JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2016. Vol. **10**(4), p. 3141-3150

Biochemical Characterisation and Responses of Resistant and Susceptible Groundnut Genotypes to Late Leaf Spot *(Phaeoisariopsis personata* (Berk. and Curt.) Von Arx.)

V.S. Shinde[1], K.S. Raghuwanshi[2], A.V. Suryawanshi[1] and R.M. Naik[3]

[1]Seed Technology Research Unit, Seed Cell, MPKV, Rahuri - 413 722, India.
[2]Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri, India.
[3]Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722 (M.S.), India.

http://dx.doi.org/10.22207/JPAM.10.4.90

(Received: 11 April 2016; accepted: 24 June 2016)

Late leaf spot (LLS) cause substantial yield losses and reduce the fodder and seed quality in groundnut (*Arachis hypogaea* L.). Knowledge on components of resistance to these diseases should facilitate the development of groundnut cultivars with enhanced resistance to LLS. The present investigation was performed to study the biochemical characterization of late leaf spot resistant groundnut genotypes *viz.,* RHRG 6083, GPBD 4, KDG 128, ICG 11426, ICG 12672, ICG 13919, ICG 14475, ICGV 94118, ICGV 13160, ICGV 13165 including two wild species ICGV 13160 (*Arachis batizocoi)* and ICGV 13165 (*A. cardenasi*) and two susceptible *viz.,* SB XI and JL 24 at AICRP on groundnut, MPKV, Rahuri during 2013-14 and 2014-15. The content of phenol and the enzyme activity of peroxidase and polyphenol oxidase was found more in resistant than susceptible genotypes, while total sugar content was found to be maximum in susceptible than resistant genotypes. There was reduction in reducing sugar content, while total sugar and total phenol and the enzyme activity of peroxidase and polyphenol oxidase was increased after inoculation of the pathogen in all groundnut genotypes. The reduction level of these biochemical constituents was less in resistant genotypes. Similarly, increased level of total sugar, total phenol, and peroxidase and polyphenol oxidase activity was more in resistant genotypes than susceptible genotypes.

Keywords: *Arachis hypogaea* L., Late leaf spot, resistance and biochemicals.

Groundnut (*Arachis hypogaea* L.), also known as peanut, originated from South America, occupies 9 per cent of the world's oilseed area, and contributes close to 5 per cent to vegetable oil production. Groundnut production in the world averages to 36.45 million tons from harvest of approximately 23.95 million ha with an average yield of 1520 kg/ha in 2009 and in India, it is, 8.26 million tons from 5.86 million ha with average productivity of 1411 kg/ha in 2010-11(Anon., 2015). The groundnut cultivation is unique in that it is being cultivated under three different production seasons like rainy (*kharif*), winter (*rabi*) and summer fitted into different cropping pattern. Hence, the problems and constraints are multi-varied and multi-faceted according to the production system involved. Among the biotic stresses; fungi, bacteria, viruses, nematodes and insects cause the losses. Fungal foliar diseases such as early leaf spot (ELS) caused by *Cercospora arachidicola* Hori., late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk and M.A. Curtis) Von Arx., webblotch (WB) caused by *Phoma arachidicola* Marasas, Pauer and Boerema, rust caused by *Puccinia arachidis* Spegazinni, collar rot caused by *Aspergillus niger* Van Tieghem, root rot caused by *Macrophomina phaseolina*

* To whom all correspondence should be addressed.
E-mail: shindevs27@gmail.com

(Tassi) Goid and stem rot caused by *Sclerotium rolfsii* Sacc. are important diseases on groundnut. Occurrences of these diseases results in reduction of quality and hamper yield upto 50-70% (Pretorius, 2005, Subrahmanyam *et al.*, 1980). Of the foliar fungal diseases, the two leaf spots together are popularly known as "*Tikka*" disease in India. Both early and late leaf spot are commonly present wherever groundnut is grown. However, the incidence and severity of each disease varies between locality and seasons.

Late leaf spot (LLS) disease caused by *Phaeoisariopsis personata* (Berk. and Curt.) von Arx. is globally wide spread and is the most important foliar disease of groundnut. Reduction in the yield due to late leaf spot is largely due to damage caused to the leaves as a result of intense spotting and consequent loss in the photosynthetic area (Gerlagh and Bokdman, 1974). The presence of variation in biochemical characters play important role in disease resistance in groundnut (Jyosthna, *et. al.,* 2004). It is essential to understand the basis of resistance operating in the newly identified genotypes and the way in which these are affected by disease development.

MATERIALS AND METHODS

One hundred fourteen groundnut genotypes including four wild species screened against late leaf spot under field conditions during kharif 2013-14 and 2014-15 at All India Coordinated Research Project on Groundnut, MPKV, Rahuri, Dist. Ahmednagar, Maharashtra. Seeds of different genotypes were procured from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, Agriculture Research Station, Kasbe Digraj, Dist. Sangali, Maharashtra and All India Coordinated Research Project on Groundnut, MPKV, Rahuri, Dist. Ahmednagar, Maharashtra.

