Indian J. Genet., 59(3): 331-336 (1999)

BIOCHEMICAL DEFENCE AND THE NATURE OF GENE ACTION AGAINST 'TIKKA' DISEASE IN GROUNDNUT

G. C. BERA, S. K. GHOSE AND P. K. DAS*

Department of Genetics,
Bidhan Chandra Krishi Viswavidyalaya Mohanpur 741 252

(Received: December 16, 1998; accepted: June 30, 1999)

ABSTRACT

Under the attack of *Cereospora Spp.* resistant genotypes as studied in groundnut are capable of maintaining a higher level of chlorophyll, soluble sugar, acid protein, RNA and total phenol content in their leaves. Gene action study revealed that both additive × dominance and dominance × dominance gene interactions are important for total phenol content in the leaves. Biparental matings followed by recurrent selection of types with increased amount of phenol in the leaves would be suggested for the development of 'Tikka' disease resistant genotypes.

Key Words : Groundnut, 'Tikka' disease, Biochemical defence, resistance, susceptible, gene interaction.

In groundnut leaf-spot disease caused by *Cercospora Spp.* and is commonly known as 'Tikka' has attracted attention for a serious scientific management [1]. Genotypes resistant to the disease would be the cheap and stable management practice [2]. The susceptibility or resistance to disease is a dynamic interaction between host and the parasite which is being expressed differentially depending upon the changes in physiological or biochemical constituents within the tissues which in turn are the resultant manifestation of the activities of genes under which they operate. The present study is concerned with the analysis of biochemical changes in different groundnut genotypes under the attack of *Cercospora Spp.* in natural environment and the elucidation of the genetic control of the interaction of host and pathogen in relation to changes in total phenol content in the leaves which is known to be a major biochemical defence [3, 4].

MATERIALS AND METHODS

Four genotypes namely 'Zuled', ICCV 87157', 'TMV2 (7731)' and 'ICGV 87121'

*Corresponding author.

were taken as experiment material. From our earlier studies in field trial for three consecutive years on scoring of leaf of the disease on 0-7 point scale as suggested by [5] revealed that genotypes 'Zuled' exhibited a very high level of field resistance, ICGV 87157 moderately resistant, while TMV2 (7731) and ICGV 8721 were highly susceptible to *Cercospora Spp.* causing Late leaf spot disease of 'Tikka'. Leaf samples from each genotype (both healthy and diseased) were collected at 75 days of crop growth because at this stage maximum disease severity was recorded [6]. Total chlorophyll content, soluble sugar content, acid protein, RNA and phenol content were estimated following biochemical methods as devised by [7].

For the study of gene action a 6 × 6 half diallel cross was done. Parents used in this crossing programme were 'Zuled' ICGV 87157, TMV2 (7731), ICGV 8721, NFSG 72, NRGS (FDRS) 6 respectively. Crossing was done at 50% flowering stage (32-36 days after sowing). The selected flower buds to be opened in the next morning were tagged following the special 'ring cut' technique [8]. The buds were emasculated in the previous evening (4 P.M. - 6 P.M.). In the next morning (6 A.M. - 8 A.M.) pollination was done. F_1 plants were raised in replicated randomised blocks. Phenol content (mg^{-1} gm fresh wt) of diseased and healthy leaves of both parents and F_1S was estimated. Genetic components were calculated according to Griffings Method-2 Model-1 [9].

RESULTS AND DISCUSSION

Chlorophyll :

Among the four genotypes, 'Zuled' recorded higher amount of total chlorophyll (both chl. a and chl. b) in the healthy leaves. In the infected leaves all the genotypes showed appreciable reduction in chlorophyll content but 'Zuled' maintained comparatively higher the amount (Table 1a) and was able to maintain greater leaf surface area supporting the findings of Brahmachari and kolte [10]. In susceptible genotypes TMV2 (7731) and ICGV 87121 chl. a level had been affected more adversely than chl. b which also corroborates the observations of Bala and Dhillon [11].

Soluble Sugar :

Two distinct different trends for soluble sugar content was observed. Susceptible genotypes showed significantly a very high level of soluble sugar content in their healthy leaves whereas resistant genotypes had lower level of sugar content in their unaffected leaves but the level was increased in their infected leaves (Table 1b) which was an agreement with the findings of [12].

