



Biochemical constituents influencing thrips resistance in groundnut germplasm

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Abstract

Field screening of 56 groundnut genotypes revealed that none reacted as highly resistant to thrips. However, 13 genotypes of 56 collected and screened genotypes acted as resistant, 25 as moderately resistant and 11 as moderately susceptible. Phenols and tannins showed significant and negative relationship with number ($r = -0.750, -0.864$) and damage ($r = -0.641, -0.784$) of thrips. Total sugar ($r = 0.313, 0.38$), amino acids ($r = 0.830, 0.723$) and reducing sugars ($r = 0.408, 0.337$) showed positive relationship with number of thrips and their per cent damage. Linear regression analysis revealed that high amount of tannins and phenols contributed for thrips resistance in groundnut.

Key words

Genotypes, Phenols, Sugars, Tannins, Thrips

Introduction

Arachis hypogaea L., 1735 (Fabaceae) is a valuable cash crop for millions of small scale farmers in the semi-arid tropics and is the principal oilseed crop in India. Its seeds are a rich source of edible oil (43-55%) and protein (25-28%), also a valuable source of vitamins viz., E, K and B. Groundnut cake after oil extraction, is a high protein animal feed and haulm provides quality fodder (Blummel *et al.*, 2005).

Of the insect pests attacking groundnut crop, thrips is an important sucking insect pest. Four genera commonly infest groundnut namely *Scirtothrips dorsalis* Distant, *Frankliniella schultzei* Trybom, *Thrips palmi* Karny and *Caliothrips indicus* Bagnall. Thrips live in young foliage especially between the folded groundnut leaflets and flowers that inhibit terminal buds and flowers. Both nymphs and adults feed by rasping the surface of rapidly growing leaf tissues and suck the released plant fluid (Chisholm and Lewis, 1984). They cause tiny scars on leaves leading to stunted plant growth. Damaged leaves may become papery and distorted, infested terminal leaves lose colour, rolled up and drop before maturity (Chisholm and Lewis, 1984). Thrips are also known to transmit tomato bud necrosis disease caused by tomato spotted wilt virus in groundnut and other several crops

(Nagaraja *et al.*, 2005). No single measure can currently provide adequate control of spotted wilt where severe epidemics occur. However, interdisciplinary investigations have resulted in development of integrated management systems that make use of moderately resistant cultivars, chemical and cultural practices, each of which helps to suppress spotted wilt epidemics (Culbreath *et al.*, 2003).

Early season moisture stress associated with thrips injury intensifies the groundnut yield and quality loss (Funderburk *et al.*, 1998). Groundnut crop contains different types of plants that exhibit variability in phenotype (morphological and anatomical differences) and/or genotype (genetically different from one another). One of the means by which thrips damage on groundnut crop can be curtailed by breeding varieties which can genetically or physically resist the feeding by thrips (Krishnaiah *et al.*, 2012). So, the first step is to screen groundnut genotypes to thrips damage in order to identify resistant genotype. While screening the groundnut genotypes showing wide variations are generally selected. For instance, genotypes with variation in biochemical constituents, leaf shape and size are selected (Ekvised *et al.*, 2006). Thus host plant resistance provides an additional measure to integrate with other management tactics to reduce yield loss in

groundnut. Considering the above facts, a study was undertaken to screen biochemical constituents influencing thrips damage on groundnut.

Materials and Methods

Screening groundnut genotypes against thrips : Experiment was conducted during *khariif* 2010 at Agriculture Research Station, Chintamani, Karnataka, India (13°24' N lat, 70° 4' E longi at 857 m AMSL.). Different types of *A. hypogaeae* obtained from different centres were screened in the field under natural thrips infestation to identify the resistant ones. Groundnut genotypes were sown in three replications at 30 cm x 10 cm between rows and plants, respectively. Group of scientists, after years of field and laboratory experimentation develop practices or crop husbandry procedures for cultivating crop commercially for a particular geographical zone (Package of practices). The crop was raised following recommended package of practices except for plant protection measures (University of Agricultural Sciences, 2012).

