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Biochemical basis of resistance in soybean genotypes against *Spodoptera litura* (Fabricius)

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Abstract

The tobacco caterpillar, *Spodoptera litura* (Fabricius), is one of the most destructive defoliating insect pests of soybean, causing substantial yield losses under field conditions. Identification of resistant genotypes based on biochemical characteristics provide an effective approach for developing insect-resistant cultivars. The present investigation was undertaken to determine the biochemical constituents, including total protein, total phenols, total sugars, reducing and non-reducing sugars. Significant variation was observed among the genotypes for all biochemical parameters. Total protein content ranged from 53.67 to 106.20 mg/g fresh weight, whereas total phenol content varied from 0.10 to 0.32 mg/g fresh weight. Total sugar, reducing and non-reducing sugar content ranged from 8.35 to 12.77, 0.22 to 1.04 and 7.72 to 11.14 mg/g fresh weight, respectively. Among the evaluated genotypes, JS-20-69 recorded the highest total protein content, while EC-396059 exhibited the highest total phenol content. In contrast, EC-251861 contained the highest level of total, reducing and non-reducing sugars. Resistant genotypes were generally characterized by higher protein and phenol contents and lower sugar concentrations than susceptible genotypes. These biochemical characteristics were associated with reduced host suitability for *S. litura*. The findings suggest that protein, phenol, and sugar profiles may serve as useful biochemical markers for identifying and developing soybean genotypes with improved resistance to *S. litura*.

Keywords: Soybean, biochemical resistance, *Spodoptera litura*, Plant protein, phenols, and sugar analysis, plant defense compound

1. Introduction

Soybean (*Glycine max* L. Merrill) is an important oilseed and protein-rich crop cultivated worldwide and serves as a major source of vegetable oil and high-quality protein (Guo *et al.*, 2022) [3]. Among the insect pests attacking soybean, the tobacco caterpillar, *Spodoptera litura* is one of the major defoliating pests causing considerable damage to the crop under field condition (Komatsu *et al.*, 2010) [4]. Host plant resistance is an important component of integrated pest management because it provides an economical, environmentally safe, and sustainable approach for managing insect pests. Biochemical constituents of plants play a crucial role in determining their resistance or susceptibility to insect attack. Among these, phenolic compounds, proteins and sugars are known to influence the feeding behavior, growth, development, and survival of insect pests. Phenolic compounds act as important defensive substances and are often associated with reduced insect feeding and infestation. They interfere with the digestive enzymes of insects and produce toxic oxidation that adversely affect insect growth (Woodhead, 1981) [14]. Similarly, defensive proteins and proteinase inhibitors can impair insect digestion and reduce larval growth and survival (Neupane *et al.*, 2019) [9]. In contrast, sugars serve as important nutritional components that influence host preference and larval performance. Higher concentrations of total, reducing and non-reducing sugar generally enhance host suitability for insect pests, whereas lower sugar content is often associated with resistance (Chowdary and Katlam, 2023; Moirangtham *et al.*, 2026) [2, 7]. Although several studies have reported the role of biochemical constituents in plant resistance against insect pests, information on the biochemical basis of resistance among selected genotypes against *S. litura* is still limited. Therefore, the present investigation was undertaken to estimate the total protein, total phenol, total sugar, reducing and non-reducing sugar contents of selected soybean genotypes and to study their association with resistance against *Spodoptera litura*.

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2. Materials and Methods

The experiment was conducted at the ICAR-Indian Institute of Soybean Research (ICAR-IISR), Indore, Madhya Pradesh, India. Eleven selected soybean exhibiting differential reactions to *Spodoptera litura* were used for biochemical analysis. The experiment was laid out in a randomized block design (RBD) with two replications. The third fully expanded healthy leaf from the top of each genotypes was collected at 50 days after sowing (DAS) from each replication. Two grams of fresh leaf tissue from each genotypes were used for the preparation of leaf extract (aliquot). The aliquot was subsequently used for the estimation of total sugars, reducing and non-reducing sugars, total phenols, and total protein following the procedure described by Mohammad *et al.*, (2019) [6].

2.1 Preparation of leaf extract (Aliquot)

The aliquot was prepared by cutting 2 g of fresh leaf sample into small pieces and immersing them in 80% ethanol. The leaf tissues were thoroughly ground using a pestle and mortar with a small quantity of ethanol and filtered through muslin cloth. The extraction procedure was repeated once and filtrate were pooled and filtered through Whatman No. 41 filter paper. The volume was adjusted to 20 ml with 80% ethanol. The filtrate was clarified by adding 2 ml of saturated lead acetate followed by 3ml of disodium hydrogen phosphate solution. The mixture was allowed to stand overnight for complete settling of impurities and then filtered through Whatman No. 42 filter paper. The final volume of the clear filtrate was adjusted to 25 ml with 80% ethanol. The resulting filtrate served as stock solution (aliquot) for the estimation of sugars, phenols, and proteins. The absorbance of each biochemical constituent was measured using a spectrophotometer.

