri and Murugesan (1996) which cause necrotic leaf spots.

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During summer crop of 2009, occurrence of *Alternaria* leaf blight disease was noticed in severe form in farmers' fields in Junagadh, Rajkot and Kuchchh districts of Gujarat. Its occurrence was also observed in some of the cultivars at Directorate of Groundnut Research (DGR) experimental fields. Surveys were carried for severity of the disease in farmers' fields. The severity of the disease in commonly grown cultivars was recorded along with variation in symptoms, and the etiological study was made. Loss in yield due to the disease was estimated and the reaction of some cultivars and advance breeding lines to the disease was recorded at DGR experimental fields.

In this article the widespread occurrence of the leaf blight disease, symptoms, causal organism, severity of the disease, yield losses, apparent resistance in cultivars and likely impact of the disease on the production of summer groundnut is discussed.

MATERIALS AND METHODS

Isolation and identification of the pathogen

The pathogen from the blighted leaf was isolated on potato dextrose agar (PDA) medium at a temperature of 28±1°C and pure culture was obtained by hyphal tip culture. Identification of the pathogen was made on the basis of its morphology and growth characteristics. Dimension of the conidia and conidiophores was measured using ocular and stage micrometer.

Pathogenicity test

Koch's postulates were followed in confirming the pathogenicity. To harvest conidia and mycelial bits, sterile distilled water was added onto a 10-day old culture growing

on PDA slants and a few drops of Tween-20 (10 drops L^{-1}) were added. The conidial suspension thus obtained by shaking was sprayed on 30-day old plants of the cultivar JL-42, grown in plantation pots (30 × 30 cm) using a hand atomizer. After spraying plants were covered with transparent plastic cover and kept in the glasshouse at a temperature ranging from 32-35°C. In case of control plants sterile distilled water containing Tween-20 was sprayed.

Survey and scoring of disease in farmers field

Groundnut fields were randomly surveyed in Junagadh and Rajkot district for the occurrence and severity of leaf blight disease. The severity of the disease was recorded towards maturity phase of the crop (80-90 days after sowing). A 9-point scale was adopted for recording the severity similar to field screening of groundnut genotypes for resistance to late leaf spot (Subrahmanyam *et al*., 1995). Thirty leaves were collected from 10-randomly selected plants from top, middle and bottom of the crop canopy in each field and scored individually taking into account the leaf area damaged by the disease and the extent of defoliation in the plants, where 1 = 0%, 2 = 1-5%, 3 = 6 -10%, 4 = 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-60%, 8 = 61-80%, 9 = 81-100% disease severity. The percent disease severity index (PDI) was then calculated. This scoring was also followed for recording disease reaction of different cultivars and advance breeding lines at DGR experimental fields where the disease developed due to natural inoculums.

Estimation of yield loss

The yield loss corresponding to the different level of disease severity was estimated in four cultivars *viz.,* JL-42, ICR-3, TG-37A and GG-2. The different levels of disease severity in the field were due to natural inoculums. Twenty plants of each cultivar were selected from fields in the categories of apparently healthy (with minimum lesions) and with high disease severity for taking observations and calculating the PDI. These plants were harvested separately and used for calculating loss of pod and haulm yield.

Statistical analysis

The data was analyzed using randomized block design. Analysis of variance was carried out to determine least significant differences (LSD) between cultivars for percent disease severity, pod yield and haulm yield.

RESULTS

Symptoms

The characteristic symptoms of the disease were blighting of the apical portions of leaflets, which turned light to dark brown colour (Fig. 1). In the later stages of infection, blighted leaves curled inward and become brittle. Adjacent lesions coalesced giving the leaf a ragged and blighted appearance. Disease developed more rapidly on the upper portion of the canopy than on the lower portion. The disease progression was so fast that the full canopy was blighted in a week covering entire field. In pathogenicity test typical symptoms of the disease appeared 12-15 days after inoculation.