The reaction of groundnut genotypes was assessed by visual grading of damage and absolute insect counts on each test entry. Visual observations were recorded on leaf damage and curling of leaves (Fig. 1) due to thrips feeding during peak infestation following standard scale 1-9 (Ranga Rao and Wightman, 1997). Categorization of genotypes was based on damage score. Absolute insect population counts were recorded as number of thrips per terminal bud leaves. Each cultivar was selected and labelled for observations. Observations were recorded on thrips population at weekly intervals from 25 days after sowing of crop to the time crop was harvested. Per cent foliage damage was calculated by the following formula.

Percent leaf damage = Total number of leaves / number of damaged leaves X 100

Collection of Samples for biochemical analysis: Tender shoot and leaves of 16 groundnut genotypes were collected, which included resistant, moderately resistant and susceptible group, and dried at 32°C in a hot-air oven for 48 hr. The samples were powdered using mixer for 3 min. The powdered samples were sieved through a 100 mesh screen and stored in sealed plastic containers (0.5 m diameter) at 4°C, for further analysis.

Sugars : Total sugar and non reducing sugars were hydrolyzed in 1.0 ml of 1.0 N H₂SO₄ to 0.5 ml of aliquot and heated over boiling water bath for 30 min. After cooling under running water, one to two drops of phenolphthalein indicator was added. Later, 1.0 N NaOH was added drop by drop to neutralize the acid in the hydrolysate till it developed pink colour. Further, 1.0 N H₂SO₄ was added to make it colourless, finally the volume was made up to 10.0 ml with distilled water and absorbance was read at 510 nm.

Reducing sugars were estimated in 0.4 ml of aliquot by

adding 1.0 ml of Nelson's reagent A (Twenty five grams of sodium carbonate (anhydrous), 25 g of Rochelle's salt (sodium potassium tartarate), 20 g of sodium bicarbonate and 200 g of sodium sulphate were dissolved in 800 ml distilled water and diluted to one liter) + Nelson's reagent-B (Fifteen grams of copper sulphate was dissolved in a small quantity of distilled water and made upto 100 ml and a few drops of concentrated H₂SO₄ was added). The mixture was heated for 20 min. After cooling in running water, 1.0 ml of arsenomolybdate solution was added and finally the volume was made upto 10.0 ml with distilled water. The absorbance was read at 510 nm. A standard graph was constructed using glucose solution as a standard (Nelson, 1944).

Amino acids : One ml of nonhydrin reagent was added to 1.0 ml of extract and boiled in a specimen tube over water bath for 20 min. The specimen tubes were cooled under running water and the volume was made up to 10 ml with diluents solution till it developed a purple colour and absorbance was read at 570 nm. A standard curve was prepared with glycine to calculate the quantity of total soluble amino acids (Moore and Stein, 1958).

Total phenol content : 100 mg of oven-dried powdered sample was extracted in 10 ml of warm 80 % ethanol for 1 hr at room temperature. The extract was centrifuged at 6000 rpm for 15 min. The supernatant was evaporated to dryness on a water bath and the residue was dissolved in 5 ml water. Alcohol free extract was used for estimation of total phenols (Mallick and Singh, 1980).

An aliquot sample of 0.1 ml was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteu reagent (FCR) was added and mixed. Exactly after 3 min, 2 ml of 20 % sodium carbonate solution was added and kept in boiling water bath for one min. After cooling under running tap water, the absorbance was read at 650 nm, against the reagent blank in a colorimeter. A standard graph was constructed with catechol as a standard. The total phenol content was expressed as mg g⁻¹ d.wt.

Total tannin content : 100 mg of oven-dried powdered sample was extracted with 5 ml of methanol for 24 hr at room temperature with occasional stirring. The extract was centrifuged at 5000 rpm for 10 min. The supernatant was used to estimate of total tannins (Burns, 1971). A standard graph was constructed using catechin as a standard. The total tannin content was expressed as mg g⁻¹ d.wt..

