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Studies on biochemical parameters responsible for resistance against okra shoot and fruit borer *Earias vittella* (FAB)

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Abstract
The genotype Parbhani Kranti recorded maximum moisture content of 90.33 % which was at par with IC-113904 (89.77 %) and IC-43746 (89.56 %) whereas the resistant genotype IC 90210 recorded a minimum moisture content of 81.16 %. This shows that moisture content was ranged from 81.16 to 90.33 %.
The chlorophyll content in selected okra genotypes was ranged from 0.18 to 0.52 mg/gm. Significant chlorophyll content of 0.52 mg/gm was noticed in the genotype EC 128888 as against minimum chlorophyll content of 0.18 mg/gm in the genotype 1557. The results indicated that there was no relation between amount of chlorophyll content in fruits of selected okra genotypes and *E. vittella* infestation. Results also indicated that, resistant genotypes contain maximum amount of total phenol as compared to susceptible and moderately susceptible genotypes. Maximum total phenol content of 1.27 and 1.17 % was recorded in the two resistant genotype i.e. IC 90210 and IC-974 respectively. However two genotypes from susceptible and four genotypes from moderately susceptible category *i.e.* IC-99724, 1557, IC-90251, IC-133336, Parbhani Kranti and 1957 recorded minimum of 0.59, 0.60, 0.61, 0.65, 0.68 and 0.69 % total phenol content respectively.
The total soluble sugar content was ranged from 1.98 to 5.91 %. Moreover, it was noticed that, susceptible genotypes i.e. 1557 and Parbhani Kranti contained maximum total soluble sugar of 5.91 and 5.84 % respectively as against minimum of 1.98 per cent and 2.13 % total soluble sugar in resistant genotypes i.e. IC-90210 and IC-974 respectively.
Ash content in the tested genotypes was varied from 1.47 to 4.64%. Least ash recorded was of 1.47 % which is in the genotype Parbhani Kranti which was at par with the genotypes 1557 and IC-90251 with 1.61 and 1.73% ash respectively. However, maximum ash content of 4.64 % was noticed in the genotype IC-974. The results showed that the genotypes with minimum ash content were found to be susceptible to *E. vittella* infestation whereas the genotypes with maximum ash content were found to be resistant to *E. vittella* infestation.

Keywords: Okra Germplasm, *Earias Vittella* (FAB), Biochemical Parameters, Spectrophotometer

1. Introduction
Okra is attacked by a number of insect pests, of which shoot and fruit borer, *Earias spp.* is one of the major constraints in achieving potential yield. [1] The infested fruits become unfit for human consumption, thus resulting in 35-76 % decrease in yield [2] and causes severe damage to the crop leading to yield losses to an extent of 3.5 to 90 % in Andra Pradess [3] and 30.81 % at Coochbehar, West Bengal [4]. In early stage of the crop growth, the larvae bore into the tender shoots tunneling downwards and the affected shoots wither and growing points are killed. The entrance whole is plugged with excreta. Later the caterpillars bore inside the developing buds, flowers, fruits and feed on inner tissues. Damaged buds and flowers fall, while the infested fruits present a deformed appearance and become unsuitable for consumption [5].
Success of breeding programme depends on screening of available germplasm for resistance source and successful use of identified resistance sources to the existing high yielding varieties [6]. Also various biophysical and biochemical characters of the plants play an important role by providing resistance against this pest [7]. However, literature on role of these biophysical and biochemical parameters imparting resistance towards different okra genotypes against *E. vittella* is scanty [8]. Some antibiosis mechanism of plant also affect on pest incidence, damage, their survival, growth and development [9]. To evolve such antibiosis reaction of okra plant on *E. vittella*, larval and post larval study having immense importance.

