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## Defensive responses of groundnut genotypes to *Aproaerema modicella* and *Spodoptera litura*

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### Abstract

Insect Pest management by adopting host plant resistance is one of the most economic and environment friendly methods in crop production. Hence, a study was undertaken to identify differential reactions and reasons behind them in certain promising groundnut genotypes with reference to most devastating leaf feeders of groundnut under field conditions. Thirty nine promising groundnut genotypes (K 1274 to K 1622, TCGS 750, DRT 24, DRT 40, DRT 43, TIR 9, ICGV 888, ICGV00350, ICGV009114, TAG 24 and JL 24) were field screened under rain-fed situation during 2013-15 crop seasons in order to identify source of resistance or tolerance to *Aproaerema modicella* (Deve.) and *Spodoptera litura* (Fab.). The field level study indicated the interaction between leaf feeders (*A. modicella* and *S.litura*) and 39 groundnut genotypes, since there was perceptible incidence of pests in all the test seasons. Twelve genotypes that reacted to pests could be categorised as resistant, while 18 genotypes were grouped under moderately resistant to *A.modicella* and *S.litura*. Leaf thickness, density of leaf laminar hairs and phenol levels showed negative relationship with larval number and foliage damage due to *A.modicella* and *S.litura*. Total sugars showed positive relationship in terms of larval numbers and foliage damage due to the insects. Linear regression indicated the role of leaf thickness and laminar hairs besides the amount of phenolic compounds for resistance to *A.modicella* and *S.litura* on groundnut.

**Keywords:** *Arachis hypogaea*, defoliating insects, morphological and chemical characters, defensive responses, pest management

### Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is one of the world's oilseed crop grown in around 26 million ha with total production of 37 million mt and average productivity of 1.4 mt ha<sup>-1</sup>. Over 100 countries, mostly developing ones depend directly or indirectly on this principal oil seed nut crop. India is one of the largest producers of oilseeds with special importance to groundnut [1]. In India Three-fourths of the area and production is concentrated in five states viz., Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka and Telangana mostly under rain-fed farming situations [2]. Naturally, the productivity in India is far inferior (1893 kg ha<sup>-1</sup>) as compared to USA (4125 kg ha<sup>-1</sup>) and China (3964 kg ha<sup>-1</sup>), the other large producers of groundnut [3]. The yield loss caused by many insect pests and diseases were identified as one of the main reasons for low yields and low returns [4]. Losses due to insect pests in groundnut ranges from 10 to 30 per cent annually depending upon the severity of pest attack [5, 6]. Leaf miner or folder, *Aproaerema modicella*, defoliator and pod eater, *Spodoptera litura* are serious on groundnut in India both in rainy and post rainy seasons on groundnut. Being oligophagous in nature, *A.modicella* on Soy-bean, pigeonpea like crops and *S.litura* on crops like tobacco (*Nicotiana rustica*), cotton (*Gossypium hirsutum*), soybean (*Glycine max* Mirr.), pigeonpea (*Cajanus cajan* Millsp.) [7], Lucerne (*Medicago sativa* Linn.) and on several weeds also, these two insects continue to be active throughout the seasons [8].

In view of environmental safety and cost effectiveness field screening of germplasm for tolerance/resistance to insect pests has received considerable attention. However, there is limited progress in assessment of morphological and biochemical mechanisms conferring tolerance/resistance to insects pests [9, 10]. Although the response of groundnut to drought stress has been well studied [11], the progress in developing tolerance/resistance to insect pests is not to the extent made possible. Plants that developed a wide range of defensive mechanisms to defend themselves against herbivore attack [12, 13]. Plant phenolic constituents, such as phenolic acids, flavonoids, isoflavonoids, tannins, lignins, etc., a part in plant defense as

phytoanticipins, phytoalexins as structural barriers besides modulators of pathogenicity, and activators of plant defense genes [14]. The present study was undertaken to compare the morphological and biochemical responses of resistant and susceptible genotypes of groundnut to the damage by *A.modicella* and *S.litura*. The study could focus on the morphological characters such as leaf thickness, leaf laminar hairs and biochemical constituents' viz., total phenols and total sugars in relation to leaf damage of the insects.