For the estimation of sugars and protein, 1 ml of the aliquot was heated in a hot water bath until the alcohol had completely evaporated to eliminate interference during estimation. The alcohol-free aliquot was then used for sugar and protein estimation. However, removal of alcohol was not required for phenol estimation, as it did not interfere with phenol determination (Mohammad *et al.*, 2019) [6].

2.2 Estimation of Reducing Sugars

A standard stock solution was freshly prepared by dissolving 100 mg of D-glucose in distilled water and making the volume up to 100 ml to obtain a concentration of 1 mg/ml. an aliquot of 10 ml from the stock solution containing 100 µg/ml D-glucose. Reducing sugar content in the filtrate was estimated following the Nelson (1944) [8] and Somogyi (1952) [13] method.

Standard solutions (0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml) and sample aliquots (0.1 and 0.3 ml) were taken in separate test tubes, and the volume was adjusted to 1 ml with distilled water. One ml of alkaline copper reagent was added and mixed thoroughly. The tubes were heated in a boiling water bath for 20 min and cooled under running tap water without shaking. Subsequently, 1 ml of arseno-molybdate reagent was added immediately, and the volume was made up to 20 ml with distilled water. The blue colour developed was measured at 510 nm using a spectrophotometer.

2.3 Estimation of Total Sugars

One ml of alcohol free aliquot was transferred into a test tube, and 1 ml of 1 N hydrochloric acid was added. The

tubes were incubated in a hot water bath at 50 °C for 20 min. After cooling, one drop of indicator was added, and the solution was neutralized with 1 N sodium hydroxide until a pink colour appeared. Excess alkali was then neutralized with 0.1 N hydrochloric acid until the solution became colourless. The volume was adjusted to 5 ml with distilled water. from this solution, 1 ml was used for estimation of total sugars following the Nelson-Somogyi method (Reddy *et al.*, 2020) [12]

2.4 Estimation of Non-reducing Sugars

Non-reducing sugar content was calculated by subtracting the reducing sugar content from the total sugar content and multiplying the difference by a conversion factor of 0.95 (Reddy *et al.*, 2020) [12]

2.5 Estimation of Total Protein

Total protein content was estimated following the method described by Lowry *et al.* (1951) [5]. The absorbance was recorded at 660 nm using a spectrophotometer. A standard curve ($R^2 + 0.989$) was prepared, and the protein content of each sample was calculated from the regression equation and expressed as mg/g fresh weight.

The reagent used were : (A) 2% Na_2CO_3 in 0.1 N NaOH: (B) 1% sodium potassium tartrate in distilled water: (C) 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in distilled water: (D) Reagent I: 48 ml of reagent A + 1 ml of reagent B + 1 ml of reagent C; and (E) Reagent II: Folin-Ciocalteu (2 N) diluted with distilled water in a 1:1 ratio.

One ml of the protein extract was transferred into a test tube and mixed with 4.5 ml of freshly prepared alkaline copper reagent (Reagent I). The mixture was incubated at room temperature for 10 min. Subsequently, 0.5 ml of diluted Folin-Ciocalteu reagent (Reagent II) was added with immediate mixing. The volume was adjusted to 15 ml with distilled water, and the absorbance was recorded at 660 nm against a reagent blank using a spectrophotometer.

2.6 Estimation of Total Phenols

Total phenol content was estimated following the Folin-Ciocalteu method described by Bray and Thorpe (1954). A standard curve ($R^2 = 0.978$) was prepared using gallic acid. One ml of alcoholic extract was transferred into a 25 ml volumetric flask, followed by the addition of 1 ml of Folin-Ciocalteu reagent. After 8-10 min, 2 ml of 20% sodium carbonate solution was added and mixed thoroughly. The flasks were heated in a boiling water bath for 1 min and cooled under running tap water. The volume was adjusted to 25 ml with double-distilled water and incubated at room temperature for 30 min for colour development. A reagent blank was prepared without the sample extract. The absorbance was measured at 740 nm using a spectrophotometer (Pinnamwar *et al.*, 2022) [11].

2.7 Statistical Analysis

The absorbance (OD) values of each sample were converted into concentration (µg) using the regression equation obtained from the respective standard curve:

$$\text{Concentration } (\mu\text{g}) = (\text{OD} - \text{Intercept}) / \text{Slope}$$

The calculated concentration was converted into biochemical content (mg/g fresh weight) by applying the appropriate conversion factor based on extraction volume,

dilution factor, aliquot volume, and sample weight. The final biochemical data were subjected to analysis of variance (ANOVA) appropriate for a Randomized Block Design (RBD).

3. Results and Discussion

3.1 Biochemical constituents of soybean genotypes

The biochemical analysis significant variation among the eleven soybean genotypes for total protein, total phenols, total sugars, reducing and non-reducing sugars (Table 1). The observed variability among the genotypes indicates considerable differences in their biochemical composition which may influence their reaction to *Spodoptera litura*.

