

Bio-physical and Bio-chemical Basis of Host Plant Resistance of Soybean [*Glycine max* (L.) Merrill] Genotypes Against *Spodoptera litura* (Fabricius)

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ABSTRACT

A field trial was conducted to evaluate the field resistance against *Spodoptera litura* in eighteen genotypes of soybean at Research Farm of Department of Entomology, ICAR- Indian Institute of Soybean Research, Indore (M.P.) during the Kharif 2024. The results revealed that six genotypes viz., CAT-146, G5P22, CAT-47, F4P21, F3P18, and CAT 2503 found effective to combat the menace of *S. litura*, resulted in minimum larval population. Further, biochemical and biophysical analysis found highest phenol content (CAT-47: 23.13 mg/g), peroxidase (CAT-146: 0.75 min⁻¹g⁻¹), polyphenol (CAT-146: 0.32 min⁻¹g⁻¹), phenylalanine ammonia lyase (F4P21: 1.73 mg/g), protein (CAT-146 and CAT-47: 3.95 mg/g), trichome density on midrib (F4P21: 35.9 mm²), leaf blade (CAT 2503: 33.5 mm²), and leaf lamina (CAT 2503: 33.1 mm²), and specific leaf weight (CAT-146 and CAT-2503: 0.41 g/cm²) for resistant genotypes. On contrary, the susceptible genotypes had higher reducing sugar (JS335: 29.36 mg/g), relative water content (JS335: 34.35 %), chlorophyll a (JS 93-05: 21.52 mg g⁻¹fw), chlorophyll b (JS 93-05: 13.59 mg g⁻¹fw) and total chlorophyll (JS 93-05: 35.11 mg g⁻¹fw). These biophysical and biochemical components in soybean can be effectively utilized in breeding for resistance against *S. litura*. The larval population of *S. litura* has shown significant positive correlation with reducing sugar (0.751**), relative water content (0.871**), chlorophyll A (0.668**), chlorophyll B (0.698) and total chlorophyll (0.707**), while significant negative correlation was observed for phenol (-0.710**), peroxidase (-0.647**), polyphenol oxidase (-0.637**), protein (-0.771**), trichome density midrib (-0.695**), leaf blade (-0.742**), leaf lamina (-0.779**) and specific leaf weight (-0.594**). Non-significant correlation was observed between *S. litura* larval population and PAL (-0.504).

Keywords: Host plant resistance, Biochemical and biophysical analysis, *Spodoptera litura*, Soybean

1 | INTRODUCTION

Soybean is the world's most important oil seed crop with highest production in the world by United States, followed by Brazil, Argentina, China and India. Globally, soybean cultivated on the area of 136.82 Mha, with production of around 353.47 MMT annually (Anonymous, 2023). In India, the soybean is being cultivated on an area of 13.06 Mha with production of 13.26 MMT and productivity of 985 Kg/ha in 2023-24 (Anon., 2024). *Spodoptera litura* is an important insect pest affecting the yields of soybean worldwide and host plant resistance is a key pest management strategy for insect pest. Hence, Understanding the mechanism of resistance to *S. litura* would help in developing resistant cultivars. Insect pests and diseases together contribute 32% of the yield losses in soybean (Sharma and Shukla, 1997) insect pests account for more than 25% of the yield loss (Harish et al. 2009, Oerke, 2006, Sharma and Shukla, 1997, Shriram, 1996). The occurrence of nine insets species on soybean in India was documented (Fletcher, 1922). As soybean crop go through distinct phases of development, thirty-nine different kinds of insect pests have been observed (Biswas, 2013). Tobacco caterpillar, *S. litura* is one of the important serious insect pests

of agricultural crops in tropical and temperate Asia, Australasia, and the Pacific Islands (EPPO, 2013). It is an important leaf feeder and polyphagous pest that has been seen on over 112 plant species (Chari and Patel, 1988). Numerous economically significant crops including cotton, groundnut, tobacco, chili, soybean, castor, okra, pulses etc. are seriously harmed by it (Armes *et al.* 1997, Niranjankumar and Regupathy, 2001). The pest is active from the end of July or the middle of August to October, when the warm and humid weather and the soybean's reproductive stages coincide, this ends up resulting in yield losses ranging from 26-29% (Punithavalliet *al.* 2014).

Numerous reports indicate that certain biochemical substances and enzymes are accountable for the pest resistance exhibited by numerous crop plants. Such reports describing the involvement of many biochemical and biophysical characters in offering resistance to tobacco caterpillar in soybean are scanty. The current study assessed the biochemical and physical changes in the susceptible and resistant genotypes of soybean by measuring the biophysical parameters such as leaf area index, relative water content, trichome density (mid rib, leaf blade and leaf lamina) and chlorophyll. These changes

were based on changes in several significant biochemical compounds such as sugar, phenol, protein, phenylalanine ammonia lyase, and enzymes like peroxidase and polyphenol oxidase.

The purpose of this study was to identify soybean resistance to *S. litura* using screening, biochemical and biophysical properties. Finding the level that is capable of resistance may then be utilized for discovering the resistance mechanism for each genotype. Furthermore, soybean resistance to *S. litura* can be boosted by using the resistant genotypes found in this research. The resistant genotypes may be further assessed for potential yield and may be used to create a new *S. litura* resistant variety in India.