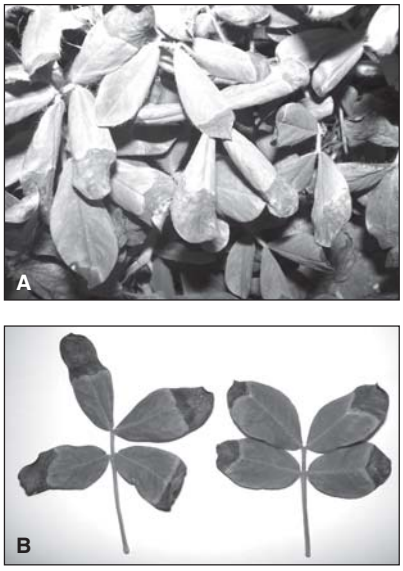

Fig. 1. Symptoms of the disease, A. Field view and B. Close up view

Isolation and identification of the pathogen

The same fungus was isolated consistently from the blighted leaf on potato dextrose agar (PDA) as a pure culture. Colonies on PDA were buff initially, later turning to grayish black (Fig. 2A), fast growing (9 cm in 10 d) and had well developed aerial mycelia. The fungus produced profusely branched, brownish, septate mycelia. Conidiophores arose singly or in small groups and were simple or branched. Conidia formed in long chains (often branched), oval to ellipsoidal, with 2-7 transverse and 1-4 longitudinal or oblique septa, tapering gradually to form a short swollen beak at the apex. Conidia had an overall length of 18-68 µm, width at broadest part of 4-12 µm and beak of 4-10 µm (Table 1; Fig. 2B). The number of conidia in chains varied from 2-8 (Fig. 2C). The characteristics of conidia from the cultures were similar to those of conidia isolated from the infected plants. On the basis of morphological characteristics, the fungus was identified as *Alternaria alternata* (Fr.) Keissler which was confirmed by a former mycologist, Indian Type Culture Collection, New Delhi. Re-

Table 1. Dimensions and characteristics of conidia of *Alternaria alternata*

Size	Length (µm)	Width (µm) (µm)	Beak length (µm)	No. of transverse septa	No. of longitudinal septa
Large	42-68	8-12	6-10	5-7	2-4
Medium	28-38	6-8	4-10	4-6	2-3
Small	18-26	4-6	4-6	2-4	1-2

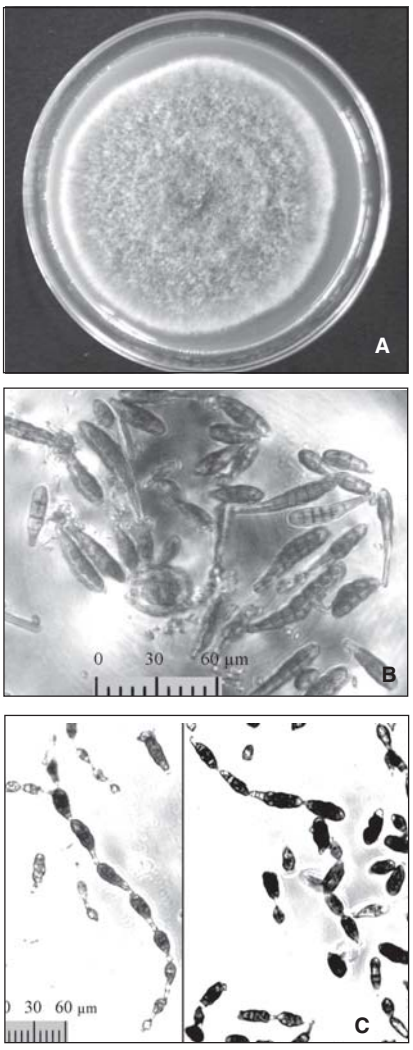

Fig. 2. A. Colony characteristics of *Alternaria alternata*, B. Conidia C. Conidia in chains

isolated fungus from the inoculated plants showing the typical blight symptoms resembled the original culture in its characteristics.

Disease severity

During 10th-15th May 2009, 15 groundnut-fields were randomly surveyed in eight villages covering three *talukas* (a unit under district) in Junagadh district, and 13 fields in eight villages covering two *talukas* in Rajkot district for the occurrence and severity of Alternaria leaf blight disease. The PDI in Junagadh in three cultivars viz. GG-2, TG-37A and TPG-41 ranged from zero to 48.9 (Table 2). Out of these 15 fields in Junagadh, two fields had cultivar GG-2 with no disease, five had TG-37A with PDI in the range of zero to 33.3 and eight had TPG-41 with PDI in the range of zero to 48.9. In Rajkot district, PDI in the four cultivars viz., GG-2, GG-20, TG-37A and TPG-41 were in the range of zero to 51.1. The PDI in the cultivar GG-2 was between zero to 24.4, in GG-20 was between zero to 15.6 and in TG-37A and TPG-41, between zero to 51.1. Out of 13 fields randomly surveyed, three were having cultivar GG-2, two GG-20, three TG-37A and five TPG-41. Except GG-20, all the cultivars belonged to Spanish bunch type.