3.2 Total Protein

Total protein content varied significantly among the soybean genotypes, ranging from 53.67 to 106.20 mg/g. among the evaluated genotypes, JS-20-69 recorded the highest protein content, whereas the lowest protein content was JS-335. The resistant genotypes generally exhibited higher protein content than the susceptible genotypes. The comparatively higher protein content observed in resistant genotypes may be associated with the presence of defensive proteins and proteinase inhibitors, which adversely affect larval growth and development, thereby contributing to host plant resistance. The results of present study are in agreement with Neupane *et al.*, (2019)^[9], who reported that elevated protein content is associated with increased resistance against insect herbivores by reducing their feeding efficiency.

3.3 Total Phenols

Total phenol content ranged from 0.10 to 0.32 mg/g, with

genotype EC-396059 recording the highest concentration, Whereas EC-251861 exhibited the lowest phenol content. Among the biochemical constituents, phenolic compounds play a vital role in imparting resistance against insect pests in plants (Painter, 1951)^[10]. The comparatively higher phenol content observed in resistant genotypes suggests an important role of phenolic compounds in resistance against *S. litura*. Phenolic compounds reduce host palatability, interfere with insect digestive enzymes, and generate toxic oxidation products that adversely affect insect growth and survival. Consequently, increased phenol accumulation is commonly associated with enhanced resistance against foliage feeding insects.

3.4 Sugars

Total, reducing, and non-reducing sugar contents differed significantly among the soybean genotypes. Total sugar content ranged from 8.35 to 12.77 mg/g, while reducing and non-reducing sugar contents varied from 0.22 to 1.04 mg/g and 7.72 to 11.14 mg/g respectively. Among the evaluated genotypes, EC-251861 recorded the highest contents of total, reducing, and non-reducing sugars, whereas EC-396059 exhibited the lowest values. Resistant genotypes consistently possessed lower sugar contents than susceptible genotypes. Sugars are important nutritional constituents that influence host preference and larval development in phytophagous insects. Higher sugar concentrations enhance host suitability by providing readily available energy for insect growth and feeding, whereas lower sugar levels reduce the nutritional quality of the host plant and are generally associated with increased resistance against insect pests.

Table 1: The biochemical basis of resistance to *Spodoptera litura* on selected soybean genotypes.

Genotypes	Protein (mg/g)	Phenol (mg/g)	Total sugar (mg/g)	Reducing sugar (mg/g)	Non-reducing sugar (mg/g)
EC-251861	76.28 (8.73)	0.10 (0.32)	12.77 (3.57)	1.04 (1.02)	11.14 (3.34)
EC-287466	54.00 (7.35)	0.14 (0.37)	12.03 (3.47)	0.75 (0.87)	10.72 (3.27)
EC-289099	56.60 (7.52)	0.14 (0.37)	11.95 (3.46)	0.72 (0.85)	10.67 (3.27)
EC-291401	81.60 (9.03)	0.18 (0.42)	10.85 (3.29)	0.74 (0.86)	9.60 (3.10)
EC-313915	90.38 (9.51)	0.21 (0.46)	12.00 (3.46)	0.73 (0.85)	10.70 (3.27)
JS-20-69	106.20 (10.31)	0.25 (0.50)	11.04 (3.32)	0.50 (0.71)	10.01 (3.16)
JS-20-29	105.37 (10.26)	0.24 (0.49)	10.56 (3.25)	0.49 (0.70)	9.57 (3.09)
JS-335	53.67 (7.33)	0.12 (0.34)	12.46 (3.53)	0.93 (0.96)	10.95 (3.31)
EC-383165	84.68 (9.20)	0.19 (0.44)	10.45 (3.23)	0.75 (0.87)	9.21 (3.04)
Cat No.-2503	99.09 (9.95)	0.29 (0.54)	8.96 (2.99)	0.28 (0.53)	8.24 (2.87)
EC-396059	89.52 (9.46)	0.32 (0.57)	8.35 (2.89)	0.22 (0.47)	7.72 (2.78)
Mean	8.97	0.44	3.32	0.79	3.14
Sem±	0.32	0.02	0.09	0.06	0.09
CD at 5%	0.72	0.04	0.20	0.13	0.21

Values in parentheses are square-root transformed values used for statistical analysis

4. Conclusion

The biochemical analysis of selected genotypes revealed significant variation in protein, Phenol, Total sugar, reducing and non-reducing sugar contents. The present investigation indicated that resistant soybean genotypes were characterized by comparatively higher protein and phenol contents together with lower concentrations of total, reducing, and non-reducing sugars than susceptible genotypes. These biochemical characteristics may contribute to resistance by reducing host suitability and adversely affecting the feeding, digestion, growth, and survival of *Spodoptera litura*.

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