2 | MATERIALS AND METHODS

The present investigation was carried out at Research farm, Department of Entomology, ICAR- National Soybean Research Institute, Indore (M.P.), during *kharif* 2023-24. In screening, biochemical and biophysical programs eighteen soybean genotypes were replicated thrice under Randomized Block Design were evaluated for resistance against *S. litura*. All the genotypes were kept unsprayed and allowed to build up the pest population and field screening technique was followed. The data were recorded on larval population of major defoliator pest like *S. litura*, at peak incidence. Similarly, the data were also collected at 50 Days after sowing (DAS) on biochemical and biophysical traits using following method to determine resistance or susceptibility of soybean genotypes against *S. litura*.

In the field screening experiment, the larval population of *S. litura* was observed at three randomly chosen sites in each plot, with a one meter row length. The plants were gently shaken over a tray that is put between the rows, measuring one meter by 0.5 meters, leaving border rows. Average numbers of larvae per meter row length (mrl) were worked out.

2.1 | Sample Collection

For biochemical and biophysical analysis, after 50 days after sowing (DAS), the third fully opened leaf from the top of each genotype was taken out for biochemical analysis from the field. Leaf aliquot was prepared and utilized in the study to estimate sugar, total phenols, phenylalanine lyase, and protein levels. Fresh samples were collected from the field on the day of the experiment to measure the enzyme activity of peroxidase and polyphenol oxidase and prepared pure extract for analysis. Biochemical analysis was performed twice for each genotype.

2.2 | Preparation of Pure Extract of Leaves

The pure extract is prepared by fresh leaves. 2 gm of leaves were taken and cutting them into small pieces and immersing in 80% ethanol. The pieces of leaf were ground thoroughly in a pestle and mortar with a little ethanol and passed through the muslin cloth twice. The extract of leaves were used for estimation of polyphenol oxidase, peroxidase.

2.3 | Preparation of Leaf Extract in Alcohol (Aliquot)

The aliquot was prepared by taking 2 gm of fresh leaves sample and cutting them in to small pieces and immersing in alcohol (distilled ethanol 80%). The pieces of leaf tissues were ground thoroughly in a pestle and mortar with a little ethanol and passed through the muslin cloth and the extraction procedure was repeated one more time. The filtrates were pooled and filtered through Whatman No. 41 and volume was made up to 20 ml using 80% ethanol. The filtrate was clarified by adding 2 ml (0.396 gm) of saturated lead acetate and 3 ml (0.117 gm) of di-sodium hydrogen phosphate which are allowed over night for setting down of the tissues and filtrate through Whatman No. 42 filter paper. The final volume of clear filtrate was made up to 25 ml by adding 80% ethanol. This was the stock solution from which an aliquot was prepared to determine the amount of reducing sugar, total phenol, PAL (phenylalanine ammonia lyase), and protein content. The absorbance of each chemical constituent in a sample was determined using a spectrophotometer.

2.4 | Estimation of Reducing Sugar

Quantitative analysis of reducing sugars was done as per Nelson- Somogyi method (Nelson 1944; Somogyi 1952). Standard curve ($R^2=0.998$) was prepared by using D- glucose. One ml of sugar extract was taken in a 25 ml volumetric flask. One ml of alkaline copper tartarate reagent was added to it. The contents were mixed and heated in a boiling water bath for 20 min. Then the flask was cooled under tap water and 1 ml of arsenomolybdate reagent was added. The mixture was then diluted to 10 ml with double distilled water and mixed thoroughly. Blank was prepared without adding the sample extract. Then the absorbance was measured on a spectrophotometer at 520 nm. The results are expressed as mg of reducing sugars/g of sample.

2.5 | Estimation of Total Phenols

Total phenols were estimated as per Folin-Ciocalteu method of Bray and Thorpe (1954). Standard curve ($R^2 = 0.994$) was prepared by using gallic acid. One mL of alcoholic extract was taken in a 25 mL volumetric flask and 1 ml of Folin-Ciocalteu reagent was added to it. After 8–10 min 2 ml of 20% Na_2CO_3 solution was added and shaken thoroughly. Then the flasks were kept in a boiling water bath for 1 min and cooled under tap water. The final volume was made to 25 ml using double distilled water and incubated at room temperature for 30 min for colour development. The blank was prepared without adding the sample extract in the flask. The absorbance was measured at 740 nm on a spectrophotometer. The results are expressed as mg of total phenols/g of sample.

2.6 | Extraction of Enzymes

Fresh leaves samples were collected from the field on the day of analysis. One gram of leaves sample was macerated with 5 ml of phosphate buffer using a pre chilled mortar and pestle. The mixture was centrifuged at $15,000 \times g$ at 4°C for 25 min and supernatant was collected which was used immediately for enzyme assay (Joshi et al 2012).

2.7 | Estimation of Peroxidase Activity

Peroxidase activity was measured by the method given by Retig (1974). Assay mixture contained 3 ml of phosphate buffer, 0.2 ml of 0.01 M guaiacol, 0.3 ml of H₂O₂, 1 ml of freshly prepared enzyme extract and 4 ml of double distilled water. The enzyme tube (containing 1 ml of freshly prepared enzyme extract) and substrate tube (containing phosphate buffer, guaiacol and H₂O₂) were prepared separately. The absorbance was recorded immediately after mixing the enzyme and substrate. Absorbance was recorded at every 30 sec. interval up to 2 min on a spectrophotometer at 420 nm. One unit enzyme activity was expressed as change in absorbance (U) /m/g fresh wt. of sample.

