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Screening of Soybean Germplasm against *Spodoptera litura* (Fab.) for the Expression of Antixenosis Resistance Through Seasonal Incidence and Dual-feeding Assay

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ABSTRACT

Background: Soybean, [*Glycine max* (L.) Merr.] is an important *kharif* growing crop and affected by a number of insect pests which are directly or indirectly decrease the yield. To increase the yield, the infestation by insect pest should be managed. *Spodoptera litura* is considered as the major pest of soybean crop and damages soybean to a very extent. Cultivation of insect resistant soybean can be the best technique for pest management program. Insect resistance is usually conferred by antixenosis *i.e.* the set of plant characteristics and insect responses that lead to undesirability of host. Thus, to find out the antixenosis resistance among 16 germplasm this study was performed.

Methods: Seasonal incidence of *S. litura* was recorded with the abiotic parameter. Antixenosis resistance was determined through non-choice and free-choice feeding assay likewise MLAC (cm^2) and C-value were calculated to find out the preference or non-preference of soybean germplasm. Trichome density and length were also examined which aids in the resistance mechanism.

Result: Incidence of pest show positive correlation with temperature and morning humidity. The data on area consumption and C-value of soybean signified the resistant germplasm (BAUS 102, DSB34, MACS 1493 and RSC 11-03) and highly susceptible germplasm (NRC 131 and RSC 11-07) against *S. litura* which also conferred indirect relationship with trichome density and length.

Key words: Antixenosis, Non-choice and choice feeding assay, Non-preference, Preference, Seasonal incidence, Soybean germplasms, *Spodoptera litura*, Trichome density.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is economically the most essential bean in the world, supplying 25% of the global edible oil for millions of people and ingredients for hundreds of chemical products and contributes about two-thirds of the world's protein concentrate for livestock feeding (Agarwal *et al.*, 2013). Seeds of soybean contain about 42% protein and 20% oil and provide 60% of the world supply of vegetable protein and 30% of the edible oil (Fehr, 1989).

According to FAOSTAT (2020) the cultivated area under soybean in India is about 5.5 million hectares and the production was about 1.126 million metric tons. The productivity of this crop is affected by various abiotic and biotic factors. Under the biotic stress insect pest solely caused more than 25% yield loss (Harish *et al.,* 2009) and the most destructive insect pests of soybean include a variety of foliage feeders, stem borers, gram pod borer and stink bug. The tobacco armyworm, *Spodoptera litura* (Fabricius) is a polyphagous pest and known to attack soybean from the early growth up to harvesting time and it is the major defoliating pest and responsible for upto 68% yield loss (Bayu *et al.,* 2017). The larval form of this pest cause severe damage to the soybean crop by defoliating the leaves. After hatching the first and second instars gregariously feed on the leaf and completely skeletonize it then the third instars disperse and feed on leaves only remaining the veins. Thus, to protect the crop from damage the incidence of pest must be known so that proper management can be taken. Apart from this, antixenotic resistance is usually confer resistance to pest and helpful to decrease the damage. Antixenosis denotes the group of plant characters and insect responses that lead to or away from the use of a particular plant or variety, for oviposition, for food, or for shelter, or for combinations of the three (Kogan and Ortman, 1978). The trichome length and trichome density is main factor which aids in antixenosis resistance which deters the insect from feeding (Smith, 2005) and considered as a source of development of insect pest resistant cultivar.

The overall aims of the present study are as follows: 1) to identify resistance and susceptible germplasm in field condition with respect to the incidence of *S. litura*;

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2) investigation the antixenotic resistance of soybean germplasm with dual feeding assays against *S. litura*; and 3) to characterize the underlying trichome structure that provides resistance against *S. litura.*

MATERIALS AND METHODS

The experiments were carried out in G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during *Kharif*-2021.