### Materials and Methods

The field site was Agricultural Research Station, Kadiri, Anantapuramu district, Andhra Pradesh, India located at an altitude of 182.9 m above MSL and 79°E longitude and 13° N latitude in the scarce rain fall semi-arid climate zone. The climate is semi-arid dry with a mean annual precipitation of 530 mm and a prolonged dry season from February to June. Site and climate characteristics have been described elsewhere. The trial was laid out in a Randomized Block Design with 39 genotypes of groundnut obtained from different institutes in Andhra Pradesh, Telangana and Maharashtra. The genotypes were screened in field under natural infestation to identify the tolerance/resistant ones. Each genotype was sown in two rows along with a single row of susceptible check, JL 24 after two test rows in three replications at 30 cm x 10 cm between rows and plants with an area of 0.005 ha respectively. The groundnut crop was raised by following normal agronomic practices excluding plant protection measures. The responses of groundnut genotypes to target pests were assessed by visual grading of damage and absolute insect counts on each test entry. The per cent foliage damage due to *A. modicella* and *S.litura* (0-100%) during the peak infestation period was made by following the standard scale of 1-10 [15]. Per cent foliage damage was made by counting the total number of leaflets and damaged leaflets from 10 randomly selected plants of each entry. Damage levels were categorised by using the standard scale of 1-100 method [16] (Table 1).

**Table 1:** Damage score and per cent foliage damage to calculate severity index.

Foliage damage (%)	Score (B)
0	1
1-20	2
21-30	3
31-40	4
41-50	5
51-60	6
61-70	7
71-80	8
81-90	9
91-100	10

Severity index is the index showing the severity of infestation in terms of severity of burning/defoliation symptoms. The severity index was calculated by using the formula;

$$SI = \frac{A \times B}{100}$$

Where

A = mean leaflet damage score

B = mean foliage damage score [17].

Categorizations of genotypes (Table 2) were made based on

severity index by following the methodology [18]. After the crop-attained maturity, the pods were harvested separately from each screening plot, dried properly and pod weight was recorded. The response of groundnut germplasm against *A. modicella* and *S.litura* were assessed mainly by visual recording of per cent foliage damage. While calculating severity indices considered per cent leaflet damage. Observations were also recorded on absolute population of *A. modicella* and *S.litura* larvae per, per cent foliage damage in 1-10 scale along with pod yield.

**Table 2:** Categorisation of resistant levels based on per cent foliage damage.

Foliage damage (%)	Category
0	Immune
1-20	Resistant
21-40	Moderately resistant
41-60	Moderately susceptible
61-100	Highly susceptible

Fresh uniformly developed leaves were collected at 40-50 days after germination from randomly selected plants and leaf thickness, leaf laminar hairs were measured [19]. The groundnut leaves was cut into bits of 9 mm<sup>2</sup> (3x3 mm) with help of stainless steel blade and hairs present on the laminar portion of these leaves were counted under a binocular microscope (10x, 100x). Similarly, leaves thickness were measured under a compound microscope using stage and ocular micrometer. Collected the fresh leaf and shoots from resistant, moderately resistant and susceptible groundnut genotypes in field and dried at 32°C in a hot air oven for 48 hr. These leaf and shoot samples were powdered using grinder for 3 then sieved with using 100 mesh screen and stored in a sealed plastic containers (0.5m diameter) at 4°C for estimation of total sugars and total phenols. According to the method denoted as [20] total sugar content was hydrolyzed in 1.0 ml of 1.0 N H<sub>2</sub>SO<sub>4</sub> and 0.5ml of aliquot and heated over boiling water bath for 30 min. Immediate after cooling in running water, added one to two drops of phenolphthalein indicator. Later added 1.0 N NaOH drop by drop to neutralize the acid in the hydrolysate till it developed pink colour. The neutralized solution was made into colour less by diluting 1.0 N H<sub>2</sub>SO<sub>4</sub>, finally the volume was make up to 10 ml with distilled water and read the absorbance at 510 nm using spectrophotometer. As per method [21] the 100 mg of aqueous was extracted from oven dried powdered plant samples in 10 ml of 80% ethanol for 1 hour at room temperature. The extracted 100 mg aqueous was centrifuged at 6000 rpm for 15 min. After centrifuge, the supernatant was evaporated to dryness on a water bath and the residue was dissolved in 5 ml fresh water. For estimation of total phenols alcohol free extract was used. An aliquot sample of 0.1ml was diluted in 3ml water and added 0.5ml of Folin-ciocalteau reagent (FCR) mixed well. Exactly after 3 min, of 20% sodium carbonate solution was added in aliquot solution and kept in boiling water bath for one min. After under running tap water the absorbance was read at 650 nm against the reagent blank in a photo spectrometer. A standard graph was prepared while constructing with Catechol as a standard. The total phenol content in a plant sample was expressed as mg g<sup>-1</sup> d.wt. The collected data were pooled together for calculating mean and standard error. Data were statistically analyzed by two-way analysis of variance (ANOVA) using SPSS (Version 15.1). The mean values were separated by following Turkey's test.