Statistical analysis : The data was statistically analyzed by subjecting to the correlation 'r' and regression 'R' formula, between biochemical parameters and per cent foliage damage and number of thrips (Lalchand, 1981).

Results and Discussion

Thrips were found to be active throughout the cropping season from July - October, 2011. Weekly observations revealed that among 56 genotypes screened against thrips damage, 13

Table 1 : Reaction of groundnut genotypes against thrips damage, *kharif* 2010

Genotypes	Thrips population/terminal bud leaf	Foliage damage (%)	Damage score
GKVK-1	4	21.50	3
GKVK-13	4.23	22.15	3
GKVK-2	5.2	32.71	4
GKVK-12	6.6	33.13	4
CTMG-4	4.13	17.56	2
CTMG-6	4.07	20.58	3
CTMG-7	4.27	18.19	2
CTMG-3	4.07	19.23	2
CTMG 5	5	17.72	2
ICGV91114	4.4	17.84	2
GKVK-15	4.53	22.23	3
ICGV00350	4.33	18.66	2
TMV 2	6.4	42.66	5
JL 24	6.33	41.19	5
GPBD 4	4.1	18.88	2
Chintamani 2	6.07	25.17	3
ICGV-960165 X ICGV-98105(F6)	6.8	42.62	5
Sel.2-3-1 X VRI-2(F5)	4.8	31.00	4
CTMG-1 X ICG-9035(F5)	4.4	23.87	3
GPBD-4 X ICGS 76	5.0	25.33	3
TG-36 B	5.0	19.12	2
TG-37	6.2	22.42	3
M-13	6.6	27.12	3
ICGV-11717	5.6	24.51	3
JSP-37	6.6	29.00	3
ALG-234	5.2	36.96	4
JSP-36	5.4	31.25	4
ICGV-2400	4.6	30.66	4
Fes P	6.2	45.00	5
ICGV-3662	5.4	32.90	4
ICGV-7046	7.2	31.33	4
JAL-18 X ALR-2(F4)	5.2	23.03	3
JAL-31 X CO-3 (F5)	4.8	31.87	4
PSB 11039 X TAG 24 (F4)	6.4	17.61	2
GG-2 X ICGV 91114	5.0	18.70	2
Cyto-2 X VRI-2(F6)	6.0	23.33	3
CTMG-3 X ICGV-00350	6.4	24.37	3
FDR.ICG X 020063-10(F6)	5.8	37.90	4
Dh-86 X TG-39 (F6)	5.2	25.33	3
Samrat X ICGV-1114(F6)	6.2	24.37	3
ICGV 020058 (F6)	4.0	16.65	2
Dh-203	5.6	21.33	3
Dh-4-3: ICLH	5.6	24.00	3
ICGV 00350	6.2	43.93	5
KGN-37	5.6	22.50	3
JSP 33	6.8	24.51	3
ICGV-1337	8.4	31.62	4
ICGV 10471	6.8	46.12	5
ICGV-10655	4.6	22.66	3
PVK-9816	4.6	23.22	3
Dh-208	4.8	23.75	3
VLS mutant	4.4	16.77	2
GT-38 C	6.0	18.66	2
ICGV-3554	7.8	26.16	3
Dh-201	6.8	25.16	3
PBS-30073	6.6	43.22	5

$r = 0.546^{**}$; ** Significant at $P=0.01$

Table 2 : Reactions of select groundnut varieties and germplasms to thrips damage under field conditions, *kharif* 2010