Evaluation of genotypes and wild species against late leaf spot disease was carried out to identify the source of resistance under field condition by following "Infector row technique". Ten test genotypes of groundnut were sandwiched among the susceptible genotype (SB XI) and along the border were grown to maintain the effective inoculum load.

The disease infected leaves collected from the LLS infected field were soaked in water for half an hour. Then the LLS conidia were collected by rubbing the infected leaves in the water and used for inoculation on test material. Artificial late leaf spot disease epiphytotic was created in screening experiment. Thirty days after sowing, plants were inoculated uniformly in the evening with LLS for three successive days during evening hours. The inoculum contained 20,000 conidia per ml water and mixed with Tween 80 (0.2 ml/1000ml of water) as a mild surfactant and atomized on the plants using knapsack sprayer in field (Pandey *et al.,* 2002). High humidity was maintained by irrigating the field in the night by surface irrigation. The severity of late leaf spot was recorded on three compound leaves of the main stem chosen from bottom, middle and top position of five plants of each genotype with the interval of 10 days after inoculation (DAI)., using the scale 1-9 (Subramanyam *et al.,* 1995) (Table 1).

Ten resistant *viz*., RHRG 6083, GPBD 4, KDG 128, ICG 11426, ICG 12672, ICG 13919, ICG 14475, ICGV 94118, ICGV 13160, ICGV 13165 including two wild species ICGV 13160 (*Arachis batizocoi)* and ICGV 13165 (*A. cardenasi*) and two susceptible *viz.,* SB XI and JL 24 were selected for the biochemical studies. These selected genotypes sown in 12" plastic pots in triplicate under glass house conditions. Prior to sowing these pots were filled with sterilized soil, sand and compost mixture. Three plant per pot were maintained for further studies. After 30 days of sowing the plants were inoculated with LLS pathogen. One separate set of pots was maintained as uninoculated control. Sampling was done at an interval of 5 days from the date of inoculation i.e. 30 DAS and continued till 25 days after inoculation under glass house conditions. Similar sampling procedure was adopted for uninoculated control. The third leaves from apex of the LLS pathogen inoculated plants were collected and used for estimation of biochemicals. The collected leaf samples were placed in ice box containing ice cubes and brought to the laboratory for estimation of different biochemicals.

Reducing sugar

Reducing sugar content was determined by Nelson Somogyi method (Somogyi, 1952). The results were expressed by measuring absorbance at 520 nm nm using Spectronic-20

Spectrophotometer.

Total sugar

Total sugar content was determined by the method of Thimmaia (2004). Observations were taken at 490 nm by using following formula.

$$\text{Total Sugars in the sample (\%)} = \frac{\text{Sugar value from the graph}}{\text{Aliquot sample used (ml)}} \times \frac{\text{Total volume of Extract}}{\text{Wt. of sample}} \times \frac{1}{1000}$$

Total phenols

Total phenol content was determined by using Folin-Denis reagent as described by Swain and Hills (1959).The extinction measured at 650 nm on a Spectronic-20 Spectrophotometer.

Peroxidase (PO)

The peroxidase activity from the leaf tissue was assayed by the method of Maco *et al.* (1968). The absorbance was read at 420 nm on a Spectronic-20 Spectrophotometer for every 30 sec. up to 3 min.

One unit of peroxidase activity was determined as an increase in O.D. by 0.001. The enzyme activity was calculated for one g of sample by the formula:

$$\frac{1}{W} \times \frac{V_1}{1} \times \frac{1}{V_2} \times \frac{S}{I} \times \frac{T}{1}$$

$$\frac{I}{1}$$

Where,

W = Weight of sample.

V_1 = Final volume of aliquote.

V_2 = Extraction taken for activity.

S = Summation of change in OD.

I = Time in reading interval.

T = Time in minutes.

Polyphenol oxidase (PPO)

The polyphenol oxidase activity from the leaf portion was assayed by the method of Linskens *et al.* (1964). The absorbance was read at 420 nm on a Spectronic-20 for every 30 sec. up to 3 min. The PPO activity was calculated according to formula given for peroxidase.

The data generated from the various *in vitro* experiments were subjected to statistical analysis in Factorial Completely Randomized Design (FCRD) following the procedure given by Panse and Sukhatme (1985).