2. Material and methods
The biochemical constituents were estimated on % basis according to the standard of AOAC International Procedures [10] with some modifications. For the biochemical attributes, fruits from twenty okra genotypes were analyzed and following parameters were analyzed i) Moisture content ii) Chlorophyll iii) Total soluble sugar iv) Total phenol and v) Ash.
The samples were collected from earlier described field experiment wherein, all the twenty genotypes were raised in the field under uniform conditions in three replications. The uninfested healthy plants were used. Fruit samples were collected between 9.00 to 11.00 am on clear sunlight morning. All the samples were collected at the same time. For chlorophyll and moisture content analysis, fresh green samples of each genotype were used whereas, for analysis of total soluble sugars, total phenol and ash content the portions of fruit samples were completely dried at 60ºC and

stored at 4ºC until used for analysis. Each sample was analyzed in triplicate for biochemical attributes.

2.1 Preparation of samples for analysis
The dried fruits were powdered separately in multiplex grinding mill so as to pass through 60 mesh sieve. The powdered material was used for different estimations. A portion of the powdered material was defatted using carbon tetrachloride. The analysis of fruit was undertaken separately and following parameters were studied.

2.2 Moisture
Ten grams of samples of each healthy green fruits were accurately weighed and dried in oven at 100ºC for 24 hours. After cooling in desiccator samples were weighed. Drying was continued for one more hour and samples were weighed again. The drying and weighing were repeated until constant weight was obtained. The loss in weight of sample was recorded as moisture content.

2.3 Chlorophyll
Fresh, green, matured okra fruits from different genotypes were used for estimation of chlorophyll. One gram sample of healthy green okra fruits from each genotype was accurately weighed and grinded in mortar and pestle with the addition of 20 ml of 80 % acetone. Grinded tissues were centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred to 100 ml volumetric flask. The procedure was repeated until the colorless residue found and made the volume to 100 ml with 80 per cent acetone. The absorbance of solution was read at 645 nm against blank. The amount of chlorophyll present in the extract was calculated in mg per gram of tissue.

2.4 Total phenol
For estimation of total phenol from fruits of different selected okra genotypes method given by Bray and Thorpe [11] was adopted.

One ml of plant extract (alcohol evaporated after extraction with 80 % alcohol) was pipetted out into a test tube, 1 ml of Folin-ciocalteu reagent followed by 2 ml of Na_2CO_3 solution was added. Shakings were given to the tubes with automatic shaker and heated in a boiling water bath for exactly 1 min. After boiling, solutions were allowed to cool and diluted the blue solution to 100 ml with distilled water and absorbance was measured at 650 nm in a spectrophotometer. A blank containing all the reagents (without plant extract) was used to adjust the absorbance to zero. A standard graph was prepared by plotting absorbance v/s tannic acid concentration (0.2, 0.3, 0.4 and 0.5) with the help of a standard graph; total phenol content was calculated.

2.5 Total soluble sugar
Total soluble sugar was determined as per the method given by Dubois *et al.* [12]. Defatted dried fruit sample of 500 mg was weighed and 25 to 30 ml of hot 80 % ethanol was added in the boiling tube and shaking was given on a vertex mixture. Material was allowed to settle for 20 to 30 min. All the material was then filtered into a beaker through a Whatman No. 41 filter paper. Extract was kept in a hot water bath until the ethanol evaporated, then about 10 ml water was added and dissolved contents were transferred into a 100 ml volumetric flask. The contents were washed 2 to 3 times and then added to volumetric flask by making it up to 100 ml with water.

One ml aliquot from above contents and 1 ml water as blank was taken in a test tube and 1 ml of 5 % phenol was assessed and shaking was given vigorously on a vertex mixture and allowed to cool in water. Absorbance of golden yellow colour was measured at 490 nm against the blank. Standard was then run with different concentrations i.e. 10, 20, 30, 40 and 50 mg of glucose standard. Per cent total soluble sugar was calculated with the help of standard graph.

2.6 Ash
Well mixed samples weighing 5 g each were taken into pre-weighed silica crucibles. The latter were ignited in muffle furnace at 550ºC (dull red) until light grey ash resulted. After cooling in desiccators to room temperature, crucibles were weighed. The loss in weight was recorded and ash content was calculated.

