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Physio-morpho and biochemical basis of resistance in groundnut germplasm against thrips

Ramanaji Naralasetti and BP Katlam

Abstract
The screening of groundnut germplasms during *Rabi*, 2021–22, at the College of Agriculture, Raipur, farm revealed that NRCG - 134, NRCG - 8457, NRCG - 10348, NRCG – 10406 and NRCG – 10328 were the relatively resistant germplasms for thrips. The resistance in these germplasms was conferred due to higher leaf thickness (mm), trichome density per mm^2, and high chlorophyll content. The correlation studies also revealed that there was a significant negative correlation between biophysical parameters [leaf thickness (r = -0.910), trichome density (r = -0.945), and chlorophyll content (r = -0.588] and percent leaf damage by thrips. Except for phenols (r = -907), which exhibited a significant negative correlation with thrips percentage damage, other biochemical measures [total sugars (r = 0.942), reducing sugars (r = 0.914), non-reducing sugars (r = 0.709), and amino acids (r = 0.975)] showed a significant positive correlation with the percentage of leaf damage caused by thrips.

Keywords: Screening, biophysical, biochemical and thrips

Introduction
Groundnut, *Arachis hypogea* Linnaeus, is an important oil seed crop which is native to South America. It is a leguminous crop plant which is widely cultivated between 40 ° N and 40 ° S latitudes in the tropics and subtropics. It is also known for its high-oil edible seeds and is thus the fourth most important source of edible oil in the world. It is the third largest source of vegetable protein (Anonymous., 2018) [2]. It is also called the king of oilseeds, wonder nut, and poor man's cashew nut. Groundnut is the world's 13th most important food crop and the third largest oilseed produced in the world. In India, groundnut is grown in an area of 60.15 lakh ha with a production of 102.44 lakh tonnes and a productivity of 1703 kg/ha. Among the major groundnut-growing states, Gujarat occupied the first position. In Chhattisgarh, groundnut is grown in an area of 20.75 thousand ha with a production of 31.62 thousand tonnes and a productivity of 1524 kg/ha (Anon., 2022) [3]. Because of improved irrigation infrastructure and low-cost input technology, the area of groundnut crops is positively and significantly increasing in all of Chhattisgarh's districts. The introduction of high-yielding varieties (HYV) and increased fertiliser and pesticide use by farmers have both had a favourable and considerable impact on groundnut production and productivity (Agashe *et al*., 2018) [1]. There is a complex of thrips species that target the groundnut crop as sucking pests, mainly starting at the vegetative stage and continuing until the crop is harvested (Vijayalaxmi *et al*., 2009) [16]. Small insects called groundnut thrips live inside the blooms and folded terminal leaf buds of groundnut plants. Since they are small, only 2 mm long, and typically inconspicuous, they are simple to overlook. Due to an atrophied mandible, thrips have asymmetrical mouthparts and typically feed by sucking the contents of plant cells. Thrips eating is frequently marked by the "silvering" of leaves throughout the process (Srinivasan *et al*., 2018) [19]. However, farmers face huge economic losses in groundnut cultivation every year. Reduction in the groundnut yield is due to many biotic and abiotic stresses such as, pests, diseases, soil fertility, rainfall, water logging and climatic conditions. Among the major yield limiting factors pests are said to be an important one. So, therefore resistant peanut cultivars will be one of the most promising alternative control strategies since it is inexpensive, environmentally friendly, and easy to combine with other techniques. By emphasizing host plant resistance, we can reduce our reliance on chemical pesticides and eliminate residual problems.

Materials and Methods

Physio-morphological basis of resistance in groundnut germplasms against thrips

Physio-morphological characters *viz.*, leaf thickness, trichome density, leaf succulency, chlorophyll content and plant height were recorded from three randomly tagged plants in all the germplasms and data at 60 DAS is correlated with percentage damage of thrips.

Leaf thickness: Leaf thickness was recorded from five randomly selected plants and measured by Digital Vernier callipers and expressed in mm.

