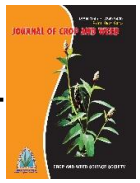
Journal of Crop and Weed, 19(3):31-36(2023)

ISSN-O : 2349 9400; P : 0974 6315



https://www.cwss.in
www.cropandweed.com

Research Article

Study on Mendelian segregation pattern of Cry1Ac gene variability and association in UASD Cry1Ac transgenic Event No. 78 based F_2 population in cotton

***N. SHILPA, [1]M. S. MARALAPPANAVAR, L. GANGAVATI AND S. S. PATIL**

Department of Genetics and Plant Breeding, [2]Agriculture Research Station, University of Agricultural Sciences, Dharwad-580005, Karnataka

Received: 29.03.2023; Revised: 05.09.2023; Accepted: 10.10.2023

DOI: https://doi.org/10.22271/09746315.2023.v19.i3.1737

ABSTRACT

During 2019–2020 at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, India, the current study sought to understand the pattern of Cry1Ac gene segregation, variability, and association study among various morphological and quantitative traits in non–Bt and Bt plants of segregating F_2 generation cotton (Gossypium hirsutum L.).The Cry1Ac gene behaved in the F2 population in a 3:1 Mendelian segregation pattern, according to the results of a chi-square test. Average boll weight, number of bolls, and seed cotton production per plant are features that showed high GCV, PCV, heritability, and genetic progress. The average boll weight, the number of bolls per plant, and the number of good opened bolls per plant all showed favourable correlations with seed cotton yield. In both the Bt and non-Bt plant groups, the number of bolls per plant and average boll weight were indirectly favourably influenced by seed cotton yield.

***Keywords*:** Bt Cotton, correlation, Cry1Ac gene, Mendelian segregation, path analysis and variability

Due to its widespread use in agriculture and the industrial sector, cotton, the "King of Fiber Crops," is renowned for its desirable qualities and is referred to as "white gold." Cotton is a significant cash crop in India where it accounts for 75% of all fibre consumption and 58% of the textile industry. It also directly supports the livelihoods of 6 million farmers and employs 40–50 million people in the trade and processing of cotton. Due to its broad pesticide tolerance, multivoltine nature, prolific feeding pattern, and polyphagous nature, *Helicoverpa armigera* is the most prevalent and challenging to control pest in the Indian cotton ecosystem. Farmers mostly relied on chemical pesticides to combat bollworms because genetic resistance, one of the key pest management measures, is not present in the cotton gene pool. Through the use of genetic engineering techniques, a substitute method of controlling insect pests in cotton has been created (Bt cotton). By incorporating Bacillus thuringiensis genes, Bt cotton is created. The three insecticidal toxin types produced by B. thuringiensis strains are crystal (Cry) toxin, cytolytic (Cyt), and vegetatively expressed insecticidal proteins (VIP).There are currently 74 Cry gene families with 770 distinct Cry genes, three Cyt gene families with 38 Cyt genes, and 139 Vip gene families. Only a few of the 67 transgenic events currently in existence—namely, COT67B, Event 1, GFM Cy1A, GHB119, MON531, MON15985, and MON1076—have gained widespread popularity (ISAAA, 2020). Monsanto 531 event has been exploited by public sector institutions like Punjab Agricultural University (PAU) to create Bt cotton cultivars. A number of Bt varieties and hybrids have also been created by ICAR, including CICR Bt-6 (RS 2013), GJHV 374 Bt, PKV 081 Bt, Rajat Bt, Suraj Bt, Bt 9, Bt 14 (CPT 2), and PAU Bt 1.The University of Agricultural Sciences, Dharwad has created a brand-new public sector event called UASD Cry1Ac transgenic Event No.78. The Cry1Ac gene from ICGEB, New Delhi, was used to create the event, which has been proven to be substantially more effective than Mon BG-II for Cry toxin expression. The occurrence is present in the genetic makeup of the *Gossypium hirsutum* L. cultivar RAH-100, a released variety.

**Email: shilpanmurthy100@gamil.com*

How to cite: Shilpa, N., Maralappanavar, M.S., Gangavati, L. and Patil, S.S. 2023. Study on Mendelian segregation pattern of Cry1Ac gene variability and association in UASD Cry1Ac transgenic Event No. 78 based F_2 population in cotton. *J. Crop and Weed*, 19(3): 31-36.