## Results and Discussion

The genotypes K 1470(FDR), K 1535 (IPR), K 1504S(LS), K 1504T(LS), K 1563(IPR), K 1564(IPR), K1571(TAF), K 1577 (LS), K 1581(LS), K 1604(HY), K 1609 (HY) and K 1520(HY) showed resistant reaction with significantly lowest foliage damage of 8.1 to 18.1 and 10.3 to 19.9 % and higher dry pod yield of 1631 to 2111 kg $ha^{-1}$  (Table 3). The genotypes K 1274, K 1320, ICGV00888, ICGV00350, K1452(DT)VG, K1454(DT)VG, K 1468(FDR)VG, K 1482(FDR)VG, K1501(LS), K1569(HY), K1570(TAF), K1574(LS), K1578(LS), K1621(HY), K1622(HY), TIR9, ICGV 0091114 and TAG24 were moderately resistant with 20.1 to 36.2 and 20.1 to 39.7 % foliage damage by *A. modicella* and *S.litura* respectively compared to highly susceptible check JL 24 which recorded higher foliage damage of 30.7 to 62.5 and 41.3 to 73.4 % . Other genotypes DRT40, K1282, K1392, TCGS750, DRT43, K1451(DT)VG, K1463 and K1576(LS) were moderately susceptible (Table 3). The present findings are in line with that of ICGV 006424, ICGV 07247, DRT 43 was

reported as highly susceptible groundnut genotypes recorded highest leaf let damage by *Spodoptera* and *Helicoverpa* [22, 23]. The resistant genotypes were least by insect damage and as resulted in lowering *Helicoverpa* larval survival and less weights than those larvae fed on the susceptible check JL 24. The number of aphids were significantly lower on insect-resistant genotypes compared to susceptible check JL 24 [24]. None of the genotypes responded as a immune to *A. modicella* and *S.litura*. A significant and positive correlation were observed between *A. Modicella* ( $r = 0.740$ ), *S.litura* larval population ( $r = 0.890$ ), foliage damage ( $r = 0.957$ ) and total sugar ( $r = 0.938$ ) (Table 5). Higher morphological and biochemical constituents in plants insisted variation in damage may be due to differential load of *A. modicella* and *S.litura* population on different groundnut genotypes. Similar reports [25] indicated that resistant groundnut genotypes showed minimum weight gain in *H. armigera* larvae compared to susceptible genotypes.