Trichome density: The observations of trichomes were recorded under microscope on abaxial side of leaf. Five leaves were collected from each germplasm at 60 DAS and were kept overnight in an acetic acid: alcohol (2:1). After removal of chlorophyll, leaves were transferred into 90 percent lactic acid in small vials for recording the observations. For microscopic examination, the leaves were mounted on a slide in a drop of lactic acid and observed under a microscope at 20X magnification. The trichomes on abaxial leaf surface were counted from randomly selected microscopic fields and were expressed as trichomes density (no. /mm^2) as described by Sasane *et al*., 2018 [17].

Leaf succulency: Leaf succulence is expressed as relative water content (RWC). The Kramer method was used to assess relative water content. Healthy leaves were used to estimate this parameter. The leaves weight was measured and expressed as fresh leaf weight (g). After being submerged in water for 4 hours to absorb the water, the leaves were removed, weighed, and expressed as turgid leaf weight (g). Following that, leaves were taken and dried in an oven for 48 hours at 70 °C. Subsequently, the weight of the leaves was measured and given as its dry weight (g). The relative water content of the leaf was estimated using the procedure below and expressed as a percentage (%) by Saleem *et al*., 2019 [15].

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

SPAD chlorophyll meter readings (SCMR): The SPAD meter (Soil Plant Analytical Development) is a simple handheld and portable instrument which provides information on the relative amount of leaf chlorophyll. The SCMR was measured on all leaflets of third leaf from the top on the main axis using SPAD meter of Minolta Company, NJ, US (SPAD502) and it is a unitless value.

Plant height: Plant height was recorded at harvest from the base of the plant to the auxiliary tip and measured with the help of scale and expressed as cm.

Biochemical basis of resistance in groundnut germplasms against thrips

Procedure for preparing the stock solution

For preparing the aliquot, one gram of fresh leaf sample was taken, chopped into little pieces, and submerged in distilled ethanol (80%). Using a pestle and mortar and some ethanol, the leaf tissue fragments were thoroughly pulverised before being centrifuged at 10,000 rpm for 30 minutes. Supernatant liquid was separated and volume was made to 10 ml with 80 percent ethanol. Then it was evaporated to dryness and leftover residues were dissolved in 10 ml of distilled water. This served as the stock solution, and an aliquot was taken in order to estimate the concentrations of phenol, sugar, and amino acids. Using a spectrophotometer, the absorbance of each chemical component in a sample was calculated.

In order to avoid inaccuracy in the estimate of sugars, a sufficient amount of aliquot was obtained from the filtrate and placed in the hot water bath until the alcohol smell of the sample was fully dissipated. The estimation of sugar was then done using the sample. However, as alcohol presence had no impact on the phenol content of the sample, its removal was not required for phenol quantification.

- Estimation of the total phenol – by Folin Ciocalteu's method (Bray and Thorpe 1954)
- Estimation of reducing sugars – by Nitro salicylic acid method (Miller, G. L. 1972) [10].
- Estimation of total sugars – by Anthrone method (Dubois *et al*., 1951) [6].
- Estimation of non-reducing sugars –Total sugars - reducing sugars
- Estimation of amino acids – Ninhydrin method (Moore and Stein 1948) [11].

Result and Discussion

Physio-morphological basis of resistance in groundnut germplasms against thrips

Plants respond to herbivory not just through biochemical mechanisms, but also by inducing morphological characteristics such as leaf thickness, trichome density, leaf succulency, chlorophyll content and plant height in following plant growth phases by Jasani *et al*., 2018 [8]. Physio-morphological basis of resistance in groundnut germplasms against thrips are shown in table 1.

Leaf thickness

The leaf thickness in our study ranged from 0.230 mm to 0.422 mm. Germplasm NRCG-134 (0.422 mm) had the thickest leaves, followed by NRCG-10406 (0.371 mm), NRCG-8457 (0.348 mm), and NRCG-10348 (0.343 mm). The germplasm with the least leaf thickness was NRCG-9481 (0.230 mm), followed by Tag 24 (0.242 mm), NRCG-10280 (0.254 mm), and NRCG- 9272 (0.256 mm). Correlation studies found a significant and negative (r = -0.910) relationship between thrips percentage damage and leaf thickness (mm) as shown in table 4.12. The regression equation between percentage leaf damage and leaf thickness is $Y = -230.21X + 99.74$, $R^2 = 0.827$.