Further, during 25th 30th May 2009, 20 fields in Junagadh district of Gujarat covering nine villages in five *talukas* were randomly surveyed for severity of the leaf blight disease. The results indicated that the PDI were in the range of 22.0 to 66.7. The severity of leaf blight was relatively more in the cultivars TPG-41 (26.7-66.7%) and TG-37A (30-56.7%), than in the cultivar GG-2 (22.0-33.0%) (Table 3). In another survey, similar disease severity of leaf blight was also observed in the farmers' field in summer crop of groundnut in Kuchchh district of Gujarat. Discussions with the farmers revealed that progression of the disease at near-maturity

Table 2. Disease situation in farmers' fields in Junagadh and Rajkot districts of Gujarat during 10th to 15th May 2009*

District: Junagadh					District: Rajkot				
Field No.	Taluka	Cropped area (ha)	Cultivar	Disease severity index (%)	Field No.	Taluka	Cropped area (ha)	Cultivar	Disease severity index (%)
1	Junagadh	0.4	TG-37A	33.3	1	Jetpur	0.5	TPG-41	51.1
2	Junagadh	0.4	TPG-41	0.0	2	Jetpur	0.4	TPG-41	48.9
3	Junagadh	0.3	TG-37A	0.0	3	Jetpur	0.5	GG-2	0.0
4	Junagadh	0.2	TPG-41	48.9	4	Jetpur	0.6	GG-2	24.4
5	Mendarda	0.5	TPG-41	24.4	5	Jetpur	0.4	TG-37A	37.8
6	Mendarda	0.4	TG-37A	31.1	6	Jetpur	0.3	TPG-41	37.8
7	Mendarda	0.3	TPG-41	0.0	7	Dhoraji	0.2	TG-37A	51.1
8	Mendarda	0.5	TPG-41	33.3	8	Dhoraji	0.5	GG-2	0.0
9	Mendarda	0.3	TG-37A	0.0	9	Dhoraji	0.4	TG-37A	0.0
10	Vanthali	0.3	TPG-41	35.6	10	Dhoraji	0.6	GG-20	15.6
11	Vanthali	0.4	TG-37A	26.7	11	Dhoraji	0.6	GG-20	0.0
12	Vanthali	0.4	TPG-41	0.0	12	Dhoraji	0.5	TPG-41	51.1
13	Vanthali	0.5	TPG-41	0.0	13	Dhoraji	0.4	TPG-41	0.0
14	Vanthali	0.5	GG-2	0.0					
15	Vanthali	0.3	GG-2	0.0					

*two weeks before harvest in early sown crop

Table 3. Disease situation in farmers' fields in Junagadh district of Gujarat during 25th to 30th May 2009*

Field No.	Taluka	Cropped area (ha)	Cultivar	Disease severity index (%)
1	Junagadh	0.4	TG-37A	56.7
2	Junagadh	0.4	TPG-41	53.3
3	Junagadh	0.3	TG-37A	60.0
4	Junagadh	0.2	TPG-41	50.0
6	Mendarda	0.4	TG-37A	46.7
7	Mendarda	0.3	TPG-41	63.3
8	Mendarda	0.5	TPG-41	56.7
9	Junagadh	0.6	TPG-41	26.7
10	Junagadh	0.7	TPG-41	60.0
11	Junagadh	0.5	TPG-41	66.7
12	Junagadh	0.4	GG-2	33.3
13	Junagadh	0.5	TG-37A	30.0
14	Junagadh	0.6	GG-2	26.7
15	Talala	0.5	TG-37A	40.0
16	Talala	0.3	TG-37A	30.0
17	Keshod	0.6	TPG-41	56.7
18	Keshod	0.3	GG-2	22.0
19	Visavdar	0.5	TG-37A	36.3
20	Visavdar	0.6	TG-37A	50.0
21	Visavdar	0.5	GG-2	30.0

*one week before harvest in late sown crop ** Field no. 1-8 of Junagadh are same as given in table 2. Field no 5 was harvested before this survey.

stage of the crop was very fast and within 7-10 days the disease attained the highest observed severity in fields.

