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Susceptibility of soybean varieties to infestation of cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) and their relation to climatic factors with emphasis on leaves characteristic

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Abstract:

Susceptibility of five soybean varieties (Giza 21, Giza 22, Giza 35, Giza111 and Crawford) to infestation of cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) were studied under natural field conditions at the experimental farm of the Faculty of Agriculture, Benha University, Egypt during two successive seasons, 2015 and 2016. The relationship between phytochemical components and anatomical characters of leaves were also studied. Results showed that, Crawford variety was highly infested by *S. littoralis* as number of larvae/100 plants and rate of leaflets soybean feeding damage. While the susceptibility of Giza 21 variety recorded the lowest number of larvae/100 plants and rate of leaflets soybean feeding damage during the two successive seasons. Statistical analysis showed positive correlation between population of *S. littoralis* with minimum and maximum temperature during the two seasons. On the other hand, the relative humidity had insignificant negative effects in both seasons. Phytochemical analysis had significant positive effect among the leaflet of the five studied varieties and the total of protein, carbohydrates and phenols. While there was insignificant negative effect between leaflet contained of phosphate and potassium and insect population. The anatomical characters of the leaflet of five soybean varieties and the population of *S. littoralis* were correlated significantly positive for all leaf morphological and anatomical characteristics on the five soybean varieties.

Introduction

Soybean (*Glycine max* (L.) Mirrill) is one of the most important leguminous crops in many countries and reached a prominent position among other crops in the world. Seeds contain high nutritional value about 40% protein and 20% edible oil, besides minerals and vitamins, It also supports many industries because of soybean oil is used as raw material in manufacturing of antibodies, paints, varnishes, adhesives, lubricants etc. and used as protein supplement in human diet, cattle and poultry feed.

In 2015, in Egypt, the soybean cultivated area reached about 33.974 feddans which produced about 46.843 tons of grains with an average yield of 3.6 ton/feddan. In Qalyoubia Governorate, the soybean cultivated area reached about 132 feddans which produced about 204 tons of grains with an average yield of 1.5 ton/feddan (Anonymous, 2015).

Under field conditions, soybean plants are subjected to be attacked by many pests, among the most common and important pests, cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered the main leaf feeding insect attacks soybean plants, causing large loss in yield . Lutfallah *et al.* (2003) showed that 12 soybean genotypes studied could be grouped into three categories: High resistant to the cotton leaf worm (2genotypes, H2L24 and L86-K73, while intermediate resistant genotypes were (8 genotypes, Giza 83. H1L2/10, H11L6, H1L3/12, H16, H1L4/32, Giza 21 and Lamar variety and highly susceptible genotypes (2 genotypes, Crawford and Clark). Genotypes were significantly different in almost all studied traits in both seasons, the most tolerant genotype to cotton leaf warm (10.0 and 9.9%) were L159L2 and L159L7 in 2011 and 2012 growing seasons, respectively, While the lowest tolerant genotypes were L132 and Black Seed in both two seasons (El-Garhy *et al.*,2013). Alakhder *et al*. (2015) results revealed significant differences among the tested soybean genotypes for all studied traits. They found that three genotypes H 19 L 96, H 4 L 24 and H 32 were considered as the best with high yield and lowest pests' infestation; in contrast H 1 L 1, L 127 with highest infestation and lowest yield genotype.

The present work is to study the population fluctuation of *S. littoralis* on five soybean varieties during two seasons 2015 and 2016. As well as susceptibility of these soybean varieties to infestation of *S. littoralis* and their relationship to climatic factors and some leaflet characteristics such as (phytochemical components and anatomical characters).