Damage score	Genotypes	Damage (%)	Reactions
1	—None—	—	Immune
2	CTMG-4,CTMG-3,CTMG5, CGV91114, ICGV00350, GPBD 4, CTMG 7, TG-36 B, PSB 11039 X TAG 24 (F4), GG-2 X ICGV 91114, ICGV 020058 (F6), VLS mutant, GT-38 C	16.65 – 19.23	Resistant
3	GKVK-1, GKVK- 13, CTMG-6, GKVK-15, Chintamani 2, CTMG-1 X ICG-9035(F5), GPBD-4, ICGS 76, TG-37, M-13, ICGV-11717, JSP-37, JAL-18 X ALR-2(F4), Cyto-2 X VRI-2(F6), CTMG-3 X ICGV-00350, Dh-86 X TG-39 (F6), Samrat X ICGV-91114(F6), Dh-203, Dh-4-3: ICLH, KGN-37, JSP 33, ICGV-10655, PVK-9816, Dh-208, ICGV-3554, Dh-201	20.58 – 29.00	Moderately resistant
4	GKVK-12,GKVK-2s, sel.2-3-1 X VRI-2(F5),ALG-234, JSP-36, ICGV-2400, ICGV-3662, ICGV-7046, JAL-31 X CO-3 (F5), FDR.ICG X 020063-10(F6), ICGV-1337	31.00 – 37.90	Moderately Susceptible
5	TMV 2, JL 24, ICGV-960165 X ICGV-98105(F6), Fes P, ICGV 00350, ICGV 10471, PBS – 30073	41.19 – 46.12	Susceptible
6-7	—None—	—	Highly susceptible
8	—None—	—	—
9	—None—	—	—

**Fig. 1** : Curling and crinkling of young leaf (circled portion) of groundnut due to thrips damage

were categorized as resistant, 25 as moderately resistant, 11 as moderately susceptible and 7 as susceptible. However, none of the genotypes reacted as completely resistant to thrips damage (Table 1 and 2). There was a significant and positive correlation between thrips density and foliage damage ($r = 0.546$) indicating that this parameter can be used to assess thrips resistance in groundnut genotypes.

The plant breeders document all growth parameters of every groundnut genotype in pedigree sheet, identify and designate the genotype. The numbers of thrips on groundnut

genotypes varied from 4.0 to 8.4 thrips/terminal bud. On resistant genotypes thrips number varied from 4.0 (ICGV 020058 (F6)) to 6.4 (PSB 11039 X TAG 24 (F4)), moderately resistant category 4.0 (GKVK-1) to 7.8 (ICGV-3554), while moderately susceptible genotypes ranging from 4.6 (ICGV-2400) to 8.4 (ICGV-1337) thrips/terminal bud. Susceptible genotypes harboured numbers ranging from 6.2 (ICGV 00350) to 6.8 (ICGV 10471) thrips/terminal bud and none of the variety showed high level of susceptibility (Table 1). The results are in confirmation with Nagaraja *et al.* (2005) who recorded 2.84, 7.13 and 13.37 at 45 DAS thrips/terminal bud on GPBD-4, TMV-2 and JL-24.

The relationship between thrips population and per cent foliage damage was significant ($p < 0.05$) and positively correlated at five per cent level with $r = 0.546$. The foliage damage of resistant category ranged from 16.65-19.23% with lowest in 16.65% (ICGV 020058) and highest in 19.23% (CTMG-3) and moderately resistant group ranging from 20.58% (CTMG-6) to 29% (JSP-37). Under moderately susceptible category, minimum damage of 31.00% was recorded in sel. 2-3-1 X VRI-2(F5) and maximum of 37.90% in FDR.ICG X 020063-10(F6). In susceptible category, the foliage damage ranged from 41.19% (JL-24) to 46.12% (ICGV 10471) (Table 2). This is in confirmation with Anonymous (2002) who recorded 47.33% leaf damage. The variation in damage may be due to differential load of thrips population on different genotypes based on the morphological/biochemical variations in plants.