Plant material and Screening of *S. litura* in field

Sixteen soybean germplasms (AMS 100-39, BAUS 102, DS 3108, DSB 34, MACS 1493, NRC 128, NRC130, NRC 131, NRC 132, NRC 136, NRC 137, NRC SL1, PS 1613, RSC11-03, RSC 11-07 and SKF-SP-11) with susceptible check variety (JS-335) were screened in Norman E. Borlaug Crop Research Centre (NEBCRC), G.B.P.U.A.T. Pantnagar, Udham Singh Nagar during *Kharif* season of 2021 and in laboratory of Morphology at Department of Entomology, G.B.P.U.A.T. Pantnagar, Udham Singh Nagar for the resistance against *S. litura*. Soybean germplasms were screened in 3 replications by using randomized block designs (RBD). The seasonal incidence of *S. litura* was examined every week after 35 days after germination in the prevailing environmental conditions.

Insect material and determination of antixenotic resistance through feeding assays

Egg mass of *S. litura* was collected from Norman E. Borlaug Crop Research Centre, GBPUAT, Pantnagar during *kharif*-2021 and then it was kept in a plastic jar (12 × 6 cm). After hatching castor leaves were provided as food and the culture was maintained in the laboratory (27± 1°C and 65 ±5% RH). Third instar larvae were used for the lab screening of soybean germplasm for antixenotic resistance. The study was performed through two feeding assays *viz.* free-choice test and non-choice test by using completely randomized design (CRD). For these assays fresh and matured leaves soybean were plucked, washed and after drying cut the leaf disc.

Non-choice test

The leaf disc were kept separately in the center of sterilized petri-plates and pre-starved (3 hours) larvae were released in each petri-plate and allowed to feed. All treatments were replicated 3 times along with the control. The leaf area consumed by larvae after 8 hours was measured using graph sheet. The determination of resistance of soybean germplasms against *S. litura* based on the intensity of leaf damage which was calculated by using C-value provided by Kogen and Goeden (1970) and presented in Table 2.

C- Value

Where,
M Eaten area of treated leaf disc.
B Eaten area of control leaf disc.

If C- Value,
>1 Indicated a preference for the test plant.
1 Feeding on test plant equal to standard plant.
<1 Lesser acceptance of test plant (Slightly antifeedent).
<0.5 Lower limit of acceptance of the test plant (Moderately antifeedent).
<0.1 Extremely antifeedent test plant.

Free-choice test

For this test, leaf disc of 16 germplasms with control was placed together in a tray (20 × 12 cm) with three replications and separated with a thermocol sheet and opened at centre. Ten larvae were released in each replication at the centre of the tray to feed freely among the given disc. The movement and feeding of *S. litura* was observed and recorded as described before.

Trichome density conferring resistance to *S. litura*

The trichome density of different germplasms was observed in 1mm^2 leaf area through compound microscope. The trichome length and width was observed through Scanning electron microscope (Fig 1) at Electron Microscopy Laboratory in the College of Veterinary, GBPUAT Pantnagar.

Data analysis

The data obtained from field was statistically analyzed using SPSS software. Field and lab data including seasonal incidence, MLAC, trichome length and density were transformed then subjected to analysis of variance.

RESULTS AND DISCUSSION

Seasonal incidence of *S. litura* in field correlated with weather parameter

Seasonal incidence of *S. litura* was studied soybean germplasm in the *kharif*-2021 by counting the population of pest at weekly interval also correlated with some environmental factors like temperature and relative humidity (Table 1) as from previous study it effects incidence of the pest the most (Suyal *et al.,* 2018). The field results depicts that initially the pest populations arise during 36th standard metrological week (SMW) which gradually increases till 39th SMW where it is on peak. On 39th SMW the incidence of *S. litura* was ranging from 0.23 (RSC 11-03) to 3.06 (NRC 131) per meter row length (mrl) and lowest on 42th SMW (Fig 2). The weather prevailing during that week were found maximum (33.4°C), minimum (24.2°C) and average (28.8°C) while the morning (91%), evening (61%) and average (76%) relative humidity. Results also depicts that four soybean germplasm (BAUS 102, DSB34, MACS 1493 and RSC 11-03) were resistant towards the *S. litura* while NRC131 and RSC 11-07 were highly susceptible in comparison to susceptible check (JS-335). The correlation between pest population (larvae/mrl) and weather parameter are presented in Table 1. The larvae/mrl in different soybean germplasm showed positive correlation with minimum temperature, maximum temperature and maximum