Materials and methods

1. Field experiments:

Field experiments were carried out at the experimental farm of Faculty of Agriculture, Moshtohor, Benha University, Qalyoubia Governorate. An area of about 1/2 feddan was divided into 20 equal plots of about 10.5 m^2 each. All plots were arranged in complete randomized design. Each variety was divided into four replicates. The five studied soybean varieties (Giza 21, Giza 22, Giza 35, Giza111 and Crawford) were sown at 25th May in 2015 season and at the first of June, 2016 to evaluate it against *S. littoralis* under natural infestation. All the tested varieties were exposed to normal field condition without using insecticide during the experimental period. The population densities of *S. littoralis* were estimated.

2. Population densities of cotton leaf worm, *Spodoptera littoralis*:

The population densities of *S. littoralis* on five soybean varieties were counted after 18 days from planting. Sampling of 25 plants taken at random from each plot to calculate the number of larvae and percentage of leaf feeding damage of leaflet area with *S. littoralis* infestation (Figure,1) according to Hunt and Jarvi (2009).

$$\text{Percentage of leaflet area damage} = \frac{\text{Score1}\times\text{No. of leaflets} + \text{Score2}\times\text{No. of leaflets} + \ldots\ldots}{\text{Total No. of infested leaflets}}$$

The rating system uses levels from 1 to 6 where:

Score (1)= few number of pin holes to 10%of leaflet area damage.

Score (2) =upto 20 %leaflet area damage.

Score (3) =21-30% leaflet area damage.

Score (4) =31-40% leaflet area damage.

Score (5) =41-50% leaflet area damage.

Score (6) =more than 50% leaflet area damage.
Soybean Defoliation Levels

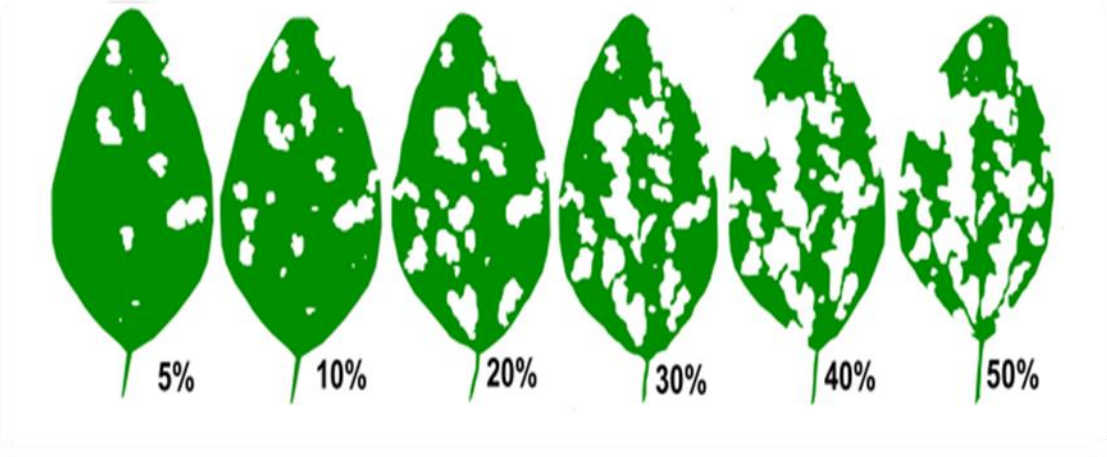

Figure (1): Graphic representations of various levels of soybean leaf defoliation (Hunt and Jarvi, 2009).

3.Phytochemical components:

The relationship between infestation rate of *S. littoralis* and phytochemical components on five soybean varieties through vegetative growth stage during 2015 and 2016 seasons, to determine carbohydrates and total protein contents according to the methods of Pregl (1945) and Michel *et al*. (1956). Also, the amount of total phenolics was determined by Folin-Ciocateu method as modified by Singleton and Rossi (1965). Moreover, inorganic phosphate was determined as described by Rockstein and Herron (1951), potassium was described by Amin and El-Halafawy (2001 and 2002).

4. Anatomical characteristics:

The anatomical characteristics of the tested varieties were studied by using the methods described by **Jackson (1973).** To study the different measurements (in micron mm) of thickness of cuticle layer, thickness of epidermis layer, thickness of central tissue (palisade tissue and spongy tissue), were determined at Botany Department, Faculty of Agriculture Moshtohor, Benha university.