Table 1. Screening of groundnut genotypes in field to the LLS

SN	Scale	Reaction	Name of genotype
1.	> 3 (12)	Resistant	RHRG 6083,GPBD 4, KDG 128, ICG 11426, ICG 12672, ICG 13919, ICG 14475, ICGV 94118, ICGV 13160, ICGV 13165, ICGV 4983 and ICGV 8193 (12)
2.	4-5(51)	Moderately resistant	ICG 4492, KDG 123, KDG 160, KDG 171, KDG 178, KDG 187, KDG 204, KDG 209, RHRG 1007, ICG 2857, ICG 8760, ICG 11337, ICG 12625, ICG 13787, RHRG 6097, ICGV 86855, ICGV 99001, ICGV 99002, ICGV 86590, ,ICGV 86699, ICGV 94108, ICGV 96283, ICGV 96284, RHRG 6021, ICG 6421, ICG 3899, ICG 6323, ICG 5663, ICG 6223, ICG 4975, ICG 6080, ICG 5052, ICG 6330, ICG 5618, ICG 3992, ICG 6466, ICG 6519, ICG 6638, ICG 6552, ICG 6388, ICG 7766, ICG 5001, ICG 4581, ICG 5222, ICG 7815, ICG 6375, ICG 6331, ICG 6243, ICG 4230, ICG 7358 and ICG 5663 (51)
3.	6-7 (41)	Susceptible	ICG 5276, ICG 2252, ICG 5610, ICG 2186, ICG 6390, ICG 5963, ICG 6263, JL 501, RHRG 6110, ICG 5210, ICG 5636, ICG 2320, ICG 6381, ICG 6221, ICG 3294, ICG 5320, ICG 6550, ICG 5240, ICG 3585, ICG 6201, ICG 2367, ICG 6497, ICG 3136, ICG 6249, ICG 6224, ICG 5490, ICG 5256, ICG 5310, ICG 6592, ICG 8695, ICG 5195, ICG 3785, ICG 8264, ICG 6372, ICG 7406, ICG 6238, ICG 5387, ICG 3515, ICG 6487, ICG 6357 and ICGV 350 (41)
4.	8-9 (10)	Highly susceptible	SB XI, JL 24, TAG 24, ICG 6615, ICG 4508, ICG 6479, ICG 6168, ICR 48, WRGS 15 and K 8808 (10)

RESULTS

Screening of groundnut genotypes for Late leaf spot disease

Out of 114 groundnut genotypes screened against LLS under field conditions, 12 genotypes showed resistant and 51 genotypes showed the moderately resistant reaction to LLS disease. The 41 entries were showed the susceptible and 10 genotypes showed the highly susceptible reaction to the LLS disease (Table 1). The intensity of the LLS disease was recorded by 1-9 scale. On the basis of this screening and earlier reports ten resistant including two wild genotypes i.e. ICGV 13160 and ICGV 13165and two susceptible genotypes were chosen for intensive screening against LLS under pot condition. The results from field screening and pot culture revealed that resistant cultivated genotypes such as RHRG 6083, GPBD 4, KDG 128, ICG 11426, ICG 12672, ICGV 86590, ICGV 94118 and ICGV 96283 had shown very slow LLS progress as against high rate of disease progress on the susceptible, SB XI and JL 24 cultivars. From the results it was also evident that absolute resistance or immunity in cultivated genotypes is not available but high degree of resistance was available in many groundnut lines. The level of LLS resistance was similar to that found in slow rusting type reaction of the cereal crops. Infection by late leaf spot pathogen brings about lot of changes in respiratory pathway and photosynthesis which are the vital processes taking place in the plant leading to wider fluctuation in biochemical components. This inturn alters the resistance of the host. Some studies on biochemical components in resistant and susceptible

Table 2. Influence of late leaf spot on the reducing sugar content (µg/g fresh wt.) of resistant and susceptible groundnut genotypes

Sr. No.	Genotypes	DAI	0	5	10	15	20	25
1	ICG 13165	UNI	134.11	359.20	514.74	648.56	706.24	548.30
		INO	134.11	323.06	428.51	579.11	486.25	322.14
2	ICG 13160	UNI	135.59	352.56	511.09	640.15	699.40	534.59
		INO	135.59	328.59	429.00	578.59	480.35	325.49
3	RHRG 6083	UNI	145.38	362.38	541.38	670.44	739.90	654.88
		INO	145.38	330.36	437.44	622.38	496.71	306.50
4	KDG 128	UNI	153.31	386.45	544.24	679.31	724.40	654.34
		INO	153.31	344.70	459.28	607.35	506.55	304.31
5	GPBD 4	UNI	156.22	378.15	532.40	665.72	725.16	656.22
		INO	156.22	355.17	456.23	615.22	503.68	305.15
6	ICG 11426	UNI	141.71	360.58	525.60	650.71	725.74	655.71
		INO	141.71	330.74	435.58	609.74	504.42	319.21
7	ICG 12672	UNI	149.31	383.25	526.34	657.61	736.38	666.71
		INO	149.31	348.30	459.44	618.19	518.48	308.31
8	ICGV 94118	UNI	138.87	348.52	523.55	660.60	731.85	655.77
		INO	138.87	340.10	443.25	621.07	525.59	326.80
9	ICG 13919	UNI	144.11	372.24	530.0	659.45	734.37	659.61
		INO	144.11	341.12	449.06	630.11	548.35	328.21
10	ICG 14475	UNI	148.85	377.85	527.45	666.25	751.61	653.30
		INO	148.85	319.68	445.15	634.62	534.25	322.65
11	SB XI	UNI	165.49	306.99	434.40	579.32	644.15	454.49
		INO	165.49	235.46	360.56	527.04	384.39	204.44
12	JL 24	UNI	170.39	319.09	445.39	586.25	651.38	465.29
		INO	170.39	250.85	370.91	540.55	391.20	206.25
		SE±	0.033	0.029	0.030	0.030	1.77	0.032
		CD 5%	NS	0.083	0.087	0.085	5.03	0.091

(UNI : Uninoculated and INO : Inoculated)

groundnut genotypes were carried out as described in material and methods and the results are presented hereunder.