Groundnut genotype screening work against thrips damage was conducted and reported by several workers but the genotypes involved in screening were different. For instance,

Table 3. Relationship between biochemical constituents on thrips numbers and foliage damage, *kharif* 2010

Varieties	Thrips/top bud leaf	Foliage damage (%)	Total sugars (mg g ⁻¹)	Reducing sugars(mg g ⁻¹)	Phenols (mg g ⁻¹)	Tannins (mg g ⁻¹)	Amino acids (mg g ⁻¹)
GKVK-1	4	21.50	4.42	0.45	0.54	0.0037	3.80
GKVK- 13	4.23	22.15	3.46	0.47	0.48	0.0038	3.79
GKVK-2	5.2	24.71	4.13	0.53	0.29	0.0018	4.52
GKVK-12	6.6	33.13	5.15	0.82	0.26	0.0021	5.26
CTMG-4	4.13	15.56	3.36	0.24	0.36	0.0032	4.23
CTMG-6	4.07	20.58	4.32	0.40	0.45	0.0028	3.79
CTMG-7	4.27	12.19	3.36	0.19	0.36	0.0038	3.41
CTMG-3	4.07	19.23	4.13	0.41	0.38	0.0035	3.58
CTMG 5	5	17.72	2.89	0.37	0.44	0.0028	4.25
ICGV91114	4.4	17.84	6.52	0.24	0.32	0.0027	4.37
GKVK-15	4.53	19.01	5.60	0.24	0.38	0.0028	4.88
ICGV00350	4.33	18.66	4.72	0.26	0.32	0.0029	3.83
TMV 2	6.4	42.66	7.9	0.19	0.20	0.0014	5.10
JL 24	6.33	41.19	3.21	0.45	0.23	0.0018	4.81
GPBD 4	4.1	18.88	2.89	0.45	0.35	0.0035	3.82
Chintamani 2	6.07	29.17	5.63	0.50	0.29	0.0018	4.52

Rohilla *et al.* (1999) tested twenty-eight groundnut genotypes, of which six (MH-11, MH-92, MH-46, MH-51, MH-53 and MH-55) showed resistance to thrips. Ekvised *et al.* (2006) tested 8 groundnut genotypes; IC 10 showed the lowest thrips numbers and plant damage. Nugrahaeni *et al.* (1997) recorded moderate resistance to thrips in ICGV 90265, ICGV 91167 and ICGV 91176. Boicajunior *et al.* (2004) found that Peru Branco was the most susceptible genotype, while Makap, Peru Amarelo and Altika were resistant. The lowest damage was observed in genotype Peru Amarelo.

Total sugar content of different groundnut genotypes varied from 2.89 mg (CTMG 5, GPBD 4) to 7.9 mg (TMV 2) per gram of leaf sample. The highest quantities were noticed in susceptible genotypes. Reducing sugar content of different genotypes varied from 0.19 mg (CTMG 9, TMV 2) to 0.82 mg (GKVK 12) per gram of leaf sample. Similarly, amino acid content of different genotypes varied from 3.41mg (CTMG 7) to 5.26 mg (GKVK 12) per gram of leaf sample (Table 3). These contents were positively correlated with thrips population and foliage damage, total soluble sugar ($r = 0.313, 0.380$), reducing sugar ($r=0.408, 0.337$) and amino acid ($r=0.830, 0.723$) (Table 4). These results are in confirmation with Somasekhar *et al.* (2003) where workers recorded positive correlation between thrips population and foliage damage. Nanda *et al.* (2000) observed low level of total soluble sugar in the leaf sheath of resistant genotype Ptb-33 (1.45%) compared with the susceptible genotype TN-1 (2.05%).

The data recorded on phenol content of different genotypes varied from 0.20 mg (TMV-2) to 0.54 mg (GKVK-1) per gram of leaf sample among the susceptible and resistant group, respectively. The highest quantities were noticed in moderately resistant and resistant group. However, lower quantities of phenols were noticed in susceptible groundnut genotypes. These