Table 1a. Chlorophyll content (mg 100 mg^{-1} fresh wt.) of healthy and diseased leaves of different genotypes

Genotypes	Chlorophyll - a		Chlorophyll - b		Total Chlorophyll	
	HL	DL	HL	DL	HL	DL
Zuled	1.27 ± 0.03	0.69 ± 0.02	0.65 ± 0.02	0.45 ± 0.01	1.92 ± 0.04	1.44 ± 0.05
ICGV 87157	0.66 ± 0.02	0.37 ± 0.01	0.35 ± 0.03	0.19 ± 0.01	1.01 ± 0.02	0.56 ± 0.02
TMV2 (7731)	0.72 ± 0.02	0.61 ± 0.02	0.55 ± 0.03	0.43 ± 0.02	1.27 ± 0.01	1.04 ± 0.01
ICGV 87121	0.48 ± 0.01	0.15 ± 0.01	0.22 ± 0.02	0.08 ± 0.001	0.70 ± 0.01	0.23 ± 0.001

Table 1b. Soluble Sugar, RNA and Acid Protein content (mg 100 mg^{-1} fresh wt.) of healthy and diseased leaves of different genotypes

Genotypes	Soluble Sugar		RNA		Acid Protein	
Zuled	9.44 ± 0.5	16.34 ± 0.4	0.387 ± 0.01	0.527 ± 0.01	2.750 ± 0.19	3.850 ± 0.23
ICGV 87157	5.04 ± 0.4	11.52 ± 0.5	0.350 ± 0.01	0.450 ± 0.02	3.010 ± 0.21	4.130 ± 0.30
TMV2 (7731)	13.14 ± 0.6	10.26 ± 0.6	0.275 ± 0.01	0.355 ± 0.01	2.310 ± 0.15	2.750 ± 0.35
ICGV 87121	16.20 ± 0.5	14.88 ± 0.7	0.375 ± 0.02	0.440 ±0.02	2.750 ± 0.18	3.010 ± 0.41

HL = Healthy leaf; DL = Diseased leaf

Table 2. Total phenol content of healthy and diseased leaves of different genotypes

a. Total phenol content (mg 100 mg^{-1} fresh weight)

Genotypes	HL	DL
Zuld	1.80 ± 0.02	6.30 ± 0.04
TCGV 87157	1.90 ± 0.03	5.90 ± 0.02
TMV2 (7731)	1.60 ± 0.02	3.40 ± 0.02
ICGV 87121	1.80 ± 0.01	3.90 ± 0.02

b. Phenol content in relation to disease severity

Genotypes	Healthy leaves	One spot per leaflet	5 spots per leaflet	20 spots per leaflet
Zuled	1.80 ± 0.02	5.20 ± 0.01	6.00 ± 0.02	8.00 ± 0.01
ICGV 87157	1.90 ± 0.02	2.08 ± 0.01	6.20 ± 0.03	6.80 ± 0.01

HL = Healthy leaf; DL = Diseased leaf

RNA and acid Protein :

Following infection the resistant genotype 'Zuled' and 'ICGV87157' recorded relatively high level of RNA and acid protein than susceptible genotypes 'TMV2 (7731)' and 'ICGV - 87121' (Table 1b). Such a situation in wheat infected with *Puccinia graminis* was also reported by Bhattacharya et al. [13].

Phenol Content :

Matern and Kneusel [3] reported that phenolic compounds which are very toxic to the pathogen and produced at a faster rate after infection in the resistant genotypes. In our study, phenol content did not show significant variation in the healthy leaves of all the four genotypes but the level had increased after infection (Table 2a). The resistant genotype 'Zuled' recorded around 4.5 times increase from Zero leaf spot level to 20 leaf spots per leaflet, while in 'ICGV 87157' the increase was around 3.5 times. Even during the appearance of a single leafspot 'Zuled' recorded a phenol turnover as high as 2.5 times of ICGV87157' (Table 2b).

The study by and large emphasizes that leaves in resistant genotype are capable of maintaining higher level of chlorophyll content (both a and b) under the attack of *Cercospora*. More importantly, the resistant genotypes maintain high level of RNA protein and phenol content in their infected leaves. This biochemical manifestation would serve as valuable parameter for the selection of resistant genotypes in groundnut.

Gene action :

The ANOVA for combining ability (Table 3) for phenol content in the leaves revealed that mean squares due to gca and sca were highly significant. The estimated ratio of additive to total genotypic variance was 0.15 which suggests that the phenol content is controlled predominantly by non-additive gene action.

Table 3. ANOVA for phenol content in diseased leaves

Source	Phenol df.	Content in diseased leaves Ms.
gca	5	2.23**
sca	15	1.65**
Error	20	0.02
$\frac{2\sigma^2 gca}{2\sigma^2 gca + \sigma^2 sca}$		0.15

**Significant at P = 0.01

The gca values showed wide variation amongst parents. 'Zuled' and ICGV 87157' recorded significantly high positive gca value while the same was significantly negative for 'ICGV-87121', 1NRGS(FDRS)6', 'NFSG72' (Table 4).