Sugar content

The data on the reducing sugar and the total sugar content in resistant and susceptible genotypes of groundnut influenced by the late leaf spot infection showed that under LLS free condition the difference in the reducing sugar between resistant genotypes were non significant. The reducing sugar content of the susceptible genotypes was higher than the resistant genotypes at 0 stage but subsequently it was found to significantly lower than the resistant genotypes.

LLS inoculation decline the reducing sugar content in susceptible and resistant genotypes 15 DAI but the rate of reduction was slightly more in susceptible lines. The rate of reduction of reducing sugars was more (55 per cent) in susceptible genotypes as compared to 39- 54 per cent in resistant genotypes (Table 2).

The total sugar content of susceptible was higher than the resistant genotypes. The initial high total sugar content of susceptible lines increased from 698.34 and 728.99µg/g fresh wt to 2401.79 to 2496.46µg/g fresh wt in SB XI and JL 24 respectively within 10 days. Subsequently there was reduction in the total sugars in all the genotypes (Table 3).

The LLS inoculations brought about significant reduction in the total sugar level of resistant genotype at the initial stage after inoculation i.e. 10 DAI but later there was a very high accumulation of sugars reaching to double the quantity of sugars than in the uninoculated samples. In susceptible lines the pathogen brought about significant accumulations of total sugars from the very beginning which reached at the level

Table 3. Influence of late leaf spot on the total sugar content (µg/g fresh wt.) of resistant and susceptible groundnut genotypes

Sr. No.	Genotypes	DAI	0	5	10	15	20	25
1	ICG 13165	UNI	536.25	949.57	1656.05	1484	1435.54	1230.53
		INO	536.25	748.54	1648.5	2081.07	1869.49	1746.51
2	ICG 13160	UNI	514.12	950.21	1691.49	1602.5	1427.76	1223.99
		INO	514.12	799.96	1620.07	2069.99	1901.59	1820
3	RHRG6083	UNI	598.84	1183.48	1899.78	1609.78	1457.96	1253.48
		INO	598.84	1005.03	1704.29	2227.04	2131.99	1912.04
4	KDG 128	UNI	601.12	1050.1	1991	1608.97	1450.27	1250.81
		INO	601.12	1016.99	1886.01	2186.05	2021.01	1985.58
5	GPBD 4	UNI	584.19	1191.98	1888.42	1608.42	1438.79	1238.42
		INO	584.19	1006.05	1717.02	2115.52	2000.52	1910.28
6	ICG 11426	UNI	558.18	993.71	1696.51	1315.04	1446.47	1228.71
		INO	558.18	990.84	1699.81	2099.84	1823.61	1794.64
7	ICG 12672	UNI	610.15	1165.11	1907.45	1613.01	1431.96	1193.62
		INO	610.15	1013.24	1544.14	2128.05	2053.04	1894.01
8	ICGV94118	UNI	568.18	1006.94	1807.28	1608	1465.97	1226.87
		INO	568.18	991.98	1742.62	2192.97	1898	1784.97
9	ICG 13919	UNI	541.18	1036.04	1885.98	1425.96	1436.04	1234.51
		INO	541.18	1022.02	1845.04	2203.74	2002.64	1752.61
10	ICG 14475	UNI	592.12	1110.08	1899.05	1614.98	1449.08	1233.55
		INO	592.12	1048.06	1753.95	2112	1806.99	1780.06
11	SB XI	UNI	698.34	1580	2401.79	2076	1917.04	1587.04
		INO	698.34	1364.03	2096.49	2320.05	2901.39	3608.96
12	JL 24	UNI	728.99	1535.16	2496.46	1860.05	1850.79	1468.42
		INO	728.99	1450.01	2179.96	2414.92	3103.03	3394.1
		SE±	0.055	0.046	0.043	0.040	0.083	0.035
		CD 5%	NS	0.132	0.124	0.114	0.236	0.101

(UNI : Uninoculated and INO : Inoculated)

of 70-106 per cent more than the resistant ones at the end of sampling.

Total phenol content

The data on total phenol content of the resistant and susceptible genotypes under LLS free and LLS pathogenesis conditions revealed that under LLS free conditions the total phenol content of resistant genotypes was significantly higher (50 per cent) than the susceptible genotypes at all the stages of observations. It was observed that quantity of total phenol accumulated in the seedling with the growth of plants irrespective of the genotypes and the inoculations. (Table 4).