3. Results and discussion
Biochemical parameters also played important role in imparting resistance to *E. vittella* in okra genotypes. The data pertaining attributes of okra genotypes associated with resistance to shoot and fruit borer is presented in Table 1.

Table 1: Biochemical parameters observed in selected okra genotypes

Sr. No.	Genotypes	Moisture (%)	Chlorophyll (mg g^{-1})	Total Phenol (%)	Total Soluble Sugar (%)	Ash (%)
1	IC-43746	89.56	0.51	1.06	2.83	4.30
2	EC-941647	87.45	0.38	0.81	4.35	3.17
3	EC-329407	83.51	0.30	0.77	4.68	2.90
4	EC-128888	87.89	0.52	0.75	4.19	3.70
5	1552	87.19	0.22	0.80	4.79	2.85
6	IC-974	81.57	0.41	1.16	2.13	4.64
7	1557	85.85	0.34	0.62	5.91	1.61
8	IC-282229	87.58	0.48	0.89	3.63	4.36
9	Parbhani Kranti	90.33	0.22	0.68	5.84	1.47
10	RHR-102	85.12	0.41	0.82	5.08	2.83
11	IC-90251	87.97	0.31	0.61	5.71	1.73
12	IC-90210	81.16	0.50	1.27	1.98	4.25
13	1957	82.97	0.18	0.69	5.65	1.90
14	IC-113904	89.77	0.33	0.91	3.74	4.10
15	EC-133408	81.78	0.41	0.72	4.86	2.96
16	IC-282284	88.54	0.37	0.87	3.99	3.73
17	IC-133336	87.86	0.29	0.65	5.45	2.07
18	IC-218903	85.23	0.21	0.69	5.27	1.99
19	IC-282280	82.57	0.46	0.79	3.38	4.00
20	IC-99724	81.80	0.36	0.59	4.52	2.04
S.E.		0.38	0.02	0.03	0.15	0.12
CD 0.01%		1.17	0.08	0.10	0.55	0.38

3.1 Moisture content
The moisture content in the fruits of selected okra genotypes was ranged from 81.16 to 90.33 %. The cultivar Parbhani Kranti recorded maximum moisture content of 90.33 % which was significantly higher than that observed in rest of the entries except IC-113904 (89.77 %) and IC-43746 (89.56 %) and were at par with each other. Resistant genotype IC-90210 recorded a minimum moisture content of 81.16 % which was significantly lower than that recorded in rest of the entries. This was followed by the genotype IC-

974 (81.57 %), EC-133408 (81.78 %) and IC-99724 (81.80 %) and were statistically at par with each other. The result of the present findings are in line with those Singh (1987) who reported that moisture content positive correlation with *E. vittella* survival and Bag (2007) who also reported that moisture was varied from 72.54 to 88.10 per cent in highly resistant to susceptible genotypes.

3.2 Chlorophyll
The chlorophyll content in selected okra genotypes was ranged from 0.18 to 0.52 mg/gm. The significantly maximum chlorophyll content of 0.52 mg/gm was noticed in genotype EC-128888 and was followed by the genotypes EC-43746, IC-90210 and IC-282229 with 0.51, 0.50 and 0.48 mg/gm chlorophyll content, respectively. On contrary significant minimum chlorophyll content of 0.18 mg/gm was recorded in genotype 1557 and it was at par with genotypes namely, IC 218903 (0.21 mg/gm), Parbhani Kranti and 1552 (0.22 mg/gm each). Thus from the data it was concluded that there was no relation between amount of chlorophyll content in the fruits of selected okra genotypes and *E. vittella* infestation and there by indicated negligible role of chlorophyll content in fruits and infestation of *E. vittella*. Results of the present findings are in line with those Banger *et al.,* (2012) who reported that total chloropyll in fruits of okra was ranged from 0.18 to 0.56 mg/gm with negligible role on infestation of *E. vittella*.