**Table 3:** Reaction of groundnut genotypes against *A. modicella* and *S.litura* damage, kharif 2013 to 2015.

Genotypes	<i>A. modicella</i> (Foliage damage %)##	Score (B)	<i>S.litura</i> (Foliage damage %) ##	Score (B)	Pod yield (kg ha $^{-1}$ )
DRT40	36.2(19.5) <sup>k</sup>	4	20.9(27.2) <sup>e</sup>	3	1613
K1274	12.0(20.3) <sup>c</sup>	2	24.3(29.6) <sup>f</sup>	3	1818
K1282	26.6(20.0) <sup>g</sup>	3	39.7(35.5) <sup>k</sup>	4	1428
K1320	11.3(19.6) <sup>b</sup>	2	20.8(27.1) <sup>d</sup>	3	1260
K1392	32.1(20.4) <sup>j</sup>	4	38.2(32.1) <sup>k</sup>	4	1822
K1463	30.7(19.1) <sup>i</sup>	4	44.4(34.1) <sup>m</sup>	5	1299
K1470(FDR)	10.2(18.7) <sup>b</sup>	2	19.5(26.3) <sup>d</sup>	2	2009
K1535(IPR)	11.2(19.6) <sup>b</sup>	2	18.6(25.5) <sup>c</sup>	2	2020
ICGV00888	12.0(20.3) <sup>c</sup>	2	21.3(27.4) <sup>e</sup>	3	1529
ICGV00350	11.1(19.4) <sup>b</sup>	2	20.1(26.6) <sup>d</sup>	3	1678
TCGS00750	34.8(22.6) <sup>k</sup>	4	32.4(32.2) <sup>i</sup>	4	1697
TIR9	17.1(24.5) <sup>e</sup>	2	25.5(30.3) <sup>g</sup>	3	1770
ICGV91114	15.2(22.9) <sup>d</sup>	2	27.2(31.4) <sup>h</sup>	3	1840
TAG24	30.0(21.1) <sup>i</sup>	3	23.6(29.1) <sup>f</sup>	3	1903
DRT43	32.7(20.8) <sup>j</sup>	4	23.8(29.1) <sup>f</sup>	3	1633
K1451(DT)VG	28.8(23.4) <sup>h</sup>	3	35.4(32.2) <sup>j</sup>	4	1376
K 1452(DT)VG	14.6(22.5) <sup>d</sup>	2	26.9(31.2) <sup>g</sup>	3	1303
K 1454(DT)VG	12.2(20.4) <sup>c</sup>	2	20.5(27.0) <sup>d</sup>	3	1649
K1468(FDR)VG	15.0(22.8) <sup>d</sup>	2	26.1(30.7) <sup>g</sup>	3	1809
K1482(FDR)VG	15.0(22.8) <sup>d</sup>	2	26.9(31.2) <sup>g</sup>	3	1546
K 1501(LS)	9.8(18.2) <sup>b</sup>	2	21.6(27.7) <sup>e</sup>	3	1386
K 1504S(LS)	9.7(18.2) <sup>b</sup>	2	19.8(26.4) <sup>d</sup>	2	1859
K 1504T(LS)	8.3(16.7) <sup>a</sup>	2	18.9(25.8) <sup>d</sup>	2	1631
K 1563(IPR)	8.1(16.5) <sup>a</sup>	2	19.9(26.5) <sup>d</sup>	2	2110
K 1564(IPR)	8.4(16.8) <sup>a</sup>	2	18.2(25.3) <sup>c</sup>	2	2041
K 1569(HY)	8.6(17.0) <sup>a</sup>	2	20.4(26.8) <sup>d</sup>	3	1639
K 1570(TAF)	9.1(17.5) <sup>a</sup>	2	21.8(27.8) <sup>e</sup>	3	1702
K 1571(TAF)	9.8(18.2) <sup>b</sup>	2	18.6(25.6) <sup>c</sup>	2	2104
K 1574(LS)	14.5(22.4) <sup>d</sup>	2	23.2(28.8) <sup>f</sup>	3	1049
K 1576(LS)	17.3(24.6) <sup>e</sup>	2	41.3(32.2) <sup>l</sup>	5	1964
K 1577(LS)	15.5(23.2) <sup>d</sup>	2	19.8(26.4) <sup>d</sup>	2	1761
K 1578(LS)	20.6(27.0) <sup>f</sup>	3	26.3(30.8) <sup>g</sup>	3	1591
K 1581(LS)	14.8(22.6) <sup>d</sup>	2	14.8(22.6) <sup>b</sup>	2	1752
K 1604(HY)	18.1(25.2) <sup>e</sup>	2	10.3(18.7) <sup>a</sup>	2	2055
K 1609(HY)	16.9(24.3) <sup>e</sup>	2	17.2(24.5) <sup>c</sup>	2	1933
K 1520(HY)	15.5(23.2) <sup>d</sup>	2	18.5(25.5) <sup>c</sup>	2	2111
K 1621(HY)	15.5(23.2) <sup>d</sup>	2	22.4(28.3) <sup>e</sup>	3	1859
K 1622(HY)	20.1(26.6) <sup>f</sup>	3	21.9(27.9) <sup>e</sup>	3	1943
JL 24(Check)	62.5(52.2) <sup>l</sup>	7	73.4(58.1) <sup>n</sup>	8	698
S.Em+	0.4	--	0.5	--	64.0
CD (P=0.05)	1.1	--	1.4	--	181.0
CV (%)	3.1	--	3.0	--	7