LLS infection enhances the accumulations of total phenol in the leaf tissues. However, the phenol accumulation was more than 100 per cent in susceptible genotypes, after the symptoms were fully expressed. But in the resistant genotypes increase in total phenol content was only to the tune of 65 to 92 per cent over uninoculated conditions at 30 DAI in respective genotypes.

Peroxidase and polyphenol oxidase activity

The data on enzymatic activity revealed that the peroxidase activity was very high in the resistant tissues than the susceptible one. It was more than three times in resistant lines than the susceptible genotypes at the end of sampling.

The LLS infection however, enhanced the activity of peroxidase irrespective of genotypes. In susceptible line peroxidase were enhanced nearly 2 times as compared to 60 per cent increase in resistant lines (Table 5).

It was evident that differences in the polyphenol oxidase activity between the resistant and susceptible genotypes were marginal but PPO activity in resistant lines was more than susceptible ones.

Table 4. Influence of late leaf spot on the total phenol content (µg/gfresh wt.) of resistant and susceptible groundnut genotypes.

SN	Genotypes	DAI	0	5	10	15	20	25
1	ICG 13165	UNI	632.01	1258.97	2070.77	2379.71	2591.71	2643.51
		INO	632.01	2176.04	3491.04	4287.01	4389.01	4504.5
2	ICG 13160	UNI	626.99	1222.96	2160.99	2310.99	2505.99	2610.24
		INO	626.99	2298.01	3510.39	4321.39	4293.39	4412.39
3	RHRG 6083	UNI	605.98	1086.98	1636.98	1906.88	2103.88	2207.88
		INO	605.98	1990.04	3192.96	3855.68	4058.68	4121.74
4	KDG 128	UNI	600.01	1209.04	1790.01	2061.22	2161.21	2271.21
		INO	600.01	2008.01	3181.68	3808.51	3908.51	4377.47
5	GPBD 4	UNI	608.02	1089.03	1823.02	2192.55	2399.52	2494.52
		INO	608.02	1885.02	3314.47	3898.52	4002.47	4140.52
6	ICG 11426	UNI	611.01	1197.01	1611.89	2080.44	2385.41	2480.41
		INO	611.01	1926.47	3421.51	3955.51	4167.51	4268.51
7	ICG 12672	UNI	617.01	1015.01	1719.01	2189.36	2289.44	2489.35
		INO	617.01	1915.94	3406.91	3737.91	4200.25	4263.91
8	ICGV 94118	UNI	606.97	1184.97	1621.97	1896.97	2102.77	2303.77
		INO	606.97	1980	3443.97	3872.97	4015.15	4295.97
9	ICG 13919	UNI	601.01	1200.01	1812.01	2287.01	2406.11	2501.11
		INO	601.01	1959.89	3445.04	4176.51	4277.51	4312.96
10	ICG 14475	UNI	614.05	1210.05	1926.05	2197.45	2392.45	2567.45
		INO	614.05	1940.01	3234.99	4077.05	4172.05	4396.05
11	SB XI	UNI	214.99	417.05	523.69	987.48	1236.4	1360.48
		INO	214.99	827.93	1049.05	1725.77	1985.53	2760.85
12	JL 24	UNI	275.99	517.99	600.03	1095.78	1196.94	1289.45
		INO	275.99	749.99	1209.49	1699.89	2085.79	2596.85
		SE±	13.44	13.49	11.98	10.04	9.93	8.97
		CD 5%	NS	38.37	34.07	28.55	28.25	25.50

(UNI : Uninoculated and INO : Inoculated)

The PPO activity was generally increased by the LLS infection in all the genotypes. However, the activity was found to be fairly low in the susceptible genotypes than the resistant ones (Table 6).

DISCUSSION AND CONCLUSION

In the present studies, among the 114 groundnut genotypes screened against late leaf spot disease, 12 genotypes were showed the resistant reaction and 51 entries were showed the moderately resistant reaction. The 41 genotypes were susceptible and 4 entries were highly susceptible to the LLS disease. The LLS resistance *viz.,* RHRG 6083, GPBD 4, KDG 128, ICG 11426, ICG 12672, ICG 13919, ICG 14475, ICGV 94118, ICGV 13160, ICGV 13165, ICGV 4983 and ICGV 8193 and susceptible *viz.,* SB XI and JL 24 genotypes of groundnut was confirmed at AICRP on Groundnut, MPKV, Rahuri under field and glasshouse conditions. The LLS progress and severity which brought out the fact that the type of resistance was not necrogenous or hypersensitive but partial type resistance (Nevill, 1981; McDonald *et al.*, 1985).