2.8 | Estimation of Polyphenol Oxidase Activity

Polyphenol oxidase activity was measured by the method described by Kumar and Khan (1982). Assay mixture contained 3.5 ml of phosphate buffer, 1 ml of 0.01 M pyrogallol and 1 ml of freshly prepared enzyme extract. The enzyme tube containing 1 ml freshly prepared enzyme extract and substrate tube containing phosphate buffer and pyrogallol were prepared separately. The absorbance was recorded immediately after mixing the enzyme and substrate after every 30 sec. interval up to 2 min on a spectrophotometer at 420 nm. One unit enzyme activity was expressed as change in absorbance (U) /m/g fresh wt. of sample.

2.9 | Estimation of Phenylalanine Ammonia Lyase

Dickerson *et al.* (1984) gave this method to analyze Phenylalanine ammonia lyase. Take 200 µl of enzyme extract added with 0.5ml of Sodium borate buffer (P^H 8.7) and incubate at 30°C for 5 min in water bath. After over incubation period 1.3 ml of distilled water added in sample or 0.5 ml of 0.1 mm L-Phenylalanine (P^H 8.5) in each test tube and incubates at 30°C for 30 min in water bath. After incubating, add 0.5 ml of 0.1 mm TCA to stop the reaction. Take OD at 290 nm and the activity is measured in only one reading.

2.10 | Estimation of Protein

Protein content was measured by lowry's method (1951). To estimate the protein content 1 ml of aliquot were taken in 25 ml volumetric flask, add 4.5 ml of reagent I and incubate in BOD at 28°C for 10 min. After incubation add 0.5 ml of reagent II and incubate for 30 min for colour development. After it measure the absorbance at 660 nm and plot the standard graph.

2.11 | Biophysical Components of Resistance

Biophysical traits *viz.*, trichome density on leaf blade, midrib and leaf lamina, specific leaf weight, relative water content and chlorophyll content (chl. a, chl. b, total chlorophyll) were studied from the top fully opened leaflets of these selected genotypes at 50 DAS.

2.12 | Trichome Density on Midrib, Leaf Blade and Leaf Lamina

Trichomes on midrib were analyzed by following the procedure Maitiet *al.* (1980). To the study of trichome density,

5 fully developed leaves from each genotype were heated with 20 ml of distilled water in a petri plate for 5 min. in hot air oven at 85°C. After five minutes, water of petri plates was decanted and added with 20 ml of 96% ethyl alcohol and the samples were heated again for 10 minutes at 80°C. The alcohol was decanted and the same procedure was repeated 3 times so that entire chlorophyll content was removed. Finally 20 ml of 90% concentrated lactic acid was added and heated again at 85°C for 45 minutes upon which. The leaf segments were cleared. Later, the petri plates were cooled and store for further observation. To observe the Trichome density, leaf segments were mounted on glass slide and a drop of lactic acid was put on it and observed under stereoscopic binocular microscope (Scope imager, version 9.0) at 10X magnification and numbers of Trichome on midrib, leaf blade and leaf lamina were counted and expressed as number of trichomes on one mm square of mid rib, leaf blade and leaf lamina.

2.13 | Specific Leaf Weight

Specific leaf weight (SLW) is expressed as Leaf thickness. It was estimated as per the formula given by Radford (1967). On the day of the experiment, three fresh trifoliate (9 leaves) of each genotype were taken. The leaf area was measured using the leaf area index. Then, dry the leaves for 72 hours at 70°C in hot air oven. Later, leaves were collected after 72 hours and measured for dry weight. SLW of the leaf was calculated by using the following formula.

$$SLW = \frac{\text{Dry leaf weight (gm)}}{\text{Leaf area (cm}^2\text{)}} \times 100$$

2.14 | Relative Water Content (Leaf Succulence)

Relative water content was estimated by the method of Kramer (1983). This parameter was estimated in the healthy leaf and 10 discs were made by using cylinder of 0.75 cm² area. Weight of the discs was taken and expressed as fresh leaf weight (g). Then discs were immersed in distilled water for 4 hrs to absorb water and later, discs were taken out and turgid leaf discs were weighed and expressed as turgid leaf weight (g). After that discs were taken and kept in hot air oven for 48 hrs at 70°C for drying. Later disc weight was taken after 24 hrs and 48 hrs respectively and expressed as dry weight (g). Relative water content of the leaf in percentage (%) was calculated by using the following formula.

$$RWC (\%) = \frac{(\text{fresh disc weight} - \text{dry disc weight})}{(\text{Turgid disc weight} - \text{dry disc weight})} \times 100$$