To successfully utilize transgenic technology for agricultural enhancement, the research on transgene inheritance in following progeny generations is of utmost importance. In light of this, the current study was conducted to evaluate the pattern of Cry1Ac gene inheritance in the F_2 population of the cross DSH-114 UASD Cry1Ac transgenic Event No. 78.

MATERIALS AND METHODS

An experiment was carried out at the University of Agricultural Sciences, Dharwad's Agricultural Research Station, Hebballi, during kharif season of 2019 and 2020. Through the use of an ELISA assay, the Cry1Ac gene segregation pattern in the F_2 population of the cross DHS-114 UASD Event No.78 was determined. In the present investigation, each individual F_2 plant of cross DHS 114 × UASD Event No. 78 was subjected to detection and qualitative assessment of Cry1Ac gene using ELISA QualiPlate kit following the protocol mentioned below-

i. Two to three young leaf discs from each of the plants at 60 days after sowing were collected in an Eppendorf tube.
ii. The collected samples were added with 800µl of extraction buffer and homogenized using a bead crusher.
iii. The tissue extract was centrifuged in a chilled centrifuge for 10 minutes at 10,000 rpm, and the supernatant was then put into the wells of the ELISA plate.
iv. The sample-loaded ELISA plate was mixed thoroughly in a circular motion for 20-30 sec. and covered with parafilm and incubated for 45 min.
v. The sample extracts were discarded and 100 µl of Cry1Ac enzyme conjugate was added in all the wells and mixed in a circular motion for 20-30 sec.
vi. Fresh parafilm was applied to the plate, which was then incubated for an additional 45 minutes at room temperature.
vii. After 1 hour of incubation, the cover was removed and the wells were flooded with wash buffer after vigorous shaking. The wash buffered plate was shaken and emptied, and the process of washing was repeated another three times.
viii. To the plate, about 100 µl of the substrate was added and the contents were mixed in a circular motion. The plate was covered with parafilm and incubated for 30 min
ix. The results of the assay were visualized with a color development (blue color) which indicated the presence of the Cry1Ac gene.

The Chi-square test was used to determine the degree of fit. In the current work, analysis was carried out to compare observed frequency to expected frequency in the segregation of the Cry1Ac gene in the F_2 population, and to determine the significance of the difference (Gomez *and Gomez,* 1984).

The F_2 population was divided into two groups, containing Bt and non-Bt plants, based on the qualitative assessment. In the F_2 population of the cross DHS-114 Event No. 78, the estimations of mean, range, variation, heritability, and genetic advance over mean were independently calculated for Bt and non-Bt plant groups.

In accordance with the formula provided by Weber and Moothy, the correlation study was conducted on Bt and non-Bt plant groups (1952). According to Wright, path co-efficient analysis was carried out on Bt and non-Bt plant groups to determine the direct and indirect effects of component qualities on yield (1921). The statistical programme WINDOSTAT was used for the analysis.

RESULTS AND DISCUSSION

To understand the stability of the gene altered, the inheritance of transgene in a transgenic event has been studied. There were 227 plants in the F_2 population. One hundred fifty-nine plants were identified by the ELISA assay as being positive for Cry1Ac protein expression, while 68 plants were identified as being negative and lacking the transgene (Fig. 1). The ratio of the 68 Cry1Ac negative plants to the 159 positive plants for Cry1Ac was 2.8:1.2. According to the Chi-square test, the calculated chi-square value was 2.94, which was lower than the computed chi-square value in the table, which was 3.84.This demonstrated that the observed data's departure from the predicted frequencies was entirely attributable to chance, and the difference was not statistically significant (Table 1). In the F_2 population, the Cry1Ac gene displayed a 3:1 Mendelian pattern of segregation. Figure 1 shows how the ELISA data were obtained. The Mendelian segregation of 3:1 was validated by Rashid *et al*. (2008) by ELISA investigation on the transmission of the Cry1Ac gene in transgenic cotton. In a similar vein, Hussain *et al*. (2014) revealed that the progeny population of their independently created transgenic lines had a Mendelian pattern of inheritance for Cry1Ac. Regarding the inheritance and expression stability of foreign genes in transgenic crops, there are numerous further publications. In transgenic plants, Mendelian inheritance of foreign genes has been seen in cotton, maize, and rice (Duan *et al*., 1996; Fearing *et al*., 1997; Canming *et al*., 2000).