Under LLS free conditions, the susceptible lines significantly differed in these metabolic processes over resistant ones. The susceptible lines SB XI and JL 24 exhibited higher amount of reducing sugars *viz.,* 165.49 & 170.39µg/g fresh wt., respectively and total sugars 698.34 & 728.99 µg/g fresh wt., respectively than the resistant genotypes during early growth phases under LLS free conditions. In contrast, the resistant genotypes were characterized by their high total chlorophyll (0.63 to 0.90 mg/gm fresh wt.), total phenols (600.01 to 632.01 µg/g fresh wt), high

Table 5. Influence of late leaf spot on the peroxidase activity (units/g/min) of resistant and susceptible groundnut genotypes

SN	Genotypes	DAI	0	5	10	15	20	25
1	ICG 13165	UNI	5.91	8.22	10.34	12.83	13.85	12.20
		INO	5.91	10.30	13.11	17.10	19.09	18.08
2	ICG 13160	UNI	5.86	8.03	10.03	12.74	13.58	12.09
		INO	5.86	9.99	12.89	16.88	18.80	17.69
3	RHRG 6083	UNI	5.63	7.53	9.65	11.97	12.85	10.51
		INO	5.63	9.94	12.73	16.04	17.59	16.38
4	KDG 128	UNI	5.52	7.42	9.44	11.82	12.82	10.42
		INO	5.52	9.74	12.64	15.75	17.46	16.37
5	GPBD 4	UNI	5.73	7.63	9.75	12.07	13.03	10.63
		INO	5.73	9.94	12.50	15.82	17.70	16.69
6	ICG 11426	UNI	5.59	7.49	9. 49	11.81	12.80	10.48
		INO	5.59	9.80	12.70	15.72	17.43	16.15
7	ICG 12672	UNI	5.67	7.57	9.69	11.80	12.52	10.28
		INO	5.67	9.89	12.76	16.08	18.03	16.52
8	ICGV 94118	UNI	5.73	7.63	9.75	11.81	12.50	10.21
		INO	5.73	9.73	12.72	15.78	17.50	16.49
9	ICG 13919	UNI	5.60	7.64	9.66	12.05	12.84	10.29
		INO	5.60	9.91	12.81	15.95	17.59	16.18
10	ICG 14475	UNI	5.69	7.59	9.71	12.14	12.90	10.52
		INO	5.69	9.98	12.98	16.01	17.46	16.29
11	SB XI	UNI	4.67	5.87	8.01	9.05	7.81	5.96
		INO	4.67	8.33	11.96	14.40	13.26	12.19
12	JL 24	UNI	4.76	6.05	8.17	9.28	7.90	5.92
		INO	4.76	8.53	12.23	14.34	13.06	12.11
		SE±	0.033	0.033	0.033	0.038	0.036	0.032
		CD 5%	NS	0.095	0.010	0.109	0.102	0.092

(UNI : Uninoculated and INO : Inoculated)

peroxidase activity (5.52 to 5.91 units/g /min) and high polyphenol oxidase activity (8.94 to 10.46 units/g/min) than the susceptible genotypes.

When these genotypes were subjected to LLS inoculation, minimum alterations in metabolism were evident in all the resistant genotypes but marked alterations occurred in the susceptible (SB XI and JL 24) varieties. It was found that LLS normally reduce the chlorophyll content and reducing sugars. Similarly, LLS infection enhanced the activities of all the oxidative enzymes and increased the accumulation of phenols and total sugars during the pathogenesis.

In the present studies, however, it was evident that there were significant correlations between the levels of total sugars and phenols with the host resistance. In the resistant genotypes any decrement or enhancement due to LLS was negligible with a low response up to 12 to 18 days after inoculation, which indicated less cellular activity of LLS during early phases of infection.

There was drastic reduction in reducing sugar with LLS infection in both resistant as well as susceptible genotypes. The rate of reduction was more (55%) in susceptible genotypes than in the resistant (39-54%) genotypes. These results are in conformity with those of Mahapatra, 1982; Patel and Vaishnav 1986 and Li *et al.,* 1991. Total sugar content was increased in the resistant as well as susceptible genotypes after LLS infection but rate of increase in total sugar content was more in the susceptible than in the resistant genotypes. The level of total phenols increased after LLS inoculation in both the resistant and susceptible genotypes. Brahmachari and Kolte (1983) and Jyosthana *et al.,* 2004 reported biochemical differences in leaf spot resistant and susceptible varieties. They found more total chlorophyll and

Table 6. Influence of late leaf spot on the polyphenol oxidase activity (units/g/min) of resistant and susceptible groundnut genotypes