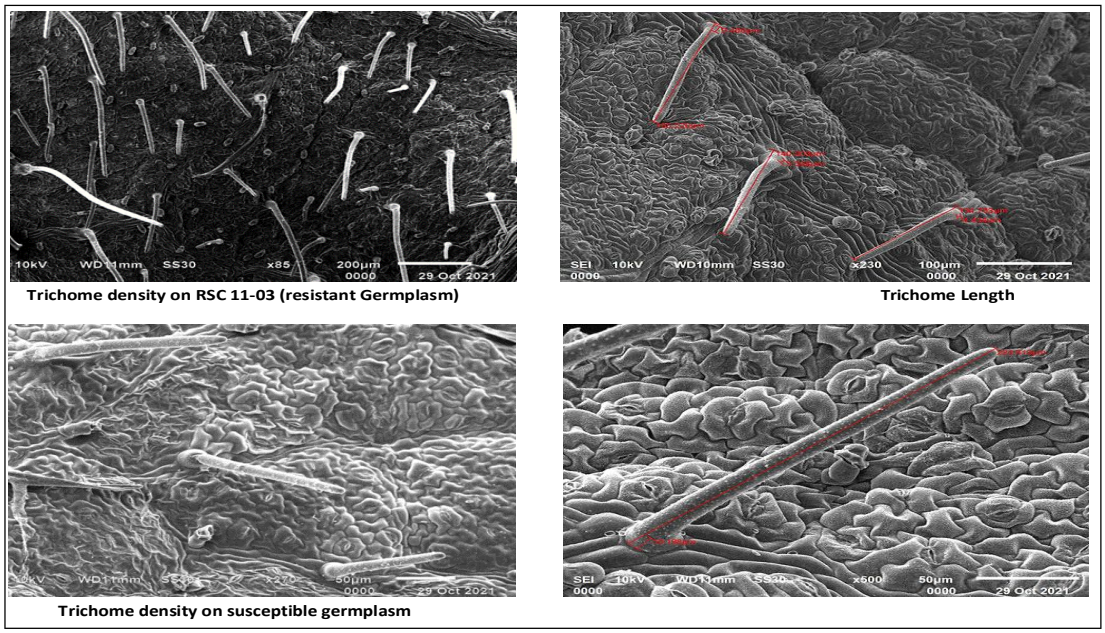

Fig 1: Scanning Electron microscopic images of trichome.