2.15 | Chlorophyll Content

Chlorophyll content was estimated by the method of Yang *et al.* (2009). For chlorophyll estimation leaf samples were cut in small pieces and 50 mg of leaf sample of every genotype were weighted. Each sample was placed in the different test tubes containing 10 ml of (80% Acetone) solvent. The sample tubes covered with aluminium foils and kept for overnight in a dark setting until the full decolourisation of the leaf tissues. The chlorophyll extract was transferred to a cuvette and the absorption measurements were then carried out in a spectrophotometer at different wavelengths of 666, 665, 655, 649, 510, 480 nm and the readings were noted down against

blanks prepared with 10 ml acetone. Then the chlorophyll and other pigments content are measured according to the following equations:

$$\text{Chl 'a'} \text{ (mg g}^{-1}\text{fw)} = (12.47 \times \text{A}_{665\text{nm}}) - (3.62 \times \text{A}_{649\text{nm}})$$

$$\text{Chl 'b'} \text{ (mg g}^{-1}\text{fw)} = (25.06 \times \text{A}_{649\text{nm}}) - (6.5 \times \text{A}_{665\text{nm}})$$

$$\text{Total chlorophyll} = \text{chl 'a'} + \text{chl 'b'}$$

3 | RESULTS AND DISCUSSION

The result of field screening (Table 1.1) revealed that six genotypes out of eighteen genotypes viz., CAT-146 (0.90 larvae/mrl), G5P22 (0.90 larvae/mrl), CAT-47 (0.83 larvae/mrl), F4P21 (0.86 larvae/mrl), F3P18 (0.90 larvae/mrl) and CAT 2503 (0.83 larvae/mrl) were found resistant (R) against *S. litura*. Eight genotypes i.e. NRC142 (1.26 larvae/mrl), NRC130 (1.20 larvae/mrl), JS 22-18 (1.23 larvae/mrl), JS 20-69 (1.23 larvae/mrl), NRC138 (1.20 larvae/mrl), JS 22-16 (1.26 larvae/mrl), NRC165 (1.23 larvae/mrl) and JS 22-12 (1.26 larvae/mrl) were found moderately resistant (MR) to *S. litura*. One genotype viz., JS 20-34 (1.33 larvae/mrl) was found low resistant (LR) to *S. litura*. JS 95-60 (1.73 larvae/mrl) and JS 93-05 (1.66 larvae/mrl) were found susceptible (S) and JS 335 (3.20 larvae/mrl) was found highly susceptible (HS) to *S. litura*.

Resistant genotypes had significantly less amount of reducing sugar (17.14-19.81 mg/g) as compared to susceptible check JS 335 (29.36 mg/g, Table 1.2). Total phenol content was significantly higher in resistant genotypes viz., CAT-47 (23.13 mg/g) compared to susceptible genotype JS 335 (16.40 mg/g) (Table 1.2). Peroxidase activity was found higher in soybean resistant genotypes CAT-146 (0.75 min⁻¹ g⁻¹) in comparison to susceptible genotypes JS 93-05 (0.15 min⁻¹ g⁻¹) (Table 1.2). Polyphenol oxidase activity was found higher in resistant genotypes CAT-146 (0.32 min⁻¹ g⁻¹) in comparison to susceptible genotypes JS 95-60, JS 93-05, JS 335 (0.17 min⁻¹ g⁻¹) (Table 1.2). The highest phenylalanine ammonia lyase content was found in resistant genotype F4P21 (1.73 mg/g) and lowest phenylalanine ammonia lyase content was found in susceptible genotype JS 95-60 (0.58 mg/g) (Table 1.2). Higher protein content of 3.40-3.95 mg/g was recorded in resistant genotypes as compared to lower 2.23 mg/g in susceptible genotypes JS 335 (Table 1.2).

Resistant genotypes had higher trichome density on midrib (F4P21: 35.9 mm²), leaf blade (CAT 2503: 33.5 mm²) and leaf lamina (CAT 2503: 33.1 mm²) and lowest trichome density was found on midrib, leaf blade and leaf lamina on JS 335 19.8 mm², 20.3 mm² and 20.5 mm² respectively (Table 1.3). Resistant genotypes viz., CAT-146 and CAT 2503 had higher specific leaf weight (0.41g/cm²) compared to susceptible genotype JS 93-05 had less specific leaf weight (0.23g/cm²) (Table 1.3). Among the resistant genotypes, CAT-47 (18.99%) had significantly less relative water content as compared to susceptible genotype (34.35%) (Table 1.3). Resistant soybean genotypes had lowest chlorophyll content i.e. chlorophyll a, chlorophyll b and total chlorophyll in CAT-

146 (7.18 mg g⁻¹fw), CAT-47 (4.40 mg g⁻¹fw) and CAT-47 (11.75 mg g⁻¹fw) respectively compared to susceptible genotypes had highest chlorophyll a, chlorophyll b and total chlorophyll in JS 93-05 21.52 mg g⁻¹fw, 13.59 mg g⁻¹fw and 35.11 mg g⁻¹fw, respectively (Table 1.3).

These observations were in accordance with the studies reported by who observed the largest number of defoliator larvae on JS 335 Ahirwar *et al.* (2015), and the soybean variety JS-335 has the highest reported prevalence of tobacco leaf-eating caterpillars (Kalyan and Ameta, 2017). In comparison to susceptible lines, resistant genotypes had the fewest larvae/mrl (Awasthi *et al.* 2003). Similarly, Martoliya *et al.* (2013) examined several soybean germplasms against soybean insect pests and found that highly susceptible germplasms had the highest defoliator larvae population compared to least susceptible germplasms at peak activity. These workers' results are well aligned with current research about the maximum number of defoliator larvae recorded on susceptible genotypes.

Sugar, protein or phenol constitutes in plant parts are important biochemical characteristics that contribute to insect resistance. A genotype is classified into either susceptible or resistant based on the variation in the quantity on biochemical constituents. As a result, analyzing the biochemical characteristics of resistant genotypes will help us understand the process of resistance to a certain insect pest. This knowledge would assist breeders in successfully incorporating this crucial strategy against a specific insect problem.