3.3 Total Phenol
From the results it was revealed that, total phenol content in selected okra genotypes was varied from 0.59 to 1.27 %. Further it was also noticed that maximum total phenol content of 1.27 and 1.17 % was recorded in the two resistant genotypes i.e. IC-90210 and IC-974, respectively. However, four genotypes from moderately susceptible category and two genotypes from susceptible category i.e. IC-99724, 1557, IC-90251, IC-133336, Parbhani Kranti and 1957 recorded minimum of 0.59, 0.60, 0.61, 0.65, 0.68 and 0.69 % total phenol content respectively. From the data it was concluded that, the resistant genotypes contained maximum amount of total phenol as compared to susceptible and moderately susceptible genotypes. These findings agree with Sharma and Singh (2010), Haldar *et al.,* (2013) who reported that phenol content was highest in resistant and lowest in susceptible varieties/genotypes.

3.4 Total Soluble Sugar
From the data it was noticed that the total soluble sugar content in selected okra genotypes was ranged from 1.98 to 5.91 %. Moreover, it was found that the genotype from susceptible category i.e. 1557 and Parbhani Kranti contained maximum total soluble sugar of 5.91 and 5.84 %, respectively. Whereas, minimum total soluble sugar was recorded in the genotypes from resistant category i.e. IC-90210 (1.98 %) and IC-974 (2.13 %) and were at par with each other. From the result it was found that the resistance genotypes i.e. IC-90210 and IC-974 contained minimum total soluble sugar as against maximum in susceptible ones i.e. 1557 and Parbhani Kranti. Chaudhari [13] reported that varieties with highest amount of sugar were most susceptible to the attack of shoot and fruit borer.
The results of the present findings are in good line with Jat and Pareek [14], Chandrashekhar *et al* [15] and Haldhar *et al* [16] who reported that total sugar was lowest in resistant and hightes in susceptible genotypes.

3.5 Ash
From the data it was revealed that the per cent ash content in the tested genotypes was varied from 1.47 to 4.64 %. From data it was also observed that the least of 1.47 % ash content was recorded in genotype Parbhani Kranti and it was followed by the genotypes 1557 and IC-90251 with 1.61 and 1.73 % ash respectively which were at par with each other. From the results it was concluded that the genotypes with minimum ash content were found to be susceptible to *E. vittella* infestation whereas the genotype with maximum ash content were found to be resistant to *E. vittella*. The results of the present findings are in accordance with Banger *et al.* [17] who reported per cent ash content ranged from 1.36 to 2.41 % and stated that an ash content in fruits were significantly negatively correlated with *E. vittella* infestation. They further indicated that increase in ash content in fruit, infestation of *E. vittella* decreased. Thus the higher amount of ash was responsible for imparting resistance to *E. vittella.*

4. Conclusion
The study on biochemical characters i.e. moisture content, chlorophyll, total phenol, total soluble sugar and ash content of different twenty okra genotypes in relation to shoot and fruit borer infestation during summer revealed that, moisture content was varied from 81.16 to 90.33 %. Susceptible genotype Parbhani Kranti recorded maximum both moisture content and total soluble sugar (90.33 % and 5.84 % respectively) as against minimum of 81.60 and 1.98 % moisture content and total soluble sugar respectively in susceptible genotype IC-90210. On the other hand, the susceptible genotype Parbhani Kranti recorded minimum total phenol and ash content (0.59 and 1.47 % respectively) as against maximum of 1.27 % and 4.25 % total phenol and ash content respectively in resistant genotype IC-90210. The correlation coefficient analysis revealed that moisture content (r=0.479*) and total soluble sugar (r=0.567*) had significant positive correlation with *E. vittella* infestation whereas total phenol (r=-0.524*) and ash content (0.598*) had significant negative correlation with *E. vittella* infestation. Chlorophyll content (r=0.246) had no relation with *E. vittella* infestation. Colour of fruit had non-significant correlation with fruit infestation and it was observed that the fruit borer preferred the dark green colour less and light green colour fruits more.

5. References
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