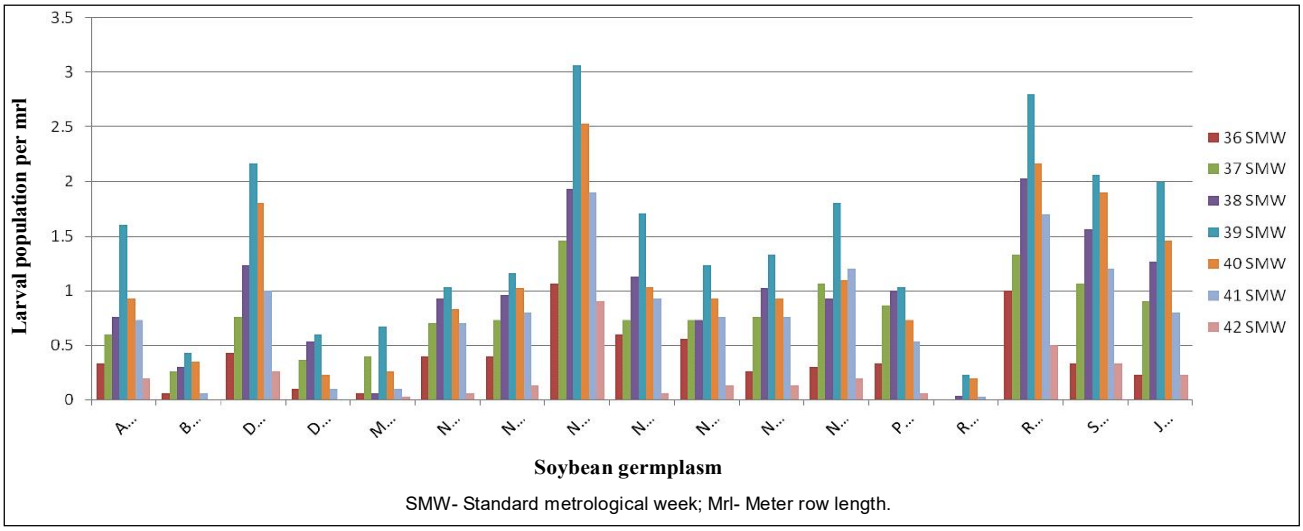

Fig 2: Screening of soybean germplasm with seasonal incidence of *Spodoptera litura* during *Kharif* - 2021.

(morning) humidity but shows negative correlation with minimum (evening) humidity (except for BAUS 102, DSB 34 and MACS 1493 which shows positive correlation) (Fig 3 i, ii, iii). Similar results were provided by Punithavalli *et al.* (2014) who studied the pest incidence for three consecutive years and found that *S. litura* larvae shows maximum infestion in soybean during early and mid September. Similarly, Brahman *et al.* (2018) reported that the peak larval population was recorded during third week of September and the larval incidence was positively correlated with maximum temperature. The more or less similar results were reported by Sundar *et al.* (2018) who stated that the correlation coefficient between abiotic factors and larval population was non-significant. They also reported a positive correlation between larval population and a negative correlation with relative humidity. Pal *et al.* (2023) also reported the similar conclusion that abiotic factors are the main determining factors of the incidence of pest population. Similarly, Umbarkar *et al.* (2010) investigated the seasonal incidence of *H. armigera* in *kharif*-2018 and found out the highest incidence was observed at 31st standard week which exhibit highly significant negative correlation with minimum temperature and evening relative humidity.

Table 1: Correlation between pest incidence and abiotic parameter.

Weather parameter	AMS 100-39	BAUS 102	DS 3108	DSB 34	MACS 1493	NRC 128	NRC 130	NRC 131	NRC 132	NRC 136	NRC 137	NRC SL1	PS 1613	RSC 11-03	RSC 11-07	SKF-SP-11	JS-335
Tem.(min)	0.202	0.49	0.203	0.427	0.299	0.411	0.311	0.127	0.406	0.387	0.277	0.101	0.520	0.128	0.278	0.175	0.254
Tem.max)	0.548	0.509	0.486	0.559	0.581	0.685	0.651	0.475	0.569	0.788*	0.575	0.668	0.618	0.335	0.576	0.477	0.474
RH (min)	-.302	0.065	-.351	0.166	0.113	-.317	-.378	-.422	-.316	-.372	-.267	-.364	-.029	-.285	-.395	-.331	-.198
RH (max)	0.468	0.236	0.343	0.518	0.196	0.279	0.259	0.219	0.510	0.200	0.408	0.334	0.286	0.260	0.406	0.315	0.407

*Significant at 5% level, Non-significant (without asteric).

