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Study of Soybean Germplasms on Biochemical Basis of Resistance against *Spodoptera litura* (Fabricius)

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ABSTRACT

Background: Soybean, *Glycine max* (L.) Merr. is an important kharif crop and affected by a number of insect pests which directly or indirectly reduces the yield. To increase the yield, the infestation by insect pest should be managed. *Spodoptera litura* is considered as one of the major pests of soybean crop and damages soybean to a very extent. Cultivation of insect resistant soybean can be the best technique for pest management program.

Methods: No choice experiment test was carried out to calculate the mean leaf area consumption of *Spodoptera litura* and the biochemical analysis is carried out for phenols, flavonoids and tannins. The correlation was studied between the mean leaf area consumption and the contents of phenols, flavonoids and tannins.

Result: The biochemical compounds show negative correlation with the mean leaf area consumption of *S. litura* in different soybean germplasms. The data shows that germplasms like DLSb1, PS 26 and JS 22-16 have high phenols, flavonoids and tannins which in turn shows high resistant against *S. litura.*

Key words: Biochemical, Flavonoid, Phenol, Soybean, *Spodoptera litura*, Tannin.

INTRODUCTION

Soybean [*Glycine max* (L.) Merill] is one of the most important oilseed crops in the world which was originated in China. The protein content in soybean is high (32-35 per cent) (Mishra *et al.,* 2020); well-balanced with vital amino acids like Arginine and Lysine and the oil content is rich (18-22 per cent) in polyunsaturated fatty acids. Additionally, it offers vitamin B-complex like thiamine (11 mg/g) and riboflavin (34 mg/g), as well as minerals like calcium, phosphorus, and others, to meet human nutritional needs (Ahriwar *et al.,* 2014). Insect pests cause more than 25 per cent of production losses (Harish *et al.,* 2009; Mathpal *et al.,* 2022). The most devastating insect pests of soybean include various foliage feeders, stem borers, gram pod borer and stink bugs.

In India, the polyphagous insect, tobacco caterpillar, *Spodoptera litura* (Fabricius), is known to cause serious damage to a variety of crops, including tobacco and groundnuts (Moussa *et al.,* 1960; Ayyanna *et al.,*1982). Fabricius reported it for the first time in 1775. It is one of the most devastating pests of agricultural crops and feeds on a variety of plants, including soybean. It is the main defoliating pest and is accountable for up to 68% of the production loss among the insect pests that attack soybean from early growth stage till harvest time (Bayu *et al.,* 2017).

Plant crude extract contains novel secondary metabolites like phenolic acid, flavonoid, alkaloid and terpenoid, momilactone, jasmonate, glucosinolate, hydroxamic acid, brassinosteriods, amino acids and carbohydrates that are primarily to blame for the inhibition of insect growth and development (Adaramola *et al.,* 2016). Similarly, these biochemical compounds impart resistant on the plant from the insect pests since, insect digestive enzymes may be impacted by allelochemicals such phenolics or their oxidation products (Woodhead, 1981).

The overall aim of the present study is to identify the resistant and susceptible germplasm on biochemical basis against *S. litura* and also to study the correlation of some biochemicals with mean leaf area consumption of *S. litura* in different soybean germplasms.

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MATERIALS AND METHODS

The experiments were carried out in G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during *Kharif*-2022.

Plant material in field

Fourteen soybean germplasms (DLSb1, JS 22-12, JS 22-16, JS 22-18, KDS 1096, PS 1092, PS 1225, PS 1569, PS 1670, PS 23, PS 24, PS 25, PS 26 and RSC 11-35) 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

2022 and in soybean entomology laboratory at Department of Entomology, G.B.P.U.A.T. Pantnagar, Udham Singh Nagar for the resistance against *S. litura*. Soybean germplasms were planted in 3 replications by using randomized block designs (RBD).

Insect material and determination of resistance through feeding assays

Egg mass of *S. litura* was collected from Norman E. Borlaug Crop Research Centre, GBPUAT Pantnagar during *kharif* 2022 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 experiment. The study was performed through 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.

No-choice test

The leaf discs were kept separately in the centre 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.