results showed significant difference at 5% level of significance. There was a negative correlation between phenols and thrips population ($r = -0.750$). A similar trend was observed between phenols content and per cent foliage damage ($r = -0.641$) (Table 4). The phenol and tannin contents showed significant negative correlation with thrips numbers. These results are in confirmation with the findings of Somasekhar *et al.* (2003) where thrips resistant groundnut varieties had higher quantities of phenols and tannins compared with the susceptible varieties. Rohini *et al.* (2011) reported that the presence of high quantity of biochemical components like tannins, phenols conferred resistance against thrips. Significant negative correlations were obtained between polyphenols and damage indices ($r = -0.57$), mean adult counts ($r = -0.56$) and mean larval counts ($r = -0.64$) of resistant cowpea cultivars, indicating that polyphenols play a significant role in cowpea thrips resistance (Alabi *et al.*, 2011).

Tannin content varied from 0.0014 to 0.0038 mg g⁻¹ of leaf sample. Lower tannin content was recorded in susceptible genotypes viz., TMV-2 (0.0014 mg g⁻¹ of leaf sample) and JL-24 (0.0018 mg g⁻¹ of leaf sample), while the resistant genotypes had higher quantities of tannins ranging from 0.0038 to 0.0027 mg g⁻¹ of leaf sample. The data showed a significant negative relationship between tannin content and thrips population ($r = -0.864$). A similar trend was observed between tannin contents and per cent foliage damage ($r = -0.784$) at 5% level of significance (Table 4). The total phenol and tannin contents in different plant parts (leaves, squares and bolls) of different cotton varieties/hybrid showed significant negative relationship with the incidence of thrips (Balakrishnan, 2006).

Multiple linear regression equation was fitted to foliage damage due to thrips population. According to regression equation, thrips population influenced foliage damage to an

Table 4 : Correlation of biochemical constituents of groundnut varieties with thrips number and per cent foliage damage by thrips of during *kharif* 2010

Host plant characters	Thrips ('r' value)	
	Thrips / terminal bud	Foliage damage (%)
Total sugars	0.313	0.380
Reducing sugars	0.408	0.337
Amino acids	0.830**	0.723**
Phenols	-0.750**	-0.641**
Tannins	-0.864**	-0.784**

** Significant at P=0.01

Table 5 : Regression equations for biochemical constituents and number of thrips

Particulars	Regression equation	R ²
Thrips	$y_1 = 4.693 - 0.036X_1 + 1.276X_2 - 2.645X_3 - 485.039X_4 + 0.496X_5$	0.856
Foliage damage (%)	$y_2 = 25.899 + 0.683X_1 + 12.710X_2 - 13.983X_3 - 5027.041X_4 + 2.172X_5$	0.670

Y_1 =Thrips (No. per top bud leaf); Y_2 =Foliage damage (%); X_1 =Total sugars (mg); X_2 =Reducing sugars (mg); X_3 =Phenols (mg); X_4 =Tannins (mg); X_5 =Amino acids (mg)

Table 6 : Stepwise regression analysis showing the significant variables in genotypes reaction against thrips in relation with biochemical characters

Variables	Regression coefficient	Standard error	't' value	'F' value	R ² value
Thrips population (per terminal bud leaf)					
Tannin	-1038.549	161.934	-0.864	41.132	0.746
Thrips damage (%)					
Tannin	-8709.617	-0.784	-4.732	22.393	0.615

** Significant at P=0.01

extent of 85.6% and 67% ($R^2 = 0.856$ and 0.670), respectively (Table 5). Stepwise regression analysis with biochemical constituents, thrips number and per cent foliage damage revealed that phenols and tannins had significant relationship with thrips numbers and per cent foliage damage. The regression equation revealed that the influence of tannin on thrips numbers was 74% ($R^2 = 0.746$) and 61% ($R^2 = 0.615$) with foliage damage (Table 6).

In the current investigation, 56 groundnut genotypes screened in field and laboratory experiment, only 13 genotypes were found resistant. These genotypes may prove promising in breeding programme concerning thrips resistance. Phenols and tannins conferred the groundnut genotype resistant to thrips

damage. This suggests that groundnut varieties with high concentration of phenols and tannins play a major role against thrips damage.

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