Reducing, non-reducing, and total sugar all significantly increase the host's vulnerability to insect pests (Nazeem, 2011). Higher sugar content in leaves and other plant components was linked to susceptibility because sugar has been shown to stimulate insect eating (Deither, 1953 and Thortsteinson, 1960). Similarly, Mohammad *et al.* (2019) and Rahman *et al.* (2021) investigated *S. litura* and discovered a statistically significant positive relationship between *S. litura* damage and lowering sugar. In comparison to the tolerant genotype, the highly susceptible genotype had the most total sugars, reducing sugars, non-reducing sugars, amino acids, and protein (Halder *et al.* 2006). Phenols are essential for restoring plants protection against insect pests among all other biochemical components (Painter, 1951). According to Munakata (1977), the phenols have antifeedant and repellent effects on tobacco caterpillar in a number of plants. In genotypes that are resistant as instead of susceptible genotypes, the more amount of phenol was found (Halder *et al.* 2006). There was a strong negative correlation between phenol concentration and defoliator damage as well as population (Nazeem, 2011). Similarly, researchers studied the biochemical resistancy in various crops, such as Haralu *et al.* (2018), Rahman *et al.* (2021) and Mohammad *et al.* (2019) discovered a substantial negative association between the amount of total phenol and pest infestation in several crops. The chemical conditions needed for peroxidases to function as anti-herbivore defenses are explained by the possibility that peroxidase may oxidize phenolics in the digestive system of an insect herbivore (Barbehenn *et al.* 2010). Plant defense

enzymes, including peroxidase, have been linked to insect genotypic resistance (War *et al.* 2015b). Enzymes that are antioxidants include superoxide dismutase, peroxidase and catalase significantly increased after *S. litura* infestation (Hafeez *et al.* 2020). PPO-mediated phenolic oxidation plays an integral role in common cutworm resistance. Manipulation of polyphenol oxidase activity may give resistance to both disease and insect pests, making it a potential component of successful integrated pest management (Mahanil *et al.* 2008). Gill *et al.* (2010) highlight the potential relevance of several oxidative enzymes in plant protection against insect herbivory. Dar *et al.*, 2017 discussed the function, relationships and significance of plant phenols and polyphenols to insect pest resistance mechanisms. In some genotypes, phenols, peroxidase and polyphenol oxidase play a major role in plant defense mechanism against certain insects. PPO / PO enzyme activity may have risen because phenolic chemicals are oxidized more often, forming poisonous quinines. The host's defensive response against insects involves the quinines. Antioxidative enzyme estimate studies found increased levels of peroxidase, catalase and superoxide dismutase, as well as decreased levels of polyphenol oxidase and phenylalanine ammonia lyase, indicating a defensive function in the plant (Rani *et al.* 2013). Insect damage produces de novo PAL production (Campos-Vergas and Saltveit 2002), which leads buildup of phenolic compounds (Bhonwong *et al.* 2009). Plants possess phenylalanine ammonia lyase (PAL), which contributes to their resistance against insect pests (Khattab & Khattab 2005, Bhonwong *et al.* 2009, War *et al.* 2011, 2012 and 2014). The resistant cultivars of crops exhibited greater levels of phenylalanine ammonia lyase (Guo *et al.* 2019). Protein levels were higher in resistance genotypes than in susceptible genotypes. They also found a strong negatively correlation among protein concentration in the leaves of soybean and *S. litura* damage. Higher protein levels might be due to higher activity of plant defensive enzymes and the synthesis of other defensive protein content (Prasad 2003). According to Mohammad *et al.* (2019) higher protein content was recorded in resistant genotypes in comparison of susceptible genotypes. Also significant negative correlation was observed between population of *S. litura* damage and protein content. Protein quality showed a reduction in injured plants compared to uninjured plants (Bi *et al.* 1994).

Plant resistance to insect pests is a complicated phenomenon that results from the interaction of several variables inside the plant. Biophysical characteristics of plants influence host selection, consuming food and insect pest behavior. Biophysical features such as trichome density on the leaf, specific leaf weight, relative water content, and chlorophyll content all play an significant role in imparting resistance to insect pests, which can have a positive or negative influence on their feeding habits or life cycles. To check the relevance of bio-physical features in resistance to *S. litura*, leaf samples from field were collected at 50 DAS, coinciding with a greater occurrence of *S. litura*. The genotypes differed dramatically in the density of laminar and midrib trichomes. Resistant genotypes showed greater midrib and leaf laminar trichome densities than susceptible genotypes. Damage from *S. litura* was significantly correlated negatively with midrib and