Biochemical analysis

The phenol content in the soybean leaf sample was estimated as per the method developed by (Malick and Singh, 1980), estimation of total flavonoids in the soybean was done as per the method developed by (Zhishen *et al.,* 1999) and estimation of tannins in the soybean leaf samples was done as per the method developed by (Burns, 1971).

Data analysis

The data obtained from field was statistically analysed using SPSS software. The data including MLAC, phenol content, flavonoid content and tannin content were transformed then subjected to analysis of variance. The simple correlation values were also calculated.

RESULTS AND DISCUSSION

Mean leaf area consumption (MLAC) of *S. litura* in different soybean germplasm

The values of MLAC in each treatment were ranged from 0.16 cm² (JS 22-16) to 1.34 cm² (KDS 1096). The highest consumption was found in germplasm KDS 1096 (1.34 cm²) among all which may be due to the reason that it might be more preferable than the susceptible check JSS-335. Apart from KDS 1096 (1.34 cm²), three germplasms namely PS 1569 (1.32 cm²), PS 23 (1.13 cm²) and PS 1092 (1.29 cm²) showed higher mean leaf area consumption than the susceptible check JS-335 (1.11 cm²) while the lowest consumption was found on JS 22-16 (0.16 cm³) followed by PS 26 (0.19 cm²), PS 25 (0.22 cm³) as given in (Table 1).

From this experiment, it was concluded that the germplasms which have high MLAC are preferably the susceptible germplasms since the larvae of *S. litura* preferred and fed on it. The results were in partial agreement with Gaur *et al.* (2018) where different soybean germplasms were screened and the results shows that higher MLAC is observed in the more susceptible germplasms. Similarly, Boica Junior *et al.* (2015) also reported that the susceptible germplasms have higher MLAC against the pest and it creates difference in consumption by pest. In another study by Mathpal *et al.* (2022), it was found that the larvae feed on those germplasms which were highly preferable while they only took a taste bite or feed very less on the undesirable germplasm.

Estimation of total phenol content in methanol leaf extract of different soybean germplasms

In this experiment, the total phenol content was determined through the Folin-Ciocalteu method and expressed as milligram per gram extract. In methanol leaf extract of soybean, the phenol content varied from 3.162 to 4.596 mg/gm of leave extract. The highest phenolic content was found in PS 26 (4.596 mg/gm) followed by JS 22-16 (4.565 mg/gm) and PS 25 (4.542 mg/gm) whereas, the lowest total phenolic content was recorded in germplasm namely KDS 1096 (3.162 mg/gm) followed by PS 1225 (3.365 mg/gm) over the susceptible check JS-335 (3.585 mg/gm). The rest of the germplasms namely DLSb1, JS 22-12, JS 22-18, PS 1569, PS 1670, RSC 11-35, PS 23, PS 24 and PS 1092 showed a range of phenol content from 4.442 mg/gm to 3.592 mg/gm (Table 2).

Table 1: Mean leaf area consumption (MLAC) of different soybean germplasm along with susceptible check (JS-335) by *S. litura.*

Germplasm	MLAC (cm²)
DLsb1	0.34±0.007
JS 22-12	1.02±0.040
JS 22-16	0.16±0.007
JS 22-18	0.73±0.018
KDS 1096	1.34±0.011
PS 1569	1.32±0.036
PS 1670	0.89±0.006
RSC 11-35	0.70±0.004
PS 23	1.13±0.016
PS 24	0.45±0.016
PS 25	0.22±0.008
PS 26	0.19±0.004
PS 1092	0.67±0.005
PS 1225	1.29±0.053
JS-335 (Check)	1.11±0.019
C.D.	0.035
SE(m)	0.012
SE(d)	0.017
C.V.	2.688

From the above finding, it was revealed that the germplasms which contain high phenol compound namely JS 22-16, PS 25 and PS 26 are categorized as antifeedant in no choice feeding experiment and less preferred by *S. litura*. Whereas, the germplasms having less phenol content in their leaf extract namely KDS 1096 and PS 1225 were highly preferred by *S. litura* during the no-choice feeding experiment (Table 2).

The above results were in accordance with the findings of Summers and Felton (1994) who reported that phenolic compounds induce the oxidative stress and provide resistance to herbivorous insects such as Helicoverpa *zea*. Quettier-Deleuer *et al.* (2000) extracted and evaluated the phenols from the seeds of Fagopyrum esculentum which provide therapeutic effects and they stated that phenolic content is directly related to their antioxidant properties which deter the feeding behaviour in insects.