Table 4. GCA effects of parents for phenol content in diseased leaves and per se performance of 4 selected cross

Parents		gca effects	per se performance of parents	Selected cross	sca effect
1.	TMV2 (7731) (P_1)	0.116	3.40	5.60 ($P_2 \times P_5$)	1.772
2.	NFSG72 (P_2)	–0.351	3.00	3.24 ($P_1 \times P_6$)	1.407
3.	ICGV 87157 (P_3)	0.794	5.90	7.40 ($P_4 \times P_6$)	2.727
4.	NRGS (FDRS) 6 (P_4)	–0.436	2.70	5.80* ($P_3 \times P_6$)	1.851
5.	Zuled (P_5)	0.0519	6.30		
6.	ICGV 87121 (P_6)	–0.410	3.90	4.80 (average of all crosses)	
	SE	0.044			7.701

The crosses P4 × P6, P3 × P6, P2 × P5 and P1 × P6 are very good specific combiners. It was also noted that these four crosses had per se performance for phenol content well above the average values of all the crosses (Table 3b). In terms of their gca effects these crosses demonstrated different gene actions. For example, the crosses 4 × 6 [NRGS (FDRS) 6 × ICGV 87121] displayed a low × low gca combinations indicating dominance × dominance gene interactions. While 2 × 5 (NFSG72 × Zuled), and 3 × 6 (ICGV87157 × ICGV87121) having low × high and high × low gca combinations demonstrated additive × dominance gene interactions. Since groundnut is predominantly a self-pollinated crop mopping up of additive genetic variance would be of major interest. A biparental matings followed by recurrent selection with these genotypes would be profitable for developing 'Tikkas' disease resistant lines. Iroume and Knauft [15] also suggested the selection among crosses for the development of high yield and disease resistance genotypes in groundnut.

REFERENCES

1. P. Subrahmanyam, D. Mc Donald, F. Waliyar, L. J. Reddy, S. N. Nigam, R. W. Gibbons, V. Ramanatha Rao, A. K. Singh, S. Pande and P. M. Reddy and P. V. Subba Rao. 1995. Screening methods and Sources of resistance to rust and late leaf spot of groundnut. ICRISAT Inf. Bullet No. 47.
2. M. A. Wells, W. J. Grichar, O. D. Smith and D. H. Smith. 1994. Response of selected peanut germplasm lines to leaf spot and southern stem rot. Oleagineux., **49**: 21-25.
3. U. Matern and R. E. Kneusel, 1989. Phenolic Compounds in plant disease resistance. Phytoparasitica., **16**: 153-170.
4. G. S. Shindhan, B. S. Jaglan and R. D. Parasar. 1987. Change in phenols and carbohydrates in resistance and susceptible cultivars of groundnut in relation to 'Tikka' disease. Plant Dis. Res., **2**: 100-101.
5. P. Subrahmanyam, D. Mc Donald and R. W. Gibbons. 1982 Variation in *Cercosporidium personatum* symptoms of certain cultivars of *Arachis hypogaea*. Oleagineux., **37**: 63-68.
6. G. C. Bera. 1994. 'Tikka' Disease in Groundnut, its Genetics and Selection of high yielding Resistant lines. Unpubl. Ph.D. Thesis. B.C.K.V., Mohanpur. W. Bengal.
7. S. Sadasivam and A. Manickam. 1996. *In*: Biochemical methods. New Age International (P) Limited, Publishers, 2nd Edn.
8. S. N. Nigam, M. J. V. Rao and R. W. Gibbons. 1990. Artificial hybridization in groundnut. ICRISAT Information Bulletin No. **29**: 27.
9. B. Griffing. 1956 b. Concepts of general and specific combining ability in relation to diallel crossing system. Aust. J. Biol. Sci., **9**: 463-493.
10. B. K. Brahmachari and S. J. Kolte. 1983. Morphological and biochemical differences in two *Cercospora* leafspot resistant and susceptible varieties of groundnut. Ind. Phytopath., **36**: 149-150.
11. M. Bala and M. Dhillon. 1987. Chlorophyll content of *Cercospora* infected groundnut leaves in susceptible and resistant varieties. Annal. Bio., **3**: 61-63.
12. S. S. Aujla and J. S. Chohan. 1984. Changes in Carbohydrate metabolism during photosynthesis in groundnut by *Aspergillus flavus*. J. Res. PAU., **21**: 49-53.
13. P. K. Bhattacharya, J. M. Naylor and M. Shaw. 1965. Nucleic acid and protein changes in wheat leaf nuclei during rust infection. Science., **15**: 1605-1607.
14. R. N. Iroume and D. A. Knauft. 1987. Selection indices for simultaneous selection for yield and leafspot resistance in Peanut. Peanut Sci., **14**: 51-54.