**Note:** Figures in parentheses are arc sine transformed values.



**Fig 1:** Scheme for identification and utilization of multiple pest resistance/tolerance groundnut genotypes

Followed Percent foliage damage 1-100 scale Induced resistance is an important component of plant defense that allows plants to be phenotypically strong in order to face different stresses and is economical, environment effective [26]. While understanding of plant response to insect pests will provide new insights into basic mechanisms of chemical communication and plant animal co-evolution and facilitate for new approaches to crop protection and improvement. Assessed the defensive biophysical and biochemical response of 39 groundnut genotypes to *A. modicella* and *S.litura* damage. Similarly explore the increased levels of defense-related proteins are a common phenomenon occurring in plants on account of biotic and abiotic stress [27]. Highest resistant response of 10 genotypes including highly susceptible response of JL-24 against *A. modicella* and *S.litura* were selected in this study, among genotypes tested exhibited leaf thickness and lamina hairs varied from 18.35 to 24.80 mm and 19.58 to 29.58 no's. The resistant genotypes

K1535 (IPR), K1563(IPR), K1564(IPR), K 1570(TAF), K 1574(LS) and K 1604(HY) had recorded higher leaf thickness (23.68 to 24.88 mm) and lamina hairs (26.90 to 29.50 no's) compared to lower leaf thicknesses (18.35 to 23.72 mm) and lamina hairs (19.58 to 25.70 no's) in susceptible genotypes viz., K 1320, DRT 40, ICGV00350 and JL 24 (Table 4). Leaf trichomes length and density, sugars, proteins and phenols were found to be accelerate resistance to *Maruca vitrata* in short duration pigeon pea genotypes [28]. Significant negative relationship were observed between leaf thickness, lamina hairs and *A. modicella* and *S.litura* larval population ( $r = -0.777, -0.929$  and  $r = -0.611, -0.779$ ). The relations between leaf thickness, lamina hairs and percent foliage damage ( $r = -0.860, -0.877$  and  $-0.822, -0.866$ ) were also existed similar trend at 1% level of significance (Table 5). Similar finding indicated that Leaf thickness and trichomes of different cotton varieties/hybrid showed significant negative relationship with the incidence of leaf feeders [29].

**Table 4:** Response of morphological and biochemical constituents in resistant and susceptible groundnut genotypes to *A. modicella* and *S.litura* damage during *kharif* from 2013 to 2015.

Varieties	<i>A.modicella</i>		<i>S.litura</i>		Leaf thickness (mm)	Laminar hairs (No.s/ 3 mm <sup>2</sup> )	Phenols (mg g <sup>-1</sup> )	Total Sugars (mg g <sup>-1</sup> )
	No.of larvae /plant	Per cent foliage damage/plant	No.of larvae/plant	Per cent foliage damage/plant				
K 1604(HY)	2.0	18.10	1.0	10.30	24.48	29.58	0.79	2.1
K 1563(IPR)	1.0	08.10	1.0	19.90	24.45	28.63	0.75	1.8
K 1564(IPR)	1.0	08.40	1.0	18.20	24.55	28.32	0.73	1.7
K 1535(IPR)	1.0	11.20	1.0	18.60	24.80	27.03	0.77	1.6
K 1571(TAF)	2.0	09.10	2.0	18.60	23.68	26.90	0.60	2.3
K 1470(FDR)	2.0	10.20	1.0	20.80	24.64	26.97	0.63	2.1
K 1320	12.0	11.30	5.0	20.90	23.72	25.70	0.61	3.2
ICGV 00350	8.0	11.10	5.0	20.10	21.84	23.74	0.53	3.5
DRT 40	5.0	36.20	7.0	19.50	21.48	24.70	0.53	3.8
JL 24(Check)	13.0	47.50	9.0	56.40	18.35	19.58	0.18	6.8

\*Significant at p=0.05

**Table 5:** Correlation between biophysical, biochemical constituents of groundnut genotypes with *A. modicella* and *S.litura* population and per cent leaf damage by *A. modicella* and *S.litura* during *kharif* from 2013 to 2015.