SN	Genotypes	DAI	0	5	10	15	20	25
1	ICG 13165	UNI	10.46	10.91	11.91	14.63	13.94	13.21
		INO	10.46	12.02	13.46	15.63	16.12	14.67
2	ICG 13160	UNI	9.97	10.77	11.45	13.97	13.67	12.54
		INO	9.97	11.98	14.2	16.16	15.02	13.59
3	RHRG 6083	UNI	8.94	10.69	11.48	13.7	12.1	10.12
		INO	8.94	11.93	13.24	15.69	14.26	11.52
4	KDG 128	UNI	9.27	10.92	11.67	13.65	13.45	10.54
		INO	9.27	11.36	13.28	14.96	14.64	12.05
5	GPBD 4	UNI	9.23	10.88	11.63	13.63	12.88	11.03
		INO	9.23	11.93	13.66	15.03	13.86	11.42
6	ICGV 11426	UNI	9.32	10.97	11.72	13.63	11.96	11.98
		INO	9.32	12.02	13.71	15.28	14.84	12.11
7	ICGV 12672	UNI	9.41	11.03	11.68	13.6	11.94	10.85
		INO	9.41	11.22	13.13	14.22	13.8	10.98
8	ICGV 94118	UNI	9.02	10.67	11.42	13.94	14.08	11.52
		INO	9.02	11.78	13.5	15.32	13.98	11.83
9	ICGV 13919	UNI	9.44	11.09	11.84	13.89	13.29	10.95
		INO	9.44	10.98	13.65	15.17	13.12	11.94
10	ICGV 14475	UNI	9.38	11.03	11.78	13.58	12.81	12.34
		INO	9.38	11.84	13.68	15.13	13.06	12.76
11	SB XI	UNI	6.94	10.12	11.18	12.62	10.03	8.94
		INO	6.94	10.82	12.11	13.76	12.68	9.35
12	JL 24	UNI	7.45	9.68	10.95	12.01	11.1	9.03
		INO	7.45	10.91	13.04	14.42	12.34	10.08
		SE±	0.034	0.034	0.043	0.052	0.039	0.047
		CD 5%	NS	0.097	0.122	0.148	0.110	0.135

(UNI : Uninoculated and INO : Inoculated)

total phenol contents in resistant varieties than in susceptible ones.

Similarly, Sindhan and Parashar (1996) reported that resistance cultivars of groundnut had higher phenol contents and lower levels of reducing and non-reducing sugars as compared to susceptible ones. Gupta *et al.,* (1992); reported that the total soluble sugar, total phenol and polyphenol oxidase activity was substantially higher in tolerant genotypes than susceptible genotypes of groundnut as influenced by leaf spot disease caused by *Cercospora.*

The activities of peroxidase and polyphenol oxidase were enhanced after the infection of groundnut leaves by LLS pathogen. Alteration in the peroxidase and polyphenol oxidase activities were more pronounced in the susceptible genotypes than in the resistant genotypes after LLS inoculations. Under LLS free conditions peroxidase activity was very high in the resistant tissues than the susceptible one. It was more than three times in resistant lines than the susceptible genotypes at the end of sampling. The LLS infection however, enhanced the activity of peroxidase irrespective of genotypes. In susceptible lines peroxidase were enhanced nearly 2 times as compared to 60 per cent increase in resistant lines. While, in polyphenol oxidase activity between the resistant and susceptible genotypes were marginal but PPO activity in resistant lines was more than susceptible ones. The PPO activity was generally increased by the LLS infection in all the genotypes. However, the activity was found to be fairly low in the susceptible genotypes than the resistant ones. Similar type of results have also been reported by Gupta *et al.,* (1992) who recorded highest polyphenol oxidase activity in the tolerant genotype and lowest in susceptible genotypes. The enzymatic activity slightly stimulated by LLS infection.

Jyosthna *et al.* (2004) reported that the higher peroxidase and polyphenol oxidase activity in resistant cultivar and low in susceptible groundnut cultivars to late leaf spot. Similar type of results were reported by several other workers *viz.,* Rathnakumar *et al.,* 2004; Sunkad and Kulkarni, 2006.

All the ten resistant genotypes considered in the studies did not vary markedly in their composition of sugars, phenols and activities of oxidative enzymes. However, the content of reducing sugars, total phenols content were higher in resistant with lower total sugars content as compared to susceptible genotypes at both the stages i.e. healthy and infected condition. There was reduction in reducing sugar content, while total sugar and total phenol and the enzyme activity of peroxidase and polyphenol oxidase was increased after LLS inoculation in all groundnut genotypes. Increased level of total phenol, peroxidase and polyphenol oxidase activity was more in resistant genotypes than susceptible genotypes. The content of phenol and the enzyme activity of peroxidase and polyphenol oxidase was found maximum in resistant genotypes. Total sugar content was found maximum in susceptible genotypes than in resistance at all the groundnut growth stages. In the resistant genotypes, however, the alteration due to LLS inoculations were always negligible upto 15 days after inoculation indicating less cellular activity of the pathogen. In susceptible genotypes, however, the changes induced by LLS pathogen were evident within 5 to 10 DAI and brought about 50 to 200 per cent variation over normal.

In conclusion, the application of these techniques either alone or in combination could help in identification and characterization of various genotypes possessing degree of late leaf spot resistance. This knowledge will help to develop cultivars with better resistance to LLS even when the environment favours rapid disease increase.