5. Statistical analysis:

The collected data were subjected to proper statistical analysis of (F) test according to **Fisher (1954)**, to compare between means L.S.D at .0.05 level of probability was used according to **Duncan (1955).**

Results and discussion

1. Population fluctuation of *Spodoptera littoralis* infested soybean varieties:

Population density of *S. littoralis* was studied under field condition on five tested soybean varieties (Giza 21, Giza 22, Giza 35, Giza 111 and Crawford) during two successive seasons 2015 and 2016. The regular weekly inspection of the number of larvae/100 plants and rate of leaflets soybean feeding damage on soybean plants were recorded during the whole period of soybean plants from the first inspection (18^{th} June) to the final one (13^{th} August) during the two studied seasons.

1.1. The first season 2015:

Data presented in Table (1) showed the fluctuations in the population densities of *S. littoralis* on five tested soybean varieties during 2015 season. The presence of *S. littoralis* larvae on five varieties extended from the 3^{rd} week of June, up to the 2^{nd} week of August. The highest peak of *S. littoralis* recorded during the 4^{th} week of July (67.5, 57.5, 55, 52.5 and 3735) larvae/100 plants on (Crawford, Giza 111, Giza 22, Giza 35 and Giza 21), respectively. The highest no. of larvae/100 plants recorded with Crawford variety with average of 44.44 larvae/100 plants., while

Giza 21 recorded the lowest number 24.44 larvae/100 plants.

Data in Table (2) summarized the weekly percentage of leaflets soybean feeding damage by *S. littoralis* larvae (in the form of holes) revealed that the highest percentage feeding damage was recorded with Crawford Variety 28.2% followed by Giza 35, Giza 111 and Giza 22 with 25, 25 and 24.4%, respectively. while the Giza 21 was the lowest one recording 23.4% of leaflets soybean feeding damage.

1.2. The second season 2016:

Data in Table (3) revealed approximately (as the first season) by the presence of *S. littoralis* larvae. The highest peak of *S. littoralis* larvae was recorded during the 3rd week of July by 230 larvae/100 plants for Giza 21 variety and 4th week of July 212.5, 207.5, 232.5 and 297.5 for Giza 22, Giza 35, Giza 111 and Crawford, respectively.

At the two studied seasons, (2015 and 2016) the abundance *S. littoralis* larvae was recorded from the beginning of vegetative stage and populations increased dramatically from the late vegetative stage until crop maturity. The highest variety harbouring *S. littoralis* larvae infestation was Crawford with 193.33 larvae/100 plants. Also, the weekly percentage of leaflets soybean feeding damage by *S. littoralis* larvae was summarized in Table (4) the data revealed that the highest percentage feeding damage was in Crawford variety with 16.9% followed by Giza 22, Giza 35 and Giza 111 were 16.5, 16 and 15.8%, respectively. These results agree with Lutfallah *et al*. (2003) evaluated 12 soybean varieties and found that the Crawford variety was highly susceptible to the cotton leaf worm. Saleh (2013) indicated that the Crawford variety was highly susceptible to the cotton leaf worm.

Statistical analysis of the data showed that the interaction of the five tested soybean varieties to infestation with *S. littoralis* larvae and the percentage of leaflets soybean feeding damage during the two seasons 2015and 2016 varied significantly from variety to another.

Table (1): Effect of certain soybean varieties on population density of *Spodoptera littoralis* larvae/100 plants at Moshtohor region, Qalyoubia Governorate during 2015 season.