Present studies were in relation with the Chandrayudu *et al*. (2016) [5] who concluded that significant and negative relationship between thrips and leaf thickness of groundnut. Rao *et al*. (2015) [13] found maximum leaf thickness in TCGS-894 (0.51 mm) which was found to be resistant and minimum thickness in susceptible germplasm ISK-I-2011-14 (0.28 mm). The feeding process may be hampered by thick leaves, which may have contributed to resistance.

Trichome density (No of trichomes/mm^2)

Trichome density in all the germplasms ranged from 9.00 trichomes/mm^2 to 53.67 trichomes/mm^2. Germplasm NRCG-134 (53.67 trichomes/mm^2) had highest number of trichomes/ mm^2, followed by NRCG-8457 (50.33 trichomes/ mm^2), NRCG-10328 (42.67 trichomes/mm^2), and NRCG-10406 (41.67 trichomes/mm^2). The germplasm with the lowest number of trichomes/mm^2 was NRCG-9481 (9.00 trichomes/m^2) followed by Tag 24 (9.34 trichomes/mm^2), NRCG-4341 (9.67 trichomes/mm^2), and NRCG-4408 (10.00 trichomes/mm^2). Correlation studies found a significant and negative (r = -0.945) relationship between thrips percentage damage and trichome density (No of trichomes/ mm^2). The regression equation between percentage leaf damage and trichome density is Y= -0.824X + 52.16, R^2 = 0.893.

One of the biophysical factors that has been linked to increased resistance to defoliators and sucking pests is trichome density on the leaf lamina. Trichomes on the leaf lamina have a crucial role in conferring resistance to insect pests, as shown by the negative and significant association between insect pests and trichomes on the leaf lamina. The current findings are consistent with those of Saleem *et al*. (2019) [15] who found that resistant genotypes of groundnut had a noticeably higher number of trichomes on the midrib as well as on the leaf lamina. Similar to this, Rahman *et al*. (2021) [12] revealed that much increased trichome density on the leaf lamina was the cause of resistance to several insect pests in germplasm lines. According to Jasani *et al*. (2018) [8] there is a substantial negative correlation between the incidence of PBND and the trichomes on the stem (r = -0.078), petiole (r = -0.318), and leaves (r = -0.455). The quantity of trichomes in the leaf lamina restricts thrips' ability to eat and restricts insect mobility. In this way, the quantity of trichomes on leaf lamina serves as a physical deterrent to insect damage.

Leaf succulency (%)

In the study we conducted, leaf succulency ranged from 42.87% to 70.33%. Germplasm NRCG-228 (70.33%) had highest succulent leaves, followed by NRCG-10328 (68.86%), NRCG-9285 (66.17%), and NRCG-10280 (65.90%). The germplasm with the least leaf succulency was NRCG-9324 (42.87%), followed by Tag 24 (48.65%), NRCG-9272 (51.89%), and NRCG-10371 (54.25%). Correlation studies found a non-significant and negative (r = -0.421) relationship between thrips percentage damage and leaf succulency (%).

The current research was in collaboration with Sonawane *et al*. (2019) [18] who discovered a negative and substantial association between leaf water content and thrips incidence (r = -0.982). According to Gadad *et al*. (2014) [7] leaf water content (r = -0.219) was negatively connected to thrips incidence.

Chlorophyll content

The chlorophyll content of different germplasms ranged from 33.16 to 45.10. Germplasm NRCG-134 (45.10) had the highest chlorophyll content, followed by NRCG-10348 (45.06), NRCG-10328 (44.76), and NRCG-228 (43.62). The germplasm with the least chlorophyll content was NRCG-10286 (33.16), followed by NRCG-4341 (35.54), Tag 24 (36.65), and NRCG-10280 (38.14). Correlation studies found a significant and negative (r = -0.588) relationship between thrips percentage damage and chlorophyll content. The regression equation between percentage leaf damage and chlorophyll content is Y= -2.339X + 125.93, R^2 = 0.346.