laminar trichome density (Mohammad *et al.* 2019). Anchala *et al.* (2015), and Sasane *et al.* (2018), who discovered a substantial negative association between leaf hair density on the abaxial surface on the leaf and percentage infestation in several crops. The results of the trichome density on the midrib, leaf blade and leaf lamina in soybean leaves show that trichomes are responsible for insect resistance, preventing damage or egg laying on the leaves. The results indicate that there are significant variations in leaf thickness amongst the genotypes of soybean. Maximum leaf thickness was considerably recorded by the resistant genotypes. Lower leaf thickness was documented by the susceptible genotypes (Mohammad *et al.* 2019). According to Prasad and Gowda (2006), the reduction in larval weight caused by ICGV 86031 might be attributed to the hardness of the leaves. In soybean, there is a negative association in between the specific leaf weight and defoliator damage. Contrasting results regarding specific leaf weight on contributing resistance might be related to *S. litura* differing eating habits (Sesane *et al.* 2018). Different plant parts thickness and resilience had a detrimental effect on insect penetration and feeding (Dhaliwal and Singh, 2005 and Lakshminarayanan *et al.* 2008). Because of thinner cell walls or enhanced turgidity of the tissues, increasing relative water content of leaves allows *S. litura* to feed more effectively (Nazeem, 2011). Sesane *et al.* (2018) also discovered a strong positive relationship between relative water content and larval population of *S. litura* damage in soybean genotypes. The results are consistent with those of Ihsan-ul-Haq *et al.* (2003), Mohammad *et al.* (2019) who discovered a substantial positive relationship between relative water content and insect infestation. Similarly, Haralu *et al.* (2018) discovered that chickpea pod borer, *Helicoverpa armigera* (Hubner) infestation and total chlorophyll content were all positively associated. All cultivar's chlorophyll pigment levels significantly decreased as a result of *S. litura* infestation (Hafeez *et al.* 2020). Under field circumstances, physio-morphological, biochemical, and percentile leaf damage resulting from *S. litura* on soybean. Correlation studies found that % leaf damage has a significant and positive relationship with chlorophyll concentration (Chowdary and Katlam 2023).

3.1 | Correlation Between *S. litura* Resistance-Contributing Biochemical and Biophysical Parameters

The data tabulated in Table 1.4 revealed the result of reducing sugar at 50 DAS recorded positive significant correlation with larval population of *S. litura* in soybean genotype was ($r=0.751$). Similarly, correlation studies revealed a negative significant correlation between *S. litura* larval population with total phenol ($r = -0.710$), peroxidase ($r = -0.647$), polyphenol oxidase ($r = -0.637$), protein content ($r = -0.771$) and phenylalanine ammonia lyase had negative non-significant correlation ($r = -0.504$). In Table 1.5 biophysical traits the result of leaf trichome density on abaxial surface at 50 DAS recorded negative correlation and significant association with larval population of *S. litura* on mid rib ($r = -0.695^{**}$), leaf blade ($r = -0.742^{**}$) and leaf lamina ($r = -0.779^{**}$). Similarly, correlation studies revealed a negative significant correlation between *S. litura* larval population with specific leaf weight

($r = -0.594^{**}$). The data revealed correlation between the relationship of relative water content (RWC) and *S. litura* larval population at 50 DAS was positive significant correlation ($r = 0.871^{**}$). The chlorophyll content show

positive significant correlation with *S. litura* larval population on chlorophyll a ($r = 0.688^{**}$), chlorophyll b ($r = 0.698^{**}$) and total chlorophyll was finding ($r = 0.707^{**}$).

Table 1.1. Larval population of *S. litura* at peak incidence and their categorization according to different level of infestation.

Sr. No.	Genotype	Mean	Category
1	CAT-146	0.90 (1.18)	R
2	G5P22	0.90 (1.17)	R
3	CAT-47	0.83 (1.15)	R
4	F4P21	0.86 (1.16)	R
5	F3P18	0.90 (1.18)	R
6	CAT-2503	0.83 (1.14)	R
7	NRC-142	1.26 (1.32)	MR
8	NRC-130	1.20 (1.30)	MR
9	JS 22-18	1.23 (1.31)	MR
10	JS 20-69	1.23 (1.31)	MR
11	NRC-138	1.20 (1.30)	MR
12	JS 22-16	1.26 (1.32)	MR
13	NRC-165	1.23 (1.31)	MR
14	JS 22-12	1.26 (1.32)	MR
15	JS 20-34	1.33 (1.35)	LR
16	JS 95-60	1.73 (1.49)	S
17	JS 93-05	1.66 (1.47)	S
18	JS 335	3.20 (1.92)	HS
SE(m) ±		(0.06)	
CD at 5%		(0.13)	
CD at 1%		(0.18)	
CV		(5.93)	

Figures in Parenthesis are $\sqrt{(X+0.5)}$ transformed values.

Table 1.2. Biochemical parameters basis of host plant resistance to *S. litura* in different soybean genotypes.