Investigation dates	Varieties					Temp.		R.H. %
	Giza 21	Giza 22	Giza 35	Giza 111	Crawford	Min.	Max.	
18/6/2015	10±0.40	15±0.28	10±0.61	15±0.28	20±0.40	22.3	39.9	45.5
25/6/2015	15±0.28	25±0.64	20±0.40	25±0.28	30±0.40	21.3	32.2	52.2
2/7/2015	20±0.40	37.5±0.47	27.5±0.47	35±0.28	42.5±0.62	22.3	33.1	59.1
9/7/2015	22.5±0.25	40±0.25	37.5±0.75	40±0.40	50±0.40	23.3	35	53.4
16/7/2015	35±0.64	52.5±0.62	50±0.70	52.5±0.85	62.5±0.85	24.1	37	54.2
23/7/2015	37.5±0.25	55±0.28	52.5±0.62	57.5±1.03	67.5±0.94	25	40.8	35.2
30/7/2015	42.5±0.85	45±1.04	42.5±0.85	45±0.28	57.5±0.75	25.5	38.4	57.8
6/8/2015	22.5±0.47	35±0.64	25±0.64	32.5±0.94	42.5±0.62	24.7	39.9	47.7
13/8/2015	15±0.28	20±0.25	15±0.28	17.5±0.47	27.5±0.47	25.9	36.2	57.6
Total	220	325	280	320	400			
Mean	24.44 d	36.11 b	31.11 c	35.56 b	44.44 a			
F. Value	24.027*							
L.S. D	4.622							

Means followed by the same letter are not significantly at 0.05 DMRT

Table (2): Percentage of leaflets soybean feeding damage in different varieties with *Spodoptera littoralis* larvae at Moshtohor region, Qalyoubia Governorate during 2015 season.

Investigation dates	Varieties				
	Giza 21	Giza 22	Giza 35	Giza 111	Crawford
18/6/2015	10.37	3.62	5	3.62	4.82
25/6/2015	10.37	11.62	3.62	6.87	13.25
2/7/2015	21.62	13.5	21.5	16.75	30.12
9/7/2015	24.25	29.62	29.87	33.12	31.25
16/7/2015	24.87	31	31.65	33.62	33.87
23/7/2015	36.25	32.25	36.75	35	36.75
30/7/2015	29.25	30.5	26.87	29.87	29.87
6/8/2015	30.87	32.62	34.75	35	35.37
13/8/2015	23.5	34.75	35.5	31.75	38.75
Total	211.35	219.48	225.5	225.6	254.05
Mean	23.4 c	24.4 c	25.0 b	25.0 b	28.2 a
F. value	9.608*				
L.S. D	7.785				

Means followed by the same letter are not significantly at 0.05 DMRT

Table (3): Effect of certain soybean Varieties on population density of *Spodoptera littoralis* larvae /100 plants at Moshtohor region, Qalyoubia Governorate during 2016 season.

Investigation dates	Varieties					Temp.		RH %
	Giza 21	Giza 22	Giza 35	Giza 111	Crawford	Min.	Max.	
18/6/2016	42.5±0.41	57.5±0.54	52.5±0.96	57.5±0.41	42.5±0.81	23.4	37.3	51
25/6/2016	87.5±1.02	80±0.61	92.5±1.13	82.5±0.41	102.5±0.96	24.4	39.4	59.9
2/7/2016	105±1.92	122.5± 1.29	117.5±1.47	125±0.90	150±0.61	24.4	36	61.2
9/7/2016	170±0.35	147.5±0.96	165±1.82	157.5±0.96	195±0.90	24.3	36.6	57.2
16/7/2016	230±1.5	172.5±0.89	195±1.82	190±0.79	230±1.27	24.2	37.2	47.1
23/7/2016	177.5±1.88	207.5±0.41	232.5±1.51	222.5±0.89	272.5±1.78	23.8	34.5	55.6
30/7/2016	137.5±1.81	212.5±1.55	207.5±0.81	232.5±1.91	297.5±0.89	24.6	35.9	60.2
6/8/2016	105±1.88	170±1.54	160±1.27	175±1.03	252.5±1.08	23.6	39.5	49.5
13/8/2016	70±1.35	125±1.55	107.5±0.75	127.7±1.49	197.5±0.85	25.2	36.3	62.7
Total	1125	1295	1330	1370.2	1740			
Mean	125.00 d	143.89 c	147.78 bc	152.24 b	193.33 a			
F.value	7.306*							
L.S. D	16.45							

Means followed by the same letter are not significantly at 0.05 DMRT

Table (4): Rate of leaflets soybean feeding damage in different varieties with *Spodoptera littoralis* at Moshtohor region, Qalyoubia Governorate, during 2016 season.