Plant height (Cm)

Different germplasms' plant heights ranged from 36.43 cm to 66.80 cm. Plant height was greatest in germplasm NRCG-134 (66.80 cm), followed by NRCG-4408 (64.60 cm), Tag 24 (60.77 cm), and NRCG-10286 (59.90 cm). Germplasm NRCG-8457 (36.43 cm) had the shortest plant height, followed by NRCG-10406 (42.80 cm), NRCG-9285 (48.43 cm), and NRCG-4341 (51.37 cm). Correlation tests revealed a non-significant and positive link (r = 0.352) between thrips percentage damage and plant height (cm) i.e., there was no relationship between percentage damage done by thrips and plant height.

Table 1: Physio-morpho and biochemical components of resistance against thrips in selected germplasms at 60 days after sowing

S. No	NRCG Germplasm No	Thrips % damage	Leaf thickness (mm)	Trichomes density	Leaf succulency	Chlorophyll content	Plant height (cm)	Phenol (mg/g of sample)	Total sugars (mg/g of sample)	Reducing sugars (mg/g of sample)	Non- reducing sugars (mg/g of sample)	Amino acids (mg/g of sample)
1	NRCG-10348	14.99	0.343	39.00	65.05	45.06	52.47	0.767	4.90	3.72	1.19	2.40
2	NRCG-10406	16.04	0.371	41.67	63.71	39.14	42.80	0.747	3.28	1.47	1.81	2.32
3	NRCG-8457	14.98	0.348	50.33	65.58	42.04	36.43	0.781	3.22	1.72	1.50	2.17
4	NRCG-134	13.95	0.422	53.67	62.95	45.10	66.80	0.940	3.90	2.12	1.78	1.98
5	NRCG-9285	22.08	0.321	32.00	66.17	39.24	48.43	0.455	5.85	3.60	2.25	2.92
6	NRCG-228	22.32	0.336	31.67	70.33	43.62	57.40	0.446	5.05	2.56	2.49	3.72
7	NRCG-10328	17.96	0.343	42.67	68.86	44.76	52.43	0.665	5.14	3.19	1.95	2.56
8	NRCG-10371	26.98	0.298	29.33	54.25	39.16	52.63	0.319	6.73	5.62	1.11	4.05
9	NRCG-9324	30.91	0.283	25.00	42.87	40.14	54.57	0.244	8.15	5.02	3.12	5.61
10	NRCG-4317	25.03	0.301	28.00	60.60	41.40	56.27	0.343	5.98	3.03	2.95	4.06
11	NRCG-203	33.65	0.268	20.67	54.70	41.52	57.13	0.210	11.36	9.13	2.23	5.94
12	NRCG-125	34.40	0.264	21.00	58.95	40.10	52.63	0.208	10.50	8.32	2.18	6.99
13	NRCG-10280	38.67	0.254	11.33	65.90	38.14	57.37	0.194	13.21	10.62	2.58	6.72
14	NRCG-4408	41.86	0.271	10.00	63.19	39.20	64.60	0.163	13.38	11.08	2.30	8.19
15	NRCG-8233	40.13	0.264	12.00	63.60	42.36	59.90	0.176	9.36	6.32	3.04	6.81
16	NRCG-10286	37.16	0.264	11.67	60.83	33.16	59.90	0.197	13.54	9.70	3.84	6.97
17	NRCG-9481	50.43	0.230	9.00	57.80	39.06	54.23	0.132	16.23	13.10	3.13	8.18
18	NRCG-4341	50.21	0.261	9.67	64.50	35.54	51.37	0.139	13.37	10.57	2.80	8.12
19	NRCG-9272	44.88	0.256	15.67	51.89	43.00	52.60	0.137	10.53	7.15	3.38	7.36
20	Tag 24	50.37	0.242	9.34	48.65	36.65	60.77	0.133	15.79	12.48	3.31	7.99