Estimation of total flavonoid content in methanol leaf extract of different soybean germplasms

Total flavonoid content was estimated through methanol leaf extract of soybean germplasm and expressed as milligram (mg) per gram. The variation in the range of flavonoid compound was found between 0.827 to 1.947 mg/gm. Highest content of flavonoid compound was found on DLSb1 (1.947 mg/gm) followed by PS 26 (1.767 mg /gm) and PS 23 (1.547 mg/gm) while, the lowest flavonoid content was found on KDS 1096 (0.827 mg/gm) followed by PS 1225 (0.897 mg/gm), JS 22-18 (1.047 mg/gm), PS 1569 (1.107 mg/gm) and JS 22-12 (1.147 mg/gm) over the susceptible check namely JS-335 (1.177 mg /gm). On the other hand, germplasms other than the above namely JS 22-16, PS 1670, RSC 11-35, PS 24, PS 25 and PS 1092 ranges from 1.277 to 1.547 mg/gm (Table 3).

From the above result, it was found that the germplasms that possess high content of flavonoid also having high content of phenol can be categorised as extremely antifeedant germplasms on the basis of no choice feeding experiment which were JS 22-16 and PS 26. Germplasms namely KDS 1096 had lowest level of flavonoid as well as phenol. Therefore, it can be categorised as highly preferred over susceptible check.

Similar results were observed by Elliger *et al.* (1980) who evaluated flavonoid compound and their role in conferring resistance against the insect pest attack. They worked on the flavonoid compound of maize plant against Heliothis zea (corn earworm) and found that these compounds inhibit the attack of pest. Similarly, Hedin *et al.* (1988) also found out that the flavonoids affect the behaviour, development and growth of a number of insects. According to Rehman *et al.* (2018) flavonoids are specific plant secondary metabolites that are mainly concerned with antiherbivore activity in soybean seed.

Estimation of tannin content in methanol leaf extract of different soybean germplasms

From the experiment conducted, it was estimated that the tannin content in different soybean germplasm through methanol leaf extract of soybean and expressed as milligram (mg) per gram. The variation in the range of tannin compound was found between 0.093 to 0.228 mg/gm.

Table 2: Estimation of total phenol from methanol leaf extract of soybean germplasms.

Germplasm	Phenol (mg/g)
DLsb1	4.504±0.172
JS 22-12	3.858±0.097
JS 22-16	4.565±0.167
JS 22-18	4.131±0.036
KDS 1096	3.162±0.109
PS 1569	3.592±0.016
PS 1670	4.050±0.070
RSC 11-35	4.285±0.160
PS 23	3.931±0.034
PS 24	4.442±0.081
PS 25	4.542±0.016
PS 26	4.596±0.008
PS 1092	4.285±0.138
PS 1225	3.365±0.046
JS-335 (Check)	3.585±0.050
C.D.	0.163
SE(m)	0.056
SE(d)	0.08
C.V.	2.404

Table 3: Estimation of total flavonoid content from methanol leaf extract of soybean germplasms.

Germplasm	Flavonoid (mg/g)
DLsb1	1.947±0.007
JS 22-12	1.147±0.004
JS 22-16	1.407±0.004
JS 22-18	1.047±0.002
KDS 1096	0.827±0.001
PS 1569	1.107±0.001
PS 1670	1.317±0.001
RSC 11-35	1.417±0.006
PS 23	1.547±0.005
PS 24	1.507±0.002
PS 25	1.217±0.002
PS 26	1.767±0.004
PS 1092	1.277±0.004
PS 1225	0.897±0.003
JS-335 (Check)	1.177±0.004
C.D.	0.003
SE(m)	0.001
SE(d)	0.002
C.V.	0.179

Table 4: Estimation of total tannin content from methanol leaf extract of soybean germplasms.