Sr. No.	Genotype	Field screening Mean	R. sugar Mean	T. phenol Mean	POD Mean	PPO Mean	PAL Mean	Protein Mean
1	CAT-146	0.90 (1.18)	17.14 (4.14)	23.01 (4.79)	0.75 (0.86)	0.32 (0.57)	1.52 (1.23)	3.95 (1.98)
2	G5P22	0.90 (1.17)	21.35 (4.62)	20.74 (4.55)	0.42 (0.65)	0.26 (0.51)	1.09 (1.04)	3.40 (1.84)
3	CAT-47	0.83 (1.15)	19.81 (4.45)	23.13 (4.80)	0.45 (0.67)	0.25 (0.50)	0.91 (0.95)	3.95 (1.98)
4	F4P21	0.86 (1.16)	19.66 (4.43)	21.28 (4.61)	0.56 (0.75)	0.29 (0.54)	1.73 (1.31)	3.54 (1.88)
5	F3P18	0.90 (1.18)	18.39 (4.28)	20.82 (4.56)	0.60 (0.77)	0.27 (0.52)	0.87 (0.93)	3.66 (1.91)
6	CAT-2503	0.83 (1.14)	19.50 (4.41)	21.25 (4.61)	0.67 (0.82)	0.25 (0.50)	1.00 (1.00)	3.56 (1.88)
7	NRC-142	1.26 (1.32)	25.47 (5.04)	19.95 (4.46)	0.34 (0.58)	0.18 (0.43)	0.83 (0.91)	2.92 (1.71)
8	NRC-130	1.20 (1.30)	24.20 (4.91)	18.95 (4.35)	0.34 (0.58)	0.23 (0.47)	0.65 (0.80)	3.16 (1.77)
9	JS 22-18	1.23 (1.31)	23.29 (4.82)	19.52 (4.41)	0.33 (0.57)	0.23 (0.48)	0.76 (0.87)	2.82 (1.67)
10	JS 20-69	1.23 (1.31)	23.81 (4.87)	18.54 (4.30)	0.29 (0.54)	0.23 (0.48)	0.65 (0.80)	3.04 (1.74)
11	NRC-138	1.20 (1.30)	25.36 (5.03)	17.25 (4.15)	0.31 (0.55)	0.19 (0.44)	0.84 (0.92)	3.15 (1.77)
12	JS 22-16	1.26 (1.32)	26.20 (5.11)	19.39 (4.40)	0.22 (0.47)	0.19 (0.44)	0.67 (0.82)	3.09 (1.75)
13	NRC-165	1.23 (1.31)	23.25 (4.82)	20.16 (4.49)	0.32 (0.57)	0.23 (0.48)	0.78 (0.88)	2.58 (1.60)
14	JS 22-12	1.26 (1.32)	26.28 (5.12)	17.83 (4.22)	0.41 (0.64)	0.22 (0.47)	0.76 (0.87)	3.00 (1.73)
15	JS 20-34	1.33 (1.35)	25.99 (5.09)	20.49 (4.52)	0.22 (0.47)	0.18 (0.43)	0.84 (0.91)	2.75 (1.65)
16	JS 95-60	1.73 (1.49)	26.70 (5.16)	16.93 (4.11)	0.16 (0.40)	0.17 (0.41)	0.58 (0.76)	2.52 (1.58)
17	JS 93-05	1.66 (1.47)	27.77 (5.26)	16.69 (4.08)	0.15 (0.39)	0.17 (0.41)	0.59 (0.77)	2.55 (1.59)
18	JS 335	3.20 (1.92)	29.36 (5.41)	16.40 (4.04)	0.17 (0.41)	0.17 (0.42)	0.62 (0.78)	2.23 (1.49)
	SE(m) ±	(0.06)	(0.24)	(0.38)	(0.04)	(0.04)	(0.11)	(0.1)
	CD at 5%	(0.13)	(0.52)	(0.8)	(0.09)	(0.1)	(0.24)	(0.21)
	CD at 1%	(0.18)	(0.72)	(1.1)	(0.13)	(0.14)	(0.33)	(0.29)
	CV	(5.93)	(5.14)	(8.68)	(7.53)	(10.26)	(12.44)	(5.81)

(R. sugar = reducing sugar, T. phenol = total phenol, POD = peroxidase, PPO = polyphenol oxidase, PAL = phenylalanine ammonia lyase.)

Table 1.3. Biophysical parameters basis of host plant resistance to *S. litura* in different soybean genotypes.