Table 2: Correlation coefficient of physio-morpho and biochemical parameters to thrips percentage damage

Parameters	Thrips % damage
Leaf thickness (mm)	-0.910**
Trichome density/ mm^2	-0.945**
Average succulency (%)	-0.421
Chlorophyll content	-0.588**
Plant height (cm)	0.352
Phenol (mg/g of sample)	-0.907**
Total sugars (mg/g of sample)	0.942**
Reducing sugars (mg/g of sample)	0.914**
Non- reducing sugars (mg/g of sample)	0.709**
Amino acids (mg/g of sample)	0.975**

*& **- Significant at 5 and 1 percent level of probability, respectively

Biochemical basis of resistance in groundnut germplasms against thrips

Total Phenols (mg/g)

Total phenols in different groundnut germplasms varied from 0.132 mg/g to 0.940 mg/g. The germplasm NRCG-134 (0.940 mg/g) showed significantly high total phenol which was resistant to thrips damage, while it was lowest in NRCG-9481 (0.132 mg/g) which was susceptible to thrips damage. The highest phenol content was observed in NRCG-134 (0.94 mg/g) followed by NRCG-8457 (0.781 mg/g), NRCG-10348 (0.767 mg/g), and NRCG-10406 (0.747 mg/g) and the least phenol content was observed in the germplasm NRCG-9481 (0.132 mg/g), Tag 24 (0.133 mg/g), NRCG-9272 (0.137 mg/g), and NRCG-4341 (0.139 mg/g) (Table 4.14).The correlation between percent leaf damage (thrips) and total phenol was highly significant and negative (r= -0.907) as shown in table 4.13. The study found that the higher the phenol levels in the germplasm, the lower the percentage of leaf damage. The regression equation between percentage leaf damage and total phenols is Y= -43.33X + 47.375, R^2 = 0.821.

The current findings are consistent with those of Chandrayadu *et al*. (2016) [5] who evaluated 39 groundnut genotypes and discovered that phenols had a substantial and negative association with the number of thrips and jassids and their damage. According to linear regression study, higher phenols contributed to thrips and jassids resistance in groundnut. The current findings are consistent with the findings of Sonawane *et al*. (2019) [18] who found that phenol concentration had a significant and negative relationship with thrips population (r = -0.830). Similarly, Gadad *et al*. (2014) [7] who discovered that plant phenol content is significant and inversely connected with thrips damage (r = -0.850).

Total Sugars (mg/g)

The present experimental result revealed that the total sugar content in different screened germplasms ranged from 3.22 mg/g to 16.23 mg/g. The germplasm NRCG-9481 (16.23 mg/g) showed significantly high total sugars which was susceptible to thrips damage, while it was low in NRCG-8457 (3.90 mg/g) which was resistant to thrips damage. The lowest total sugar content was observed in NRCG-134 (3.22 mg/g) followed by NRCG-201 (3.28 mg/g), NRCG-10406 (3.90 mg/g), and NRCG-8457 (4.90 mg/g) and the highest total sugar content was observed in the germplasm NRCG-9481 (16.23 mg/g), Tag 24 (15.79 mg/g), NRCG-10286 (13.54 mg/g), and NRCG-4408 (13.38 mg/g). The correlation between percent leaf damage (thrips) and total sugars was highly significant and positive (r = 0.942). The study found that the higher the total sugars level in the germplasm, higher will be the percentage of leaf damage. The regression equation between percentage leaf damage and total sugars is Y= 2.775X + 6.444, R^2 = 0.888.

Sonawane *et al*. (2019) [18] revealed that the total sugar content of groundnut leaves showed a positive and significant association with the thrips population (r = 0.520), which is consistent with the results of the current investigation. According to Chandrayadu *et al*. (2016) [5], total sugars (r = 0.313, 0.38) shown a positive correlation with the quantity of thrips and jassids and their percentage of damage.