Evaluation of antixenotic resistance through non-choice and free-choice feeding assay

The intensity of damage by *S. litura* was observed through non-choice and free-choice tests and found a range of differences among the germplasm. From free-choice experiment it was concluded that the larvae moves towards those plants only which were highly preferable while do not feed or only took a taste bite from the undesirable germplasm. This undesirability is known as antixenosis resistance. Table 2 indicate the mean leaf area consumed (MLAC) by larvae in both no-choice and free-choice assay. From the table it was concluded that germplasm RSC 11-03 shows minimum area consumption in both no-choice and free-choice assays (1.43±0.11 and 1.25±0.08) followed by BAUS 102 (1.87±0.04 and 1.59±0.14), DSB34 (1.94±0.04 and 1.40±0.14) and MACS 1493 (2.00±0.20 and 1.52±0.10) while maximum consumption was found in NRC131 (6.13 ±0.08 and 6.51±0.12) and in RSC 11-07 (5.84±0.11 and 6.45 ± 0.13). By comparing these results with the C-value, it was found that the soybean germplasm RSC 11-03, BAUS 102, DSB34, MACS 1493 have 0.06, 0.15, 0.14 and 0.18 C-value which depicts that these are extremely antifeedent to *S. litura,* while on the other hand NRC 131 and RSC 11-07 have 1.11 and 1.06 C-value shows extremely preferred germplasm by the pest as compared to the check (JS-335). The results are in complete agreement with the field screening where, these four germplasm were also resistant towards pest incidence. The results were in partial agreement with Gaur *et al.* (2018) where several germplasms were screened for antixenosis and results shows that the preference and non preference soybean germplasms was based on the antixenosis mechanism of resistance. Sulistyo and Inayati (2016) performed an experiment on soybean germplasm to establish the antixenotic resistance through free-choice and no-choice assay. Similarly, Boica Junior *et al*. (2015) reported that the largest consumption of the leaf (high MLAC) shows that the genotype is susceptible to the pest while resistance in plant creates difference in consumption by pest. Studies were in partial agreement with Senthilraja and Patel (2021) who performed free-choice test against pulse beetle (*Callasobruchus maculates*) to find out the morphological attributes associated with resistance. They found out that egg deposition by beetle is less on rough surface than the seed possess smooth surface.

Trichome density conferring the resistance factor in soybean germplasm

The results of density and length of trichomes on different germplasms shows that trichome act as a great physical barrier of the soybean germplasm and provide antixenotic resistance to the plant. The data on trichome density and length shows a negative relation with feeding property (C-value) (Fig 4). The graph depicts that the extremely

