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Some Physio Morphological Leaf Characters of Gamma Irradiated Cotton Lines to Resistance against Jassid (*Amrasca Devastans* Dist.)

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Abstract

The field trial was conducted to determine the effects of Physio-morphological characters expressed on leaves by application of gamma rays in cotton lines at different doses as compared of their parent lines which creating resistance against jassid, *Amrasca devastans*. The cotton line SB* (250 Gy) was found highly resistance. Whereas, cotton line SP* (150 Gy) was attained highly susceptible against jassid populations during 2008 and 2009, respectively. The trichomes density was showed highly significant and negative effects on jassid population in parent and gamma irradiated 150 Gy lines, whereas, non-significant and negative correlation in irradiated 200 and 250 Gy cotton lines. While, trichomes size was found non-significant and negative correlation with jassid populations in all parents and their irradiated with 150, 200 and 250 Gy during 2008 and 2009, respectively; except the trichomes size that had a significant and negative correlation on parent and 250 Gy cotton lines during 2009. However the total phenol concentration in plant leaves showed a significant and positive relation in parent and gamma irradiated 150 Gy cotton lines, whereas, non-significant and positive correlation with pest population in irradiated with 200 Gy and 250 Gy cotton lines during 2009, respectively. The linear regression results showed that increased trichomes density and size had decreased jassid population. Though, jassid population increased with increasing total phenol concentrations in cotton leaves. The plant leaves parameters showed best results in SB irradiated with 250 Gy to host plant resistance against jassid.

Keywords: Jassid, Trichomes, Resistance, Total Phenol, Gamma irradiation.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is an attractive food for the insects during the growing season, and about 96 species of insects and mites are recorded as the major destructive to the crop [1]. The jassid, *Amrasca devastans* (Dist.) is considered the most noxious pest of the cotton and continuously disturbing plant phenology directly and the yield losses indirectly [2]. However, [3, 4] reported that the 5-45% yield losses are caused by sucking complex. The usage of improper and non-selective insecticides causes resistance in insects and also creates environmental pollution and health hazards for human beings too [5-9]. There are common trends about usage of synthetic chemicals for pest control are noticed among the cotton growers of the Sindh region that growers are essentially spraying these insecticides to their crops without pest scouting and could not wait until the pest is touching economic injury level, not even caring about timing and dose [10]. The host plant resistance methodology is the only hope to minimize pest pressure on cotton crop. The preference and non-preferences of food depends on insects feeding behavior, responding the Physio-morphological plant characters [11]. The plant morphological and physiological characters are attributed as a plant defense mechanism against herbivore [12]. Physio-morphological changes are increasing host plant resistance against herbivore insects [13]. Jassid, *A. devastans* is a major pest of cotton, which feeds on vegetative growth from the seedling to the maturity of the crop. It injects toxic material inside the leaves and sucking the cell sap from plants [14]. The plants are getting dry, the leaves are turned downwards and finally it dies during heavy infestations of jassid which is responsible for reducing growth and yield losses [15]. Host plant resistance/susceptibility depends on the trichomes density on cotton leaves; jassid avoids reproducing and feeding on highly dense and elongated trichomes at the lower side of leaf [16-19]. Minimum density of trichomes on cotton leaves is encouraging factor for the jassid offspring's [20]. The plant secondary metabolites and trichomes plays vital role in the cotton resistance mechanism [21]. However, [22-24] were recorded jassid population were significant and negatively correlated

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with phenol concentration in different cotton genotypes. While, [25, 26] have founded phenol positively correlated with jassid populations in cotton. The purpose of this study is to find out host plant resistance against jassid infestation. The emphasis would be on developing alternate methods of pest control such as plant leaf trichomes and total phenol in the leaves which are responsible for reducing the infestation of noxious pest in cotton and to save natural ecosystem.

2. Materials and Methods

The seeds of three parent cotton lines viz; SB, SP & B-4 were irradiated (150, 200, and 250 Grays "Gy") and mutation results in the new mutant cotton lines viz SB*, SP* & B-4* were made twelve lines total in the number were studied in Cotton Research Section, Agriculture Research Institute, Tandojam Sindh, Pakistan during the year 2008 and 2009. The parent seeds were treated with Cobalt-60 irradiation at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad. The experiment was laid out in randomized complete block design (RCBD) with four replications. Each replication was comprised in four rows by keeping the distances of 30 cm and 75 cm in between plant to plant and row to row respectively, were sown on 15th May 2008 and 17th May 2009. The effects of gamma irradiation were studied for relative resistance as compared to untreated (parent) against jassid, at M4 and M5 generations. All the recommended agronomic practices were completed within time.