Reducing Sugars (mg/g)

The experimental study on reducing sugar resulted in the finding that reducing sugar content in different germplasms varied from 1.47 mg/g to 13.10 mg/g. The germplasm NRCG-9481 (13.10 mg/g) showed significantly high reducing sugars, which was susceptible to thrips damage, while it was lowest in NRCG-10406 (1.47 mg/g), which was resistant to thrips damage. The lowest reducing sugar content was observed in NRCG-10406 (1.47 mg/g) followed by NRCG-8457 (1.72 mg/g), NRCG-134 (2.12 mg/g), and NRCG-228 (2.56 mg/g) and the highest reducing sugar content was observed in the germplasm NRCG-9481 (13.10 mg/g), Tag 24 (12.48 mg/g), NRCG-4408 (11.08 mg/g), and NRCG-10280 (10.62 mg/g). The correlation between percent leaf damage (thrips) and reducing sugars was highly significant and positive (r= 0.914). The study found that the higher the reducing sugars level in the germplasm, higher will be the percentage of leaf damage. The regression equation between percentage leaf damage and reducing sugars is Y= 3.001X + 11.7, R^2 = 0.836.

The results of the current experimental trial are consistent with those of Kandakoor *et al.* (2014) [9], who claimed that amino acids had a positive association with the number of thrips and the percentage of damaged leaves (r = 0.408 and 0.337, respectively).

Non-reducing sugars (mg/g)

Non-reducing sugars in different groundnut germplasms varied from 1.11 mg/g to 3.84 mg/g. The germplasm NRCG-10286 (3.84 mg/g) showed significantly high non-reducing sugars which was susceptible to thrips damage, while it was low in NRCG-10371 (1.11 mg/g) which was resistant to thrips damage. The lowest non-reducing sugar content was observed in NRCG-10371 (1.11 mg/g) followed by NRCG-10348 (1.19 mg/g), NRCG-8457 (1.50 mg/g), and NRCG-134 (1.78 mg/g) and the highest non-reducing sugar content was observed in the germplasm NRCG-10286 (3.84 mg/g), NRCG-9272 (3.38 mg/g), Tag 24 (3.31 mg/g), and NRCG-9481 (3.13 mg/g). The correlation between percent leaf damage (thrips) and non-reducing sugars was significant and positive (r= 0.709). The study found that the higher the non-reducing sugars level in the germplasm, higher will be the percentage of leaf damage. The regression equation between percentage leaf damage and non-reducing sugars is Y= 11.878X + 2.284, R^2 = 0.502.

Amino acids (mg/g)

In various groundnut germplasm, the total free amino acid content ranged from 1.98 mg/g to 8.19 mg/g. The germplasm NRCG-4408 (8.19 mg/g) showed significantly high amino acid content which was susceptible to thrips damage, while it was lowest in NRCG-134 (1.98 mg/g) which was resistant to thrips damage. The lowest amino acid content was observed in NRCG-134 (1.98 mg/g) followed by NRCG-8457 (2.17

mg/g), NRCG-10406 (2.32 mg/g), and NRCG-10348 (2.40 mg/g) and the highest amino acid content was observed in the germplasm NRCG-4408 (8.19 mg/g), NRCG-9481 (8.18 mg/g), NRCG-4341 (8.12 mg/g), and Tag 24 (7.99 mg/g). The correlation between percent leaf damage (thrips) and amino acid was highly significant and positive (r= 0.975). The study found that the higher the amino acid level in the germplasm, higher will be the percentage of leaf damage. The regression equation between percentage leaf damage and amino acids is $Y= 5.278X + 3.626$, $R^2 = 0.901$.

The findings of present experimental trial results are in line with Kandakoor *et al.* (2014) [9] who reported that amino acids showed positive relationship with number of thrips and the percent leaf damage with correlation value (r = 0.830 and 0.723).

Conclusion

From the critical analysis of the present findings, it can be concluded that percentage damage by thrips were highly negatively correlated with leaf thickness, trichome density and phenol content and were positively associated with total sugars, reducing sugars and amino acids content.

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