Investigation dates	Varieties				
	Giza 21	**Giza 22**	**Giza 35**	**Giza 111**	**Crawford**
18/6/2015	13.5	13.87	17.1	17	10.6
25/6/2015	11.75	12.5	14.6	10.1	20.6
2/7/2015	18.37	18.25	9.6	14.5	12.5
9/7/2015	18.25	25.25	24.1	20.2	9
16/7/2015	16.12	14.12	16.3	15.3	20.6
23/7/2015	12.87	17.32	15.1	15.3	19.3
30/7/2015	17.62	12.75	17.6	23.2	21.1
6/8/2015	12.62	22	17.1	15.2	24
13/8/2015	9.5	12.75	12.5	11.5	14.2
Total	130.6	148.8	144	142.3	151.9
Mean	14.5 c	16.5 ab	16 b	15.8 b	16.9 a
F. value	2.087*				
L.S. D	7.43				

Means followed by the same letter are not significantly at 0.05 DMRT

2. Correlation of climatic factors with population density of *Spodoptera littoralis* larvae on five varieties:

This study involved the seasonal fluctuation of the investigated pest in relation to certain weekly mean of the weather factors, (maximum temperature, minimum temperature, mean relative humidity (RH %) obtained from Experimental Research Station at Moshtohor, Faculty of Agric, Benha, Univ, Qalyoubia Governorateduring two successive agricultural 2015 and 2016 seasons.

Data in Table (5) showed the simultaneous effect of the two selected weather factors on the population density of *S. littoralis* larvae on five varieties through 2015 and 2016 seasons. The results showed negative correlation between no. of *S. littoralis* larvae in different varieties and maximum temperature in the 2nd season. Also, the mean percentage of relative humidity had insignificant negative effects in the both seasons. Patait *et al.* (2008) reported that the population of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) was influenced positively by forenoon relative humidity and negatively by minimum temperature and afternoon relative humidity.Also, Khan and Talukder (2017) found positive correlation between population of *S. litura* and temperature (maximum and minimum). On the other hand, there was a negative correlation between population of *S. litura* and relative humidity.

Table (5): Correlation values between climatic factors and population density of *Spodoptera littoralis* larvae on five varieties during 2015 and 2016 seasons.

Seasons	Correlation (Temp. and RH %)		Varieties				
			Giza 21	Giza 22	Giza 35	Giza 111	Crawford
2015 season	Mean No. of *S. littoralis*		24.44	36.11	31.11	35.56	44.44
	Min Temp.	Correl.(r)	0.557	0.354	0.372	0.330	0.430
		P	0.119	0.350	0.324	0.386	0.248
	Max Temp.	Correl.(r)	0.364	0.212	0.228	0.237	0.268
		P	0.336	0.584	0.555	0.539	0.485
	RH%	Correl.(r)	-0.114	-0.195	-0.213	-0.257	-0.209
		P	0.771	0.616	0.583	0.505	0.590
2016 season	Mean No. of *S. littoralis*		125	143.89	147.78	152.24	193.33
	Min Temp.	Correl.(r)	0.000	0.085	0.012	0.087	0.200
		P	1.00	0.828	0.975	0.825	0.606
	Max Temp.	Correl.(r)	-0.322	-0.447	-0.449	-0.453	-0.353
		P	0.398	0.228	0.225	0.220	0.352
	RH%	Correl.(r)	-0.348	-0.089	-0.161	-0.102	-0.027
		P	0.359	0.820	0.679	0.795	0.945