Germplasm	Tannin (mg/g)
DLsb1	0.228±0.005
JS 22-12	0.121±0.005
JS 22-16	0.145±0.006
JS 22-18	0.128±0.005
KDS 1096	0.096±0.003
PS 1569	0.108±0.003
PS 1670	0.126±0.001
RSC 11-35	0.142±0.003
PS 23	0.144±0.003
PS 24	0.157±0.004
PS 25	0.137±0.002
PS 26	0.178±0.001
PS 1092	0.141±0.001
PS 1225	0.093±0.001
JS-335 (Check)	0.130±0.005
C.D.	0.006
SE(m)	0.002
SE(d)	0.003
C.V.	2.627

Table 5: Correlation between biochemical compounds of soybean germplasm and MLAC by *S. litura.*

Variable	Phenol	Flavonoid	Tannin
MLAC	-0.951**	-0.664**	-0.715**

Highest content of tannin was found on DLSb1 (0.228 mg/gm) followed by PS 26 (0.178 mg/gm) and PS 24 (0.157 mg/gm) while, the lowest tannin content was found on PS 1225 (0.093 mg/gm) followed by KDS 1096 (0.096 mg/gm), PS 1569 (0.108 mg/gm), JS 22-12 (0.121 mg/gm), PS 1670 (0.126 mg/gm) and JS 22-18 (0.128 mg/gm) over the susceptible check namely JS-335 (0.130 mg/gm). On the other hand, germplasms other than the above namely JS 22-16, RSC 11-35, PS 23, PS 25 and PS 1092 ranges from 0.137 mg/gm to 0.157 mg/gm (Table 4).

From the above result, it was found that the germplasms that possess high content of tannin also having high content of phenol and flavonoid can be categorised as extremely antifeedant germplasms on the basis of no choice feeding experiment which were DLSb1, JS 22-16 and PS 26. Germplasms namely KDS 1096 and PS 1225 had lowest level of tannin as well as low phenol and flavonoid. Therefore, it can be categorised as highly preferred over susceptible check.

According to Nomura and Itioka (2002), the amount of tannin consumed had a direct correlation with how much the cutworm larvae's growth was hindered by tannin. They concluded that plant tannin acts as a generalist herbivore defense agent and its effects are proportionate to its concentration in leaves and it is in accordance with the result as presented in the (Table 4). Similarly, Liu *et al.* (2010), observed that that tannic acid had a detrimental impact on newly hatched larvae, inhibiting pupae weight, pupation process, and emerging process. The mortality of the larvae also increased with higher tannic acid doses and longer treatment periods. The genotypes which contain lower amounts of tannins are more susceptible against Spodoptera littoralis as compared with the ones with higher amount of tannin (Mohamed *et al.,* 2021).

The correlation between mean leaf area consumption by *S. litura* and the biochemical compounds (phenol, flavonoids and tannin) content of methanol leaf extract was evaluated and it was found that the MLAC had highly significant negative correlation with phenol (0.951) as well as flavonoid (0.664) and tannin (0.715) content of soybean germplasms (Table 5). These results showed that the biochemical compound present in the leaves of soybean germplasms inhibit the feeding of *S. litura* and aid in the host plant resistance as total polyphenolic concentration was highest in PS 26 followed by DLSb1 then JS 22-16 while the lowest polyphenolics were present in KDS 1096 followed by PS 1225. The susceptible check JS 335 also had low biochemical compounds which suggested that germplasms having high polyphenolic content have greater resistance towards *S. litura* than those with less concentration of these compounds.

CONCLUSION

The biochemical analysis of soybean germplasms against *S. litura* revealed that the germplasms namely PS 26 (4.596 mg/gm) and JS 22-16 (4.565 mg/gm) contain high phenol compound and KDS 1096 (3.162 mg/gm) and PS 1225 (3.365 mg/gm) contain low phenol content. In case of flavonoid content, DLSb1 (1.947 mg/gm) and PS 26 (1.767 mg/gm) have highest content while KDS 1096 (0.827 mg/gm) and KDS 1096 (0.827 mg/gm) and PS 1225 (0.897 mg/gm) have lowest content. Germplasms namely DLSb1 (0.228 mg/gm) and PS 26 (0.178 mg/gm) contain high tannin content and PS 1225 (0.093 mg/gm) and KDS 1096 (0.096 mg/gm) contain low tannin content. The correlation studies between mean leaf area consumption by *S. litura* and the biochemical contents (phenol, flavonoid and tannin) showed that the MLAC has highly significant negative correlation with phenol (-0.951), flavonoid (-0.664) and tannin (0.715) content of soybean germplasms.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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