REFERENCES

1. Anonymous. 2015. Groundnut (*Arachis hypogaea* L.). ICRISAT data. http://exploreit.icrisat.org/page/groundnut/686/264 assesed on 01.12.2015.
2. Brahmachari, B.K. and Kolte, S. J. Morphological and biochemical differences in two *Cercospora* leaf spot resistant and susceptible varieties of groundnut. *Indian Phytopath.*, 1983; **36**: 149-150.
3. Gerlagh, M. and Bokdman, M. Yield of groundnut in relation to the reduction of photosynthesis by *Cercospora* leaf spot or by its simulation, *Rev. Plant Path.,* 1974; **53**: 2008.
4. Gupta, S. K.; Gupta, P. P.; Kaushik, C. D. and Chawala, H. K. L. Metabolic changes in groundnut leaf due to infection by leaf spot

pathogens. *Indian Phytopath.,* 1992; **45**(4) : pp. 434-438.

5. Jyosthna, M. K.; Eswara Reddy, N. P.; Chalam, T. V. and Reddy, G. L. K. Morphological and Biochemical Characterization of *Phaeoisariopsis personata* Resistant and Susceptible. Cultivars of Groundnut (*Arachis hypogaea*) *Plant Pathology Bulletin,* 2004; 13: pp. 243-250.
6. Li, D.; Wang, Z. Z. and Lin, K. H. Relationships between activity of several enzymes and peanut resistance to rust. *J. South China Agric. Univ.,* 1991; **12**(3): 1-6.
7. Linskens, H. F.; Sanwal, B. D. and Tracy, M.V. Modern methods in plant analysis, 1964; **7**: 681-683.
8. Maco, V.; Woodburg, W. and Stahman, M.A. The effect of peroxidase on germination and growth of mycelium of *Puccinia graminis* f.Sp. *tritici. Pytopathathology*. 1968; **58**: 1250-1254.
9. Mahapatra, N.K. Post-infection changes in sugar content of groundnut leaves infected with *Cercospora personata. Geobios,* 1982; **9**: 246-248.
10. McDonald, D.; Subrahmanyam, P.; Gibbons R. W. and Smith, D. H. Early and late leaf spots of groundnut. Information Bulletin No.21, ICRISAT, Patancheru, A.P. 502324, India 1985.
11. Nevill, D. J. Components of resistance to *Cercospora arachidicola* and C*ercosporidium personatum* in peanut genotypes in field plots. *Plant Disease*, 1981; **68** : 395-397.
12. Pandey, S.; Narayana Rao, J. and Dwivedi, S. L. Components of resistance to late leaf spot caused by *Phaeoisariopsis personata* in inter-specific derivatives of groundnut. *Indian Phytopath*., 2002; **55**(4):44-450.
13. Panse, V. G. and Sukhatme, P. V. *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research Publication, New Delhi, p. 359 1985.
14. Patel, V. A. and Vaishnav, M. U. Biochemical changes in rust infected leaves of groundnut. *Indian J .Myco. and Plant Pathol.,* 1986; **16**: 305-308.
15. Pretorius, A. E. 2005. ARC-GCI groundnut Department Progress Report, Potchestrom, South Africa.
16. Rathnakumar, A.L.; Chuni lal; Jain, V.K. and Mishra, J.B. 2004. Biochemical changes associated with rust (Puccinia arachidis Speg.) resistance in groundnut. In National Symposium Enhancing productivity of Groundnut for sustaining food and Nutritional security. October 11-13, 2004.226-227.
17. Sindhan, G. S. and Parashar, R. D. Biochemical changes in groundnut leaves due to infection by early and leaf spot pathogens. *Indian J. Mycol. and Plant Pathol*., 1996; **26**: 210-212.
18. Somogyi, M. Notes on sugar determination. *J. Biol. Chem.,* 1952; **195**: 19-23.
19. Subrahmanyam, P.; McDonald, D.; Waliyar, F.; Reddy, L. J.; Nigam, S. N.; Gibbons, R. W.; Ramanatha Rao, V.; Singh, A. K. and Pande, S. 1995. Screening Methods and Sources of Resistance to Rust and Late Leaf Spot of Groundnut. Information Bulletin No. 47. Patancheru – 502 324, Andhra Pradesh, India. 24 pp.
20. Subrahmanyam, P.; Mehan, V. K.; Nevill, D. J. and McDonald, D. 1980. Research on fungal diseases of groundnut. In ICRISAT. Proceedings of International Workshop on Groundnut, 13-17, October 1980, Patancheru, India. pp. 193-198.
21. Sunkad, G. and Kulkarni, S. Studies on structural and biochemical mechanism of resistance in groundnut to *Puccinia arachidis. Indian Phytopath*. 2006; **59**(3) : 323-328.
22. Swain, T. and Hills, W. E. The phenolic constituent of *Prunus domesiica*. The quantitative analysis of phenolic constituents. *J. Sci. Fd. Agric.,* 1959; **10** : 63-68.
23. Thimmaia, S. R. Standard methods of biochemical analysis. Kalyani publications, New Delhi; 2004; pp-323-324.