P. =Probability Correl (r)=correlation coeffecient

3. Correlation between phytochemical components in the five soybean varieties and mean infestation of *Spodoptera littoralis* larvae:

Data in Table (6) show mean count of *S. littoralis* larvae infested five soybean varieties (Giza 21, Giza 22, Giza 35, Giza 111 and Crawford) at vegetative stage during the second growing season and their relation to the level of certain phytochemical components in the leaves of the concerned varieties, total protein, carbohydrate, total phenolics, phosphate and potassium. Data showed that Crawford variety which had by the highest number of *S. littoralis* 193.3 larvae/ 100 plants contained the highest amount of carbohydrates and total phenols 15.48 and 2.05 mg/100 g**.**, respectively. While it recorded the low amount of total protein, phosphate and potassium were 17.5, 0.39 and 81.5**(**mg/100 g**)**, respectively. On the other hand, leaves of Giza 21 variety which had the lowest seasonal mean number of *S. littoralis* 125 larvae/ 100 plants contained the highest contained of potassium and phosphate 92.6 and 0.51**(**mg/100 g**)**, respectively and had the low amount of total protein 15.75, moderate rate of carbohydrates 15.26 **(**mg/100 g**)**.

Table (6): Correlation between phytochemical components in the five soybean varieties and mean infestation rate of *Spodoptera littoralis* larvae during 2016 season.

	2016 season					
Varieties	**Mean no. of insects**	**Total protein**	**Carbohydrates**	**Total phenolics**	**Phosphate**	**Potassium**
Giza 21	125	15.75 a	15.26 a	2.01 a	0.51 a	92.6 a
Giza 22	143.89	17.5 a	14.04 a	2.03 a	0.42 a	80 a
Giza 35	147.78	19.68 a	14.27 a	1.99 a	0.39 a	91.7 a
Giza 111	152.24	18.81 a	14.94 a	2 a	0.28 a	58.8 a
Crawford	193.3	17.5 a	15.48 a	2.05 a	0.39 a	81.5 a
F. value	7.306*	101.24	8.18	12.74	112.83	3034.6
L.S. D	16.45	5.88	2.44	0.68	0.32	74.2
Correl.(r)		0.251	0.386	0.645	-0.427	-0.245
P		0.684	0.552	0.240	0.473	0.692

4. Correlation between anatomical characters in the five soybean varieties and mean infestation rate of *Spodoptera littoralis* larvae during 2016 seasons:

Correlation between the anatomical characters of five tested soybean varieties and infestation rate with *S. littoralis* are in (Table, 7 and Figure, 2). The correlation between the population density of *S. littoralis* larvae infesting the five tested soybean varieties (Giza 21, Giza 22, Giza 35, Giza 111 and Crawford) and the layers of leaves, i.e. thickness of upper and lower cuticle, upper and lower epidermis, palisade tissue, spongy tissue and No. of phloem and wood in vascular bundle and their correlation coefficient values were studied.

The tabulated data in Table (7) showed the correlation values between the anatomical characters of leave fr five soybeen varities and population of *S. littoralis*. The calculated (r) values were significantly positive fr spngy tissue and number of wood vascular bundle (0.172 and 0.050, respectively). On the other hand, this relation was insignificantly negative in case of cuticle upper and lower; upper and lower epidermis, palisade tissue and phloem vascular bundle as the correlation coefficient values were (-0.581 and -0.695), (-0.396 and -0.642), (-0.268) and (-0.050), respectively.

These results are in a harmony with those obtained by Nautiyal *et al.* (2015) found a negative significant correlation was noticed between leaf thickness and per cent leaf damage of soybean.