Host plant characters	<i>A. modicella</i> (*r' value)		<i>S.litura</i> (*r' value)	
	larvae (No.s per plant)	Foliage damage (%)	Larvae (No.s per plant)	Foliage damage (%)
Leaf thickness	-0.777**	-0.860**	-0.929**	-0.877**
Laminar hairs	-0.611**	-0.822**	-0.779**	-0.886**
Phenols	-0.659**	-0.850**	-0.839**	-0.891**
Total sugars	0.740	0.957	0.890	0.938

\*\* Significant at p=0.01

The phytochemical variability in genotypes occurring in various geographical locations are the results of genotypic and environmental interactions<sup>[30]</sup>. Existing of total phenolics and sugars in plants in response to insect pests is a general phenomenon<sup>[31]</sup>. Among 10 total sugar content varied from 1.6 mg (K 1535{IPR}) to 6.8 mg (JL 24) per gram of leaf. Existed positive correlation between total sugar, *A. modicella* and *S.litura* larval population ( $r = 0.740$  and  $0.890$ ) and foliage damage ( $r = 0.957$  and  $0.938$ ) (Table 5). The findings indicated that to combat with the biotic and abiotic stresses, plants produce a number of defense-related enzymes and other protein-based defensive compounds<sup>[32]</sup>. In resistance and susceptible genotypes phenol content varied from 0.18 mg (JL 24) to 0.79 mg (K 1604{HY}) per gram of leaf sample respectively (Table 4). This is in agreement with earlier reports who indicated the increase in protein concentration following *H. armigera* damage might be due to the production of more defense related enzymes and other protein-based defensive compounds, many of which are detrimental to herbivore fitness<sup>[33, 34 35]</sup>. These results showed a significant difference at 5% level of significance (Table 4). A negative correlation were existed between phenols and *A. modicella*, *S.litura* larval population ( $r = -0.659$  and  $-0.839$ ) and % foliage damage ( $r = -0.850$  and  $-0.891$ ). *A. modicella* and *S.litura* resistant groundnut genotypes had highest leaf thickness, laminar hairs and higher quantities of phenols compared with the susceptible varieties<sup>[36]</sup>. Presence of higher quantities of trichomes and tannins, phenols conferred resistance against *A. modicella* and *S.litura*<sup>[37]</sup>. Similar reports indicated that there was a significant negative correlations between poly phenols and damage indices ( $r = -0.57$ ), mean adult counts ( $r = -0.56$ ) and mean larval counts ( $r = -0.64$ ) of resistant cowpea cultivars play a significant role in thrips and leaf feeders resistance<sup>[38]</sup>. The foliage damage due to *A. modicella* and *S.litura* larval population fitted with multiple linear regression equation. Although regression equation influenced *A. modicella* and *S.litura* larval population and foliage damage to an extent of 93.6 % and

87.6 % ( $R^2 = 0.936$  and  $0.876$ ), respectively. The present findings are in accordance with studies where reported *Scirtothrips dorsalis* and *Frankliniella schultzei* on groundnut clearly indicate a positive correlation between the phenols, leaf trichomes and damage<sup>[39]</sup>. Stepwise regression of morphological and biochemical constituents revealed that *A. modicella* and *S.litura* larval population and % foliage damage indicated significant relationship with leaf thickness, laminar hairs and phenols. Induction of enzyme activities and secondary metabolites were greater in the *H. armigera* and *A. craccivora* resistant genotypes ICGV 86699, ICGV 86031, ICGV 2271, and ICGV 1697 than in the susceptible check JL 24<sup>[40]</sup>. Thus, it can be said that higher leaf thickness, laminar hairs, decreased total sugar and increased total phenols in groundnut genotypes exhibited decreased trend of foliage feeding by *A. modicella* and *S.litura*.

As more than 70 % of the groundnut growing area comes under rainfed cultivation in India, resource poor farmers are neither adopt nor affordable to take any plant protection measures<sup>[41,42]</sup>. The situation is more alarming if the problem is viewed from the point of insecticides involved because resistance has been reported to almost every insecticide against *A. modicella* and *S.litura* that had been employed for pest control<sup>[43]</sup>.

### Conclusion

The information generated from this study would help in developing novel breeding strategies to combine both high yield groundnut genotypes and host resistance to *A. modicella* and *S.litura* diversify the genetic base in future groundnut cultivars and boon for the rainfed resource poor farmers.

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