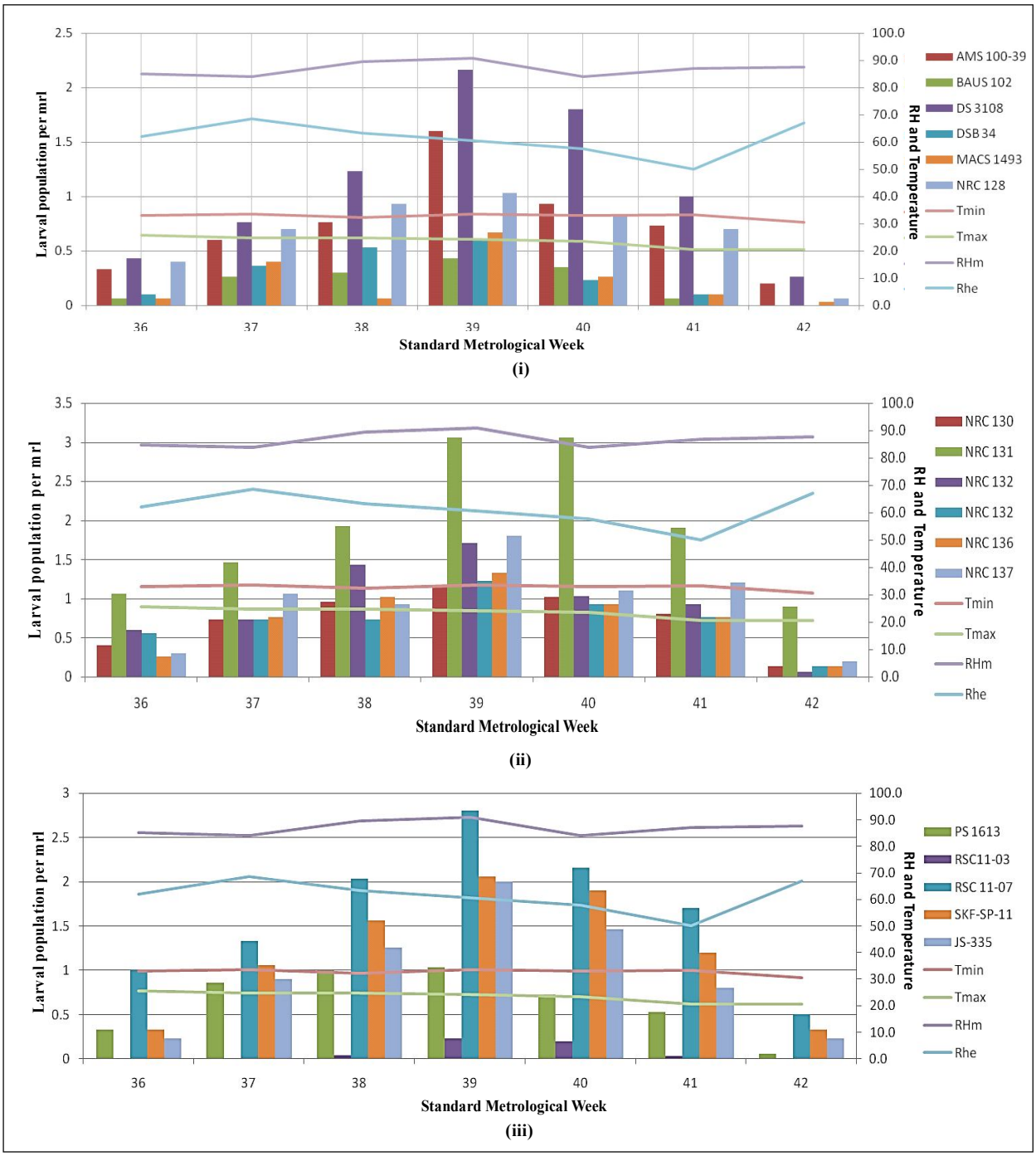

Fig 3: Influence of weather parameter on insect incidences in different germplasm.

antifeedent germplasm RSC 11-03 have high trichome density and length (3.63 and 5.73) having low C-value meanwhile, the NRC 131 have lowest value of trichome density and length (2.35 and 4.81) and have high C-value. Thus, the trichome density and length are greatly affects the pest feeding. The results are in agreement of Gowthish *et al.* (2018) who worked on Black gram to find out the antixenotic resistance against *S. litura* and reported that trichome density is the significant factor for antixenotic resistance as resistant accession contains high trichome on both adaxial and abaxial surfaces while susceptible have low trichome density. De Queiroz *et al.* (2020) also supported the results by reporting that antixenosis is a mechanism of resistance and mediated by various factor including trichome density.

Table 2: C-value and category of soybean germplasm and leaf area consumed (cm^2 ± SE) by *S. litura* prior to dual assay.

Germplasm	MLAC (cm^2)				C- value	Category
	No-choice assay		Free- choice assay			
	Mean*	SE	Mean*	SE		
AMS 100-39	2.58	0.04	2.13	0.15	0.33	Moderately antifeedent
BAUS 102	1.87	0.23	1.59	0.14	0.15	Extremely antifeedent
DS 3108	5.08	0.13	5.13	0.13	0.91	Slightly antifeedent
DSB 34	1.94	0.04	1.40	0.14	0.14	Extremely antifeedent
MACS 1493	2.00	0.20	1.52	0.10	0.18	Extremely antifeedent
NRC 128	2.49	0.14	2.43	0.19	0.30	Moderately antifeedent
NRC 130	3.30	0.17	2.82	0.15	0.50	Moderately antifeedent
NRC 131	6.13	0.08	6.51	0.12	1.11	Preferred
NRC 132	3.13	0.23	3.05	0.07	0.49	Moderately antifeedent
NRC 136	2.89	0.08	2.36	0.16	0.40	Moderately antifeedent
NRC 137	3.66	0.21	3.76	0.16	0.59	Moderately antifeedent
NRC SL1	5.23	0.16	5.56	0.21	0.95	Slightly antifeedent
PS 1613	3.10	0.11	2.72	0.08	0.46	Moderately antifeedent
RSC11-03	1.44	0.11	1.25	0.08	0.06	Extremely antifeedent
RSC 11-07	5.84	0.11	6.44	0.13	1.06	Preferred
SKF-SP-11	4.76	0.15	4.76	0.21	0.85	Slightly antifeedent
JS-335	5.48	0.07	5.89	0.16	1	Preferred