Screening of Jassid, *Amrasca devastans* (Dist.) population

The observation of jassid population (nymph and adult collectively) were recorded after 25 days of crop sowing, five plants per cotton line and five leaves per plant from each mutant and parent lines were randomly selected for counting the jassid population (nymph and adult) were seen from lower, middle and top leaves of plants and the resistance level with regard to the population of jassid was assessed according to suggested by [27] i.e. 0.1-1.0 highly resistance, 1.1-2.0 moderately resistance, 2.1-3.0 susceptible and 3.1-4.0 highly susceptible.

Trichome density measurement

Trichome density and interaction of insect population were determined in all parents and in all gammas treated lines. For the preference and non-preferences by jassid Fifteen fully expanded uniformly sized leaves per cotton line of all lines were collected from the peak blooming staged field grown plants in the laboratory and counting numbers of trichomes density/cm² of leaf was carried out by using one cm² stopper cutter/borer to punch in a fixed area at one side of the midrib and the stopper was used for tracing on the leaf then within the one cm² the number of trichomes were counted. The process of counting trichomes was done under the microscope with the aid of 10x lens and objective on microscope 10/0.25-160/0.17 Kyowa optical Co. Ltd. Japan. Ten trichomes were selected for size measurement from the midrib of the central portion of the leaf blade. Size of trichomes was measured on Microscope (Nikon Alphaphot, Ys, Japan) by ocular micrometer in micron on 5x eyepiece and objective then converted in (mm) millimeter.

Determination of total phenol content

The total phenol content was determined with some modifications on spectrophotometer by using the Folin-Ciocalteu assay by following the methods [28, 29]. Four dilutions were used 0.1, 0.5, 1.0 and 2.0 ml for calibration curve. Plant

sample consisting of 200 mg freeze-dried of the crude extract was mixed with Folin-Ciocalteu reagent (2.0 ml) and 1.25 ml of sodium carbonate solution (5%) was added [30]. The required quantity (10 ml) was measured in a volumetric flask of each sample; it was fulfilled by adding with acetone: methanol (7:3) ratio made up to 10 ml mark on volumetric flask. The material was well mixed and kept at ambient temperature (~25 °C) for 1 hour in dark. The absorbance values of the samples were measured at 750 nm against a reagent blank (except plant material) [31]. The UV/Visible spectrophotometer (Perkin Elmer Lambda 35, USA), phenol standards was used for calibration of the curve ($r^2 = 0.9986$). The total phenol of the extracts was stated as pure phenol equivalent to 1 mg/100 g of dry weight.

Data Analysis

To find out the significant differences within the cotton lines and means was compared with LSD test at 0.05% probability. Simple correlation and linear regression models were worked out amongst jassid population and leaf morphological and chemical characters. The data was analyzed statistically general (ANOVA) by using statistix software 8.1.

3. Results

3.1 Jassid Population

The results in Table 1.0 indicate that jassid population was significant ($P < 0.05$) among different cotton lines. The significantly and non-significantly differences ($F=25075.6$; $df=2$; $P < 0.0000$) and ($F=4675.64$; $df=2$; $P < 0.0000$) were generated on the general analysis of variance during 2008 and 2009, respectively. The results revealed that SB* (250 Gy) cotton line was found highly resistance during 2008 and 2009, respectively. However, moderately resistance against jassid was observed in parent cotton lines SB and B-4 followed by SB* and B-4* (150 Gy), SB* and SP* (200 Gy) and SP* and B-4* (250 Gy) respectively. Whereas, SP* (150 Gy) was found highly susceptible cotton line against jassid population during 2008 and 2009, respectively.

3.2 Trichomes Density

There were significant ($P < 0.05$) differences in trichomes density amongst untreated and in gamma irradiated cotton lines ($F=36716.0$; $df=2$; $P < 0.0000$) and ($F=30188.5$; $df=2$; $P < 0.0000$) during 2008 and 2009, respectively. Table 2.0 shows that maximum number of trichome under leaf surface per cm² was found (478.14 ± 0.608 /leaf and 473.4 ± 1.58 /leaf) in SB* (250 Gy) and minimum number was found (39.3 ± 0.433 /leaf and 40.60 ± 0.395 /leaf) in SP* (150 Gy) during both study years respectively.