Table (7): Correlation values between anatomical characters in five soybean varieties and mean infestation rate of *Spodoptera littoralis* larvae during 2016 season.

varieties	**Mean No. of *S. littoralis***	**Cuticle**		**Epidermis in**		**Palisade tissue**	**Spongy tissue**	**Vascular**	
		Upper	**Lower**	**Upper**	**Lower**			**phloem**	**wood**
Giza 21	125	9.90	8.10	16.20	16.20	58.50	81.90	90.00	365.4
Giza 22	143.89	8.10	6.30	27.00	22.50	87.30	103.50	85.50	283.50
Giza 35	147.78	6.30	5.40	18.00	13.50	58.50	63.00	141.30	369.00
Giza 111	152.24	8.10	5.40	22.50	14.40	85.50	58.50	40.50	211.50
Crawford	193.3	7.20	5.40	13.50	9.90	54.00	90.00	90.00	356.40
Correl.(r)		-0.581	-0.695	-0.396	-0.642	-0.268	0.172	-0.053	0.050
P		0.305	0.193	0.510	0.243	0.663	0.829	0.932	0.936

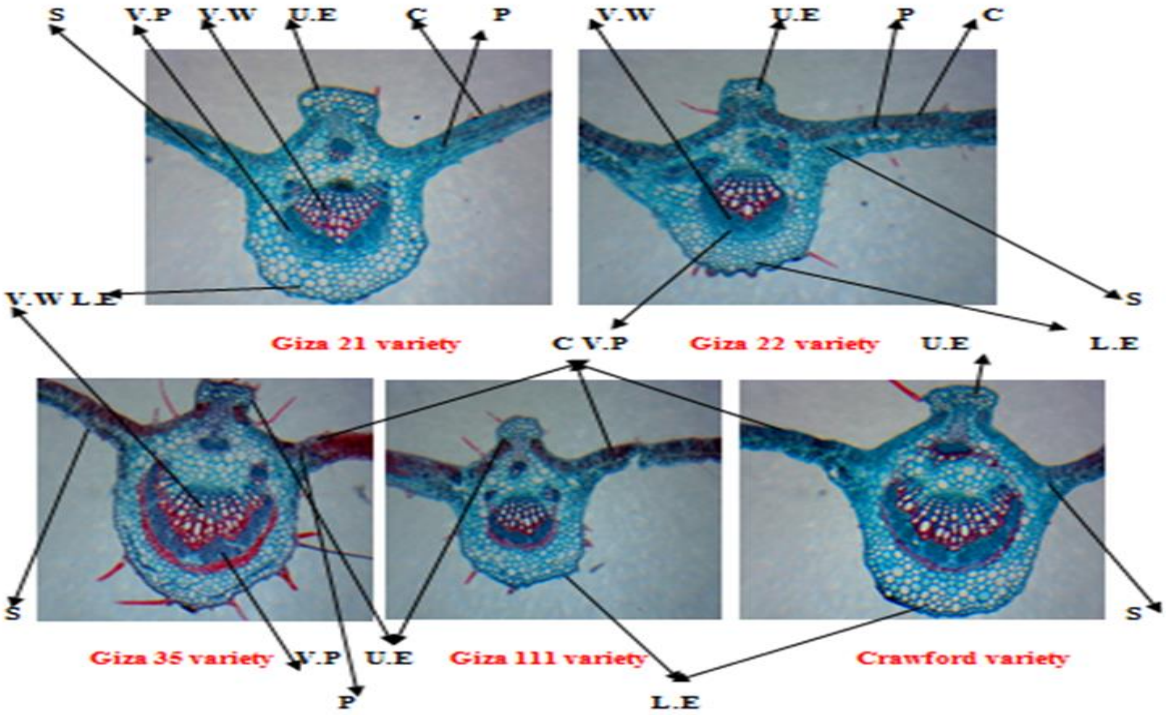

Figure (2): Anatomical characters of tested soybean varieties.
C= Cuticle U. E= Upper Epidermis L.E= Lower Epidermis in micron
P= Palisade tissue S= Spongy tissueV.P= Vascular bundle (Phloem)
V.W= Vascular bundle (Wood)

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