*Values are transformed at the 5% level.

MIAC (Mean leaf area consumption).

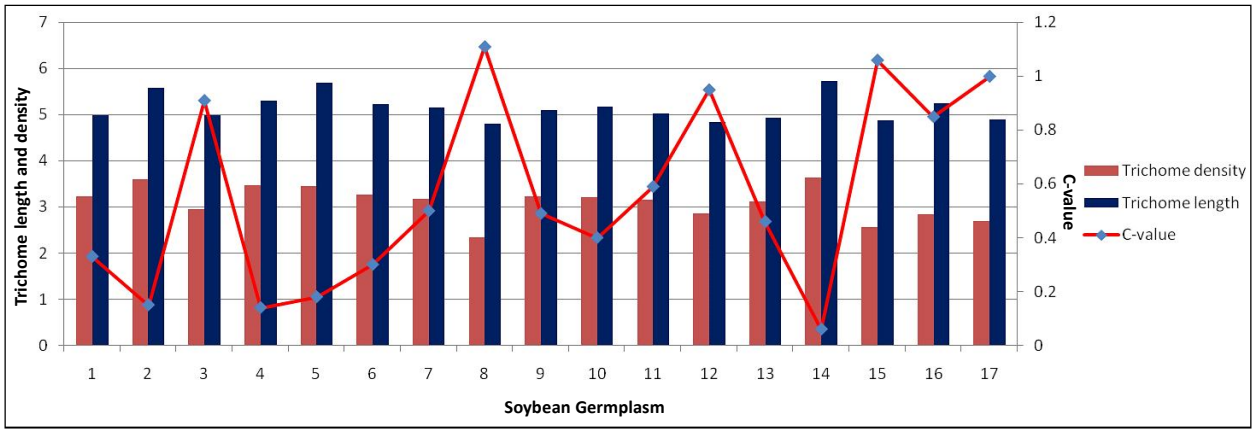

Fig 4: Trichome density and length in relation to the C-value.

CONCLUSION

The incidence of pest is directly related to the weather parameters *i.e.* positively correlated with maximum temperature, minimum temperature and morning relative humidity while negatively correlated with evening humidity. In this study four germplasm BAUS 102, DSB34, MACS 1493 and RSC 11-03 shows resistance towards *S. litura*. The pest incidence is also affected by antixenotic resistance in soybean germplasm which is further determined through non-choice and choice feeding assays. Results shows that RSC 11-03, BAUS 102, DSB34 and MACS 1493 were extremely resistant followed by AMS 100-39, NRC 128, NRC 130, NRC 132, NRC 133, NRC 134 and PS 1613 was moderately resistant. DS 3108, NRC 134 and SKF-SP-11 were susceptible and could be preferred by the pest while NRC 131 and RSC 11-07 were highly susceptible and showed maximum feeding. Trichome length and density also showed indirect relationship for the feeding preference of germplasm. Thus, the cultivation of insect-resistant soybean cultivars in integrated pest management (IPM) systems is a successful management method that reduces reliance on insecticides for insect pest control, minimizing production input costs.

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Conflict of interest

All authors declared that there is no conflict of interest.

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