3.3 Trichomes size

Table 3.0 indicates significant ($P < 0.05$) differences amongst the parent lines SB, SP and B-4 and in different gamma irradiated i.e. 150, 200 and 250 Gy cotton lines ($F=45.97$; $df=2$; $P < 0.0000$) and ($F=42.99$; $df=2$; $P < 0.0000$) during 2008 and 2009, respectively. However, there were maximum trichomes sizes observed in SB* (250 Gy) found highly resistant one against jassid and minimum size was recorded in SP* (150 Gy) which was highly susceptible cotton line against pest infestation during 2008 and 2009, respectively.

3.4 Total phenol concentration in leaves of parents and gamma irradiated cotton lines

The results in Table 4.0 represent significant differences in total phenol concentration in dried leaf powder of three parents

and their gamma irradiated cotton lines ($F=17922.6$; $df=2$; $P<0.0000$). The maximum phenol concentration (45.632 ± 0.03) was calculated in highly susceptible cotton line SP* (150 Gy)

and minimum content (11.930 ± 0.018) was observed in highly susceptible against jassid SB* (250 Gy).

Table 1: Jassid *A. devastans* population on parents and gamma irradiated cotton lines during 2008-2009

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	1.711 \pm 0.135 f	1.734 \pm 0.172 e	1.512 \pm 0.175 g	0.946 \pm 0.074 k	1.744 \pm 0.137 e	1.728 \pm 0.147 e	1.558 \pm 0.178 f	0.949 \pm 0.076 j
SP	2.156 \pm 0.214 c	4.017 \pm 0.631 a	1.972 \pm 0.205 d	1.304 \pm 0.081 h	2.119 \pm 0.216 c	4.228 \pm 0.56 a	1.994 \pm 0.172 d	1.247 \pm 0.086 g
B-4	1.037 \pm 0.1 i	1.005 \pm 0.093 j	2.489 \pm 0.315 b	1.025 \pm 0.086 i	1.091 \pm 0.1 h	1.06 \pm 0.085 h	2.636 \pm 0.254 b	1.008 \pm 0.081 i

Mean \pm S.E followed by same letters are not significantly different from each other, ($P<0.05$; LSD)

Table 2: Trichomes Density in Parent and Gamma Irradiated Cotton Line during 2008 and 2009

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	263.97 \pm 1.568 g	237.4 \pm 0.512 h	325.7 \pm 0.382 f	478.14 \pm 0.608 a	256.09 \pm 0.561 g	236.68 \pm 0.445 h	326.35 \pm 0.359 f	473.4 \pm 1.58 a
SP	154.88 \pm 0.59 j	39.3 \pm 0.433 l	165.4 \pm 1.151 i	336.08 \pm 2.28 e	156.24 \pm 1.358 i	40.60 \pm 0.395 l	150.81 \pm 0.632 j	334.63 \pm 2.047 e
B-4	427.58 \pm 1.498 d	457.74 \pm 0.717 b	130.19 \pm 0.279 k	443.39 \pm 0.383 c	432.07 \pm 2.079 d	455.17 \pm 0.996 b	129.77 \pm 1.388 k	445.03 \pm 0.173 c

Mean \pm S.E followed by same letters are not significantly different from each other, ($P<0.05$; LSD)

Table 3: Trichomes size (in mm) Parent and Gamma Irradiated Cotton Line during 2008-09

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	0.281 \pm 9.511E-03 de	0.266 \pm 5.626E-03 ef	0.293 \pm 6.626E-03 cd	0.328 \pm 4.041E-03 a	0.281 \pm 5.677E-03 de	0.265 \pm 6.380E-03 ef	0.287 \pm 5.941E-03 cd	0.327 \pm 8.893E-03 a
SP	0.253 \pm 7.765E-03 fg	0.198 \pm 3.860E-03 h	0.259 \pm 5.159E-03 fg	0.306 \pm 8.480E-03 bc	0.257 \pm 5.227E-03 f	0.197 \pm 5.443E-03 h	0.253 \pm 8.394E-03 fg	0.305 \pm 7.140E-03 bc
B-4	0.311 \pm 9.708E-03 abc	0.318 \pm 6.396E-03 ab	0.244 \pm 4.531E-03 g	0.318 \pm 6.396E-03 ab	0.321 \pm 6.635E-03 ab	0.313 \pm 8.496E-03 ab	0.233 \pm 7.130E-03 g	0.324 \pm 8.266E-03 ab