			Trichome density	Trichome density	Trichome density			Chlorophyll content	Chlorophyll content	Chlorophyll content
		Field screening	Midrib	Leaf blade	Leaf lamina	SLW	RWC	Chl. A	Chl. B	Chl. Total
Sr. No.	Genotype	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	CAT-146	0.90 (1.18)	29.3 (5.41)	31.5 (5.61)	30.8 (5.54)	0.41 (0.64)	22.73 (4.76)	7.18 (2.68)	4.69 (2.16)	11.87 (3.44)
2	G5P22	0.90 (1.17)	29.2 (5.40)	28.8 (5.36)	28.6 (5.34)	0.40 (0.63)	22.76 (4.77)	8.57 (2.92)	4.44 (2.10)	13.02 (3.60)
3	CAT-47	0.83 (1.15)	33.2 (5.76)	26.8 (5.17)	28.1 (5.30)	0.34 (0.58)	22.55 (4.74)	7.34 (2.71)	4.40 (2.09)	11.75 (3.42)
4	F4P21	0.86 (1.16)	35.9 (5.99)	30.7 (5.54)	31.8 (5.63)	0.37 (0.61)	18.99 (4.35)	8.48 (2.91)	5.42 (2.32)	13.91 (3.72)
5	F3P18	0.90 (1.18)	32.2 (5.67)	28.3 (5.31)	28.7 (5.35)	0.30 (0.54)	19.33 (4.39)	8.90 (2.98)	4.96 (2.22)	13.87 (3.72)
6	CAT-2503	0.83 (1.14)	35.6 (5.96)	33.5 (5.78)	33.1 (5.75)	0.41 (0.64)	19.55 (4.42)	8.79 (2.96)	5.17 (2.27)	13.97 (3.73)
7	NRC-142	1.26 (1.32)	27.6 (5.25)	23.9 (4.88)	26.1 (5.10)	0.27 (0.52)	26.70 (5.16)	18.03 (4.24)	10.96 (3.31)	29.00 (5.38)
8	NRC-130	1.20 (1.30)	24.1 (4.90)	25.3 (5.02)	26.0 (5.09)	0.29 (0.54)	23.67 (4.86)	9.40 (3.06)	8.83 (2.97)	18.24 (4.27)
9	JS 22-18	1.23 (1.31)	23.5 (4.84)	25.2 (5.01)	25.0 (5.00)	0.26 (0.51)	24.81 (4.98)	9.80 (3.13)	7.33 (2.70)	17.14 (4.14)
10	JS 20-69	1.23 (1.31)	27.9 (5.28)	23.8 (4.87)	23.8 (4.87)	0.25 (0.50)	27.00 (5.19)	16.44 (4.05)	6.36 (2.52)	22.81 (4.77)
11	NRC-138	1.20 (1.30)	27.3 (5.22)	25.5 (5.04)	25.0 (5.00)	0.28 (0.53)	23.96 (4.89)	17.77 (4.21)	10.18 (3.19)	27.96 (5.28)
12	JS 22-16	1.26 (1.32)	24.6 (4.95)	25.3 (5.02)	26.1 (5.10)	0.30 (0.54)	23.53 (4.85)	15.17 (3.89)	8.67 (2.94)	23.85 (4.88)
13	NRC-165	1.23 (1.31)	20.9 (4.57)	26.4 (5.13)	27.7 (5.26)	0.27 (0.52)	27.55 (5.24)	9.01 (3.00)	5.45 (2.33)	14.46 (3.80)
14	JS 22-12	1.26 (1.32)	26.6 (5.15)	25.5 (5.04)	27.5 (5.24)	0.29 (0.54)	24.31 (4.93)	10.75 (3.27)	6.87 (2.62)	17.62 (4.19)
15	JS 20-34	1.33 (1.35)	24.0 (4.89)	24.8 (4.97)	26.2 (5.11)	0.26 (0.51)	27.08 (5.20)	11.71 (3.42)	7.07 (2.66)	18.79 (4.33)
16	JS 95-60	1.73 (1.49)	20.0 (4.47)	20.4 (4.51)	22.9 (4.78)	0.24 (0.49)	28.30 (5.31)	20.03 (4.47)	12.62 (3.55)	32.65 (5.71)
17	JS 93-05	1.66 (1.47)	20.7 (4.54)	23.2 (4.81)	23.2 (4.81)	0.23 (0.48)	27.64 (5.25)	21.52 (4.63)	13.59 (3.68)	35.11 (5.92)
18	JS 335	3.20 (1.92)	19.8 (4.44)	20.3 (4.50)	20.5 (4.52)	0.24 (0.49)	34.35 (5.86)	20.14 (4.48)	11.99 (3.46)	32.13 (5.66)
	SE(m) ±	(0.06)	(0.34)	(0.31)	(0.34)	(0.03)	(0.4)	(0.41)	(0.33)	(0.27)
	CD at 5%	(0.13)	(0.73)	(0.65)	(0.73)	(0.07)	(0.85)	(0.86)	(0.71)	(0.58)
	CD at 1%	(0.18)	(1.01)	(0.9)	(1)	(0.1)	(1.18)	(1.19)	(0.98)	(0.79)
	CV	(5.93)	(6.77)	(6.13)	(6.71)	(6.89)	(8.25)	(11.8)	(12.48)	(5.47)

SLW = Specific leaf weight, RWC = Relative water content, Chl. = Chlorophyll.

Table 1.4. Correlation between tobacco caterpillar larval population and biochemical parameter.

Traits	r-value
Reducing sugar	0.751**
Total phenol	-0.710**
Peroxidase	-0.647**
Polyphenol oxidase	-0.637**
PAL	-0.504
Protein	-0.771**

**Correlation coefficients significance at $p = 0.01$ ($r = 0.575$). –ve sign: negatively correlated; no sign: positively correlated.

Table 1.5. Correlation between tobacco caterpillar larval population and biophysical parameter.

Traits	r-value
Trichome on midrib	-0.695**
Trichome on leaf blade	-0.742**
Trichome on leaf lamina	-0.779**
Specific leaf wt.	-0.594**
Relative water content	0.871**
Chlorophyll A	0.688**
Chlorophyll B	0.698**
Total chlorophyll	0.707**

Correlation coefficients significance at $p = 0.01$ ($r = 0.575$). –ve sign: negatively correlated; no sign: positively correlated.

4 | CONCLUSION

In conclusion, we can say that Studies on field screening of *S. litura* of soybean crop provide basic information about seasonal occurrence of insect pest. Soybean genotypes screened against tobacco caterpillar, minimum larval population was recorded in resistant genotypes and maximum larval population was recorded in susceptible genotype. Genotypes having highest reducing sugars were more vulnerable to *S. litura* damage. Susceptible genotypes showed more reducing sugar compared to resistant genotypes. Highest measure of phenol, enzyme activity, phenylalanine lyase and

protein content were found in resistant genotypes in contrast to susceptible genotypes indicating that they are highly induced in resistant genotype against *S. litura*. Higher trichome density (mid rib, leaf blade and leaf lamina) and specific leaf weight were analyzed in resistant genotypes in comparison with susceptible genotypes indicating that they are highly induced in resistant genotype against *S. litura*. In soybean genotypes, relative water content and chlorophyll content (a, b and total) were found lower in resistant genotypes compared to susceptible genotypes.

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