Farmers of Gujarat have been growing Spanish bunch cultivars in summer, GG-2 being the ruling cultivar in terms of acreage. However, a perusal of the data in Table 2 and Table 3 revealed that out of the 48 fields surveyed, the maximum number of fields had the cultivar TPG-41 (43.75%) followed by TG-37A (33.33%), GG-2 (18.75%) and GG-20 (4.16%). The majority of farmers preferred TPG-41 and TG-37A to GG-2. This showed a shift in choice of cultivars by the farmers in these regions during the recent years.

Disease reaction of cultivars and advance breeding lines

Differential level of resistance was apparent in the cultivars and advance breeding lines (genotypes) at DGR experimental fields. A total of 43 cultivars and 43 advance breeding lines were scored for the leaf blight severity (PDI). Twenty-two cultivars and 27 advance breeding lines were found free from infection indicating resistance. Among the cultivars, GG-2, TKG-19A, ICGV-86031, JUG-47, SB-XI, and VG-9521 were moderately susceptible, having PDI < 30. The relatively more susceptible cultivars were TG-37A, ICGS-37, JL-24, JUG-48, AK-159, ICR-3**,** JUG-22, JUG-43, TPG-41, DRG-12, JAL-13, JUG-21, TRI-14, JAL-42, and ICR-41 with PDI in the range of 30.0 to 83.3 (Table 4, Fig. 3). Among the advance breeding lines, eight lines *viz.,* NRCG-CS Nos.' -239, 332, 212, 252, 264, 237, 302, and 304 had

Table 4. Severity of leaf blight in different cultivars and genotypes (Advance breeding lines) at DGR experimental fields during summer 2009

Sl. No.	Cultivar	Disease severity index (%)	Genotype	Disease severity index (%)
1	GG-3	0.0	NRCG CS-124	0.0
2	GG-4	0.0	NRCG CS-176	0.0
3	GG-5	0.0	NRCG CS-180	0.0
4	GG-6	0.0	NRCG CS-186	0.0
5	GG-7	0.0	NRCG CS-19	0.0
6	ICGS-11	0.0	NRCG CS-195	0.0
7	ICGS-44	0.0	NRCG CS-196	0.0
8	ICGV-0350	0.0	NRCG CS-222	0.0
9	ICGV-86590	0.0	NRCG CS-240	0.0
10	ICR-19	0.0	NRCG CS-242	0.0
11	ICR-21	0.0	NRCG CS-244	0.0
12	ICR-9	0.0	NRCG CS-247	0.0
13	JAL-3	0.0	NRCG CS-249	0.0
14	JAL-47	0.0	NRCG CS-27	0.0
15	JL-286	0.0	NRCG CS-280	0.0
16	JUG-24	0.0	NRCG CS-285	0.0
17	JUG-46	0.0	NRCG CS-296	0.0
18	SG-99	0.0	NRCG CS-298	0.0
19	TAG-24	0.0	NRCG CS-349	0.0
20	TG-26	0.0	NRCG CS-363	0.0
21	TIR-17	0.0	NRCG CS-396	0.0
22	TMV-2	0.0	NRCG CS-70	0.0
23	GG-2	13.3	NRCG CS-74	0.0
24	TKG-19A	20.0	NRCG CS-77	0.0
25	ICGV-86031	26.7	NRCG CS-83	0.0
26	JUG-47	26.7	NRCG CS-85	0.0
27	SB-XI	26.7	NRCG CS-86	0.0
28	VG-9521	26.7	NRCG CS-239	20.0
29	TG-37A	30.0	NRCG CS-332	20.0
30	ICGS-37	30.0	NRCG CS-212	23.3
31	JL-24	30.0	NRCG CS-252	23.3
32	JUG-48	33.3	NRCG CS-264	23.3
33	AK-159	36.7	NRCG CS-237	26.7
34	JUG-22	36.7	NRCG CS-302	26.7
35	JUG-43	36.7	NRCG CS-304	26.7
36	TPG-41	40.0	NRCG CS-127	33.3
37	DRG-12	43.3	NRCG CS-287	33.3
38	JAL-13	46.7	NRCG CS-305	36.7
39	ICR-3	56.7	NRCG CS-241	43.3
40	JUG-21	56.7	NRCG CS-291	46.7
41	TRI-14	63.3	NRCG CS-60	53.3
42	JAL-42	66.7	NRCG CS-253	56.7
43	ICR-41	83.3	NRCG CS-307	60.0
	LSD (P=0.05)	5.36		3.31

Fig. 3. Disease reaction of the two cultivars, A. Susceptible (JAL-42), B. Resistant (TIR-17)

PDI in the range of 20.0 to 30.0. Further, eight lines *viz*., NRCG-CS Nos.' -127, 287, 305, 241, 191, 60, 153, and 307 showed relatively high PDI ranged between 33.3 to 60.0.