Mean \pm S.E followed by same letters are not significantly different from each other, ($P<0.05$; LSD)

Table 4: Total phenol concentration (mg/g) in leaves of untreated and their gamma treated cotton lines during 2009

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
SB	19.368 \pm 0.03 f	21.789 \pm 0.03 e	16.895 \pm 0.03 m	11.930 \pm 0.018 j
SP	22.947 \pm 0.03 d	45.632 \pm 0.03 a	22.737 \pm 0.03 g	40.175 \pm 0.462 b
B-4	13.211 \pm 0.03 h	13.105 \pm 0.03 h	26.895 \pm 0.03 e	12.561 \pm 0.046 i

Mean \pm S.E followed by same letters are not significantly different from each other, ($P<0.05$; LSD)

3.5 Correlation of *A. devastans* population with trichomes density and their size

As represented in Table 2.0 and 3.0 *A. devastans* population was highly significant and negatively correlated with the trichomes density in all parents and gamma irradiated cotton lines, while non-significant and negative relation was observed in irradiated 150 and 200 Gy cotton lines during 2008 and 2009. Whereas trichomes size was found non-significant and negative correlation in all parent lines and irradiated with 150, 200 and 250 Gy during 2008 and 2009, respectively. Except the trichomes size had a significant and negative correlation in parent and 250 Gy cotton lines during 2009, respectively. However, the total phenol concentration in plant leaves showed a positive and significant relation in parents and gamma irradiated 150 Gy cotton lines, whereas, non-significant and positive correlation with pest population in irradiated with 200 Gy and 250 Gy cotton lines during 2009,

respectively. The liner regression results Table 5.0-7.0 showed that increased trichomes density and size had decreased jassid population. Though, jassid population increased with total phenol concentrations in cotton leaves. The trichomes density and its size at the midrib of leaves had significant and non-significant impact on jassid population. In the view of the above results, it was marked that hair density and size at midrib of the leaves have an important resistance factor against jassid. Whereas, total phenol had no any influence in resistance development against jassid. The further study on trichomes density and size was revealed that cotton lines having maximum number of trichomes density and size had a role in keeping jassid, *A. devastans* population lessen as compared to those that having less trichomes density and size. While the highest concentration of total phenol present in leaves has no toxic effect on jassid population and reproduction.

Table 5: Pearson's correlation coefficients and the Liner regression r^2 value, among trichomes density with jassid population on parents and their three gammas irradiated cotton lines in field trial during 2008 and 2009

Parameter	r- Value	P- Value	Linear Regression		R ²
			y	x	
2008					
Trichomes Density vs Jassid on Parents	-1.000	0.002	2.792	-0.004	1.000
Trichomes Density vs Jassid on Dose-150	-0.949	0.204	3.996	-0.007	0.901
Trichomes Density vs Jassid on Dose-200	-0.926	0.247	2.89	-0.004	0.857
Trichomes Density vs Jassid on Dose-250	-0.999	0.016	2.156	-0.003	0.999
2009					
Trichomes Density vs Jassid on Parents	-1.000	0.002	2.699	-0.004	1.000
Trichomes Density vs Jassid on Dose-150	-0.938	0.225	4.183	-0.008	0.88
Trichomes Density vs Jassid Population on Dose-200	-0.859	0.341	2.936	-0.004	0.739
Trichomes Density vs Jassid on Dose-250	-1.000	0.004	1.967	-0.002	1.000

Table 6: Pearson's correlation coefficients and the Liner regression r^2 value, among trichomes size with jassid population on parents and their three gammas irradiated cotton lines in field trial during 2008 and 2009

Parameter	r- Value	P- Value	Linear Regression		R ²
			y	x	
2008					
Trichomes Size vs Jassid on Parents	-0.995	0.062	7.079	-19.331	0.991
Trichomes Size vs Jassid on Dose-150	-0.978	0.135	8.905	-25.524	0.955
Trichomes Size vs Jassid on Dose-200	-0.968	0.162	6.991	-18.844	0.937
Trichomes Size vs Jassid on Dose-250	-0.967	0.165	6.329	-16.503	0.934
2009					
Trichomes Size vs Jassid on Parents	-0.999	0.007	6.258	-16.089	0.999
Trichomes Size vs Jassid on Dose-150	-0.975	0.142	9.557	-27.941	0.951
Trichomes Size vs Jassid on Dose-200	-0.967	0.165	7.011	-19.203	0.935
Trichomes Size vs Jassid on Dose-250	-0.998	0.039	5.275	-13.201	0.996