Yield losses

The extent of yield loss depended on the time of appearance and severity of the disease. There was a reduction in pod bearing of the diseased plants (Fig. 4) and the kernels from such plants were by and large shriveled (Fig. 5). The data on estimates of losses in the four cultivars indicated that reduction in pod yield varied from 13 to 22% and the reduction in haulm yield from 24 to 63% (Table 5). The maximum reduction in yield was observed in ICR-3 which also had the highest disease severity (56.7%) amongst the selected cultivars.

Fig. 4. Reduction in pod bearing of the plant due to leaf blight: Left- healthy, Right – diseased

Fig. 5. Shriveling of kernels: Left – kernels from healthy plants, Right- kernels from diseased plants

Table 5. Estimates of loss of yield due to leaf blight disease in the four cultivars of groundnut

Cultivar	Disease severity index (%)	Reduction in pod yield (%)	Reduction in haulm yield (%)
JAL-42	34.0	19	36
ICR-3	56.7	22	63
TG-37A	30.0	17	32
GG-2	23.3	13	24
LSD (P=0.05)	2.97	3.19	4.36

DISCUSSION

The characteristic leaf blight symptoms of the disease observed was quiet different from the first report of the disease caused by *A. alternata* (Balasubramanian, 1979) where necrotic spots in the interveinal areas extending into vein and veinlets were observed. The fungus *A. alternata* is a widespread facultative pathogen of many important plants and causes disease in cotton (Patel *et al*., 1983; Singh *et al*., 1984), sesame (Rani and Thirupathaiah, 1983; Rant *et al*., 1984), sunflower (Godika *et al*., 2000) and many other crops. It has several weed hosts including aquatic weeds like water hyacinth (*Eichhornia crassipes*) and pistia (*Pistia stratiotes*). In the present studies, most of the farmers had taken cotton or groundnut in their fields during the rainy season of the previous year, and during summer farmers also grew sesame. Water hyacinth is commonly found in water reservoirs. The sources of the inoculums in epidemics of *Alternaria* leaf blight might be from sesame, sunflower or the weed hosts, besides the left over plants of previous season crops especially of cotton and groundnut. The weather parameters for the occurrence of the disease *viz.,* temperature (T) and relative humidity (RH) during the last phase of crop (from 2nd week of April to May) in the year 2008 and 2009 were almost similar (RH morning 75-81%, RH noon 36-41%, Tmin. 23-27^{0}C and Tmax. 36-42°C) except for a few degree of higher average temperature during 2009. However, the microclimate in the crop canopies during summer 2009 could have become favourable for spread and severity of the disease since summer groundnut is grown with assured irrigation, and the number of irrigation provided by flooding method by the farmers was more in 2009 than 2008. As *A. alternata* requires relative humidity of 85% and above (Reis *et al*., 2006) and optimum temperature range of 25-30°C for conidial production (the

maximum conidia being formed at 20°C and only a few above 32°C) (Stavely and Main, 1970), the possibility of a new strain which spreads fast at high temperature cannot be ruled out. Besides this the shift in choice of cultivar from GG-2 to TG-37A and TPG-41 might have provided a susceptible population of plants favouring spread of the disease. Generally Spanish bunch cultivars are grown in Gujarat in summer. Muthuswamy and Subramanian (1983) observed that most of progenies from the crosses of Virginia types cultivars were resistant to *A. alternata* whereas Spanish bunch types were susceptible. This support the fact that the leaf blight symptoms observed during summer were usually absent in rainy season crop, because mostly Virgina type cultivars are grown in rainy season in Gujarat.

The main effect of the disease was defoliation of plants due to which there was reduction in pod and haulm yield, effect on latter being drastic. Gujarat is one of the leading states of India in groundnut production. Summer groundnut is cultivated in about 1.5 lakh hectares with high average productivity (2000-3000 kg/ha). So far as summer crop is concerned, there had been a fewer incidence of diseases in Gujarat compared to rainy season crop. Alternaria leaf blight may become an important disease in summer groundnut. Further, in last few years there had been replacement of some area under groundnut by sesame crop. With this replacement of area and Alternaria leaf blight now likely to become a potential threat, it may adversely affect summer groundnut production in Gujarat. Hence, a detailed study on host range, survival and disease cycle needs to be undertaken on this disease.

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