Table 7: Pearson's correlation coefficients and the Liner regression r^2 value, among total phenol in leaves with jassid population on parents and gamma irradiated cotton lines in field trial during 2009

Parameter	r- Value	P- Value	Linear Regression		R ²
			y	x	
2009					
Total Phenol in leaves vs jassid on Parents	0.977	0.138	0.246	0.042	0.954
Total Phenol in leaves vs jassid on Dose-150	0.997	0.051	-1.429	0.142	0.994
Total Phenol in leaves vs jassid on Dose-200	0.919	0.259	-1.996	0.234	0.844
Total Phenol in leaves vs jassid on Dose-250	0.603	0.588	0.163	0.056	0.363

4. Discussion

Some plant morphological characteristics of the cotton deterred to jassid reproduction. However, the highly susceptible cotton mutant line SP* (150 Gy) having the lowest number of trichomes size and density on the lower side of the leaf surface found attractant to jassid for feeding and ovipositing. Relatively lowest jassid population against the maximum number of trichomes and size was recorded in mutant line SB* (250 Gy) found highly resistant against jassid, in the present study's results are in an agreement with Yousif and Ahmed [32], was reported that the cotton variety AU-14 with the highest mean number of trichomes 221 per cm on leaf

vein showed relatively resistant to cotton jassid. [33, 19] also reported that the trichomes density, antigenosis and antibiosis features were shown resistance and susceptibility against jassid. Slaman *et al.* [34], were recorded moderately resistance in NIAB Karishma and CIM-482 cotton cultivars against jassid infestation. The present results on trichomes size have an agreement with Khan and Agarwal [35], who had reported that hair length under surface leaves of *G. Hirsutum* varieties are longer than ovipositor of jassid, while hair length has negatively affected on the egg laying performance of jassids. The results of correlation studies of the jassid population with trichomes density and size are in full agreement with those of Ali *et al.* [18], Bashir *et al.* [36], Ahmed *et al.* [37], Ashfaq *et al.* [33], Murugesan and Kavitha [19], Naveed *et al.* [38], Ullah *et al.* [39], and Rustamani *et al.* [40] who recorded that the jassid population should be managed with highly and moderately trichomes density varieties, however, these varieties result in highly significant and negatively correlated with jassid populations. The linear regression revealed that jassid population was decreased with highly trichomes density and size, while it was increased with the lowest density and size. Bhatti *et al.* [41] reported that the trichomes density on the midrib of cotton leaf was an important feature for host plant resistance.

The results of correlation studies of total phenol concentration found non-significant and positive effects on jassid population in parent and their gamma irradiated 150, 200 and 250 Gy lines. The finding of present experiment is fully in agreement with those of Sharma and Agarwal [42], Singh and Agarwal [26] who found the phenol positively correlated with jassid populations in cotton. Chakravarthy *et al.* [43] who reported that the plant chemical total phenol in leaves had no effect on pest during vegetative and reproductive growth phase of the cotton crop. Hosagoudar and Chattannavar [44] had evaluated the total phenols in cotton lines and find out at 90 and 120 days cotton lines were decreased in total phenol found affected over healthy line. Though Mohamed *et al.* [45] had mentioned high concentration of phenol has non-significant effect on jassid population in cotton. Our result findings have not agreements with those of Balakrishnan [46] reported total phenol concentration in cotton leaves has significant negative effects on jassid populations, and with Venkatesha [23] and Shinde *et al.* [24] who was recorded jassid population significantly and negatively correlated with phenol concentrations on different cotton genotypes. The linear regression analysis revealed that increased jassid population with increasing total phenol concentration in cotton leaves.

5. Conclusion

It can be concluded from the study that maximum trichomes density and size was observed in SB* cotton line (250 Gy)

compared with a parents cotton line which was found better in reducing the population of jassids, *A. devastans* (Dist.). However, the plant chemical total phenol was not efficient to decreased pest population.

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