High PCV and GCV data were used to compute plant height, number of sympodia per plant, number of bolls per plant, average boll weight, and seed cotton yield per plant. Plant height, sympodia per plant, bolls per plant, average boll weight, and seed cotton production

showed moderate to high heritability and genetic advance over mean in both Bt and non-Bt plant groups (Table 2, 3). In comparison to the non-Bt group, the Bt group had an average of 0.05 fewer improperly opened bolls per plant. (3.0). There was a large difference in seed cotton production, good opened bolls per plant, and bad opened bolls per plant between the Bt and non-Bt groups (Table 4). The Bt gene, which provides resistance to *H. armigera*, is thought to be one requirement for the creation of a productive genotype based on the findings of the current study. The study comparing Bt and non-Bt plant groups showed that the Bt gene's presence had no effect on other yield-attributing features since the F_2 population segregates for all of the loci that affect yield. In order to create possible Bt varieties and hybrids, efforts must be made to combine all the desirable traits in later generations along with the Cry1Ac gene.

Table 1: Chi-square (χ^2) test for the segregation pattern of Cry1Ac gene in F_2 population of cross DHS-114 × Event No.78

Sample	Expected frequency	Observed frequency	Calculated χ^2	Table χ^2 at 0.05	Result
Positive plants	170.25	159	2.97	3.84	Non-significant
Negative plants	56.75	68			

Table 2: Genetic variability parameters of Bt positive plants in F_2 population of cross between DHS-114 × Event No.78

Character	Bt plants (159 plants)							
	Mean	Range	Variance	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
Days to 50% flowering	119.0	106-153	86.2	7.3	5.0	47.3	8.9	7.1
Days to boll opening	161.0	141-188	87.6	5.7	4.3	55.8	10.6	6.6
Plant height (cm)	75.3	32-143	506.6	29.8	27.0	81.7	37.8	50.2
Monopodia plant^{-1}	1.3	0-5	0.8	70.2	61.2	76.0	1.4	110.0
Sympodia plant^{-1}	17.6	6-30	20.4	25.6	20.2	62.5	5.8	32.9
Bolls plant^{-1}	9.7	1-30	33.1	59.2	51.3	75.2	8.9	91.8
Average boll weight (g)	3.4	2.1-6	0.8	26.5	18.3	47.7	0.9	26.1
Seed cotton yield plant^{-1} (g)	29.8	2-115	479.6	73.3	64.3	77.0	34.7	116.4
Good opened bolls plant^{-1}	8.8	1-28	25.9	57.5	52.9	84.6	8.8	10.4
Bad opened bolls plant^{-1}	0.05	1-6	1.0	116.2	110.5	93.5	1.9	216.7

Table 3: Genetic variability parameters of non Bt plants in F_2 population of cross DHS-114 × Event No.78

Character	Non Bt plants (68 plants)							
	Mean	Range	Variance	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
Days to 50% flowering	126.0	106-149	133.0	9.1	7.4	65.8	15.6	12.4
Days to boll opening	157.2	139-184	135.8	7.2	6.1	71.9	17.2	10.7
Plant height (cm)	74.7	34-120	425.6	27.5	24.4	78.2	33.2	44.4
Monopodia plant^{-1}	1.3	0-5	1.0	74.6	66.6	79.5	1.6	122.4
Sympodia plant^{-1}	17.4	5-28	17.4	23.9	17.9	56.1	4.8	27.6
Bolls plant^{-1}	10.2	1-29	25.0	49.0	40.2	67.2	6.9	68.0
Average boll weight (g)	3.3	1.9-6.8	0.9	29.6	21.9	54.7	1.1	33.4
Seed cotton yield plant^{-1} (g)	29.9	2.0-86.9	421.6	68.4	58.8	73.9	31.2	104.2
Good opened bolls plant^{-1}	7.1	1-22	14.4	52.8	44.9	72.4	5.6	78.8
Bad opened bolls plant^{-1}	3.0	0-7	2.1	49.0	47.8	95.4	2.9	96.3

Table 4: Comparison of the average yield and yield-related features of Bt and non-Bt plants

Characters	Bt positive (159 plants)	Bt negative (68 plants)	t test
Number of bolls per plant	9.7	10.2	0.41
Average boll weight (g)	3.4	3.3	1.06
Seed cotton yield per plant (g plant^{-1})	29.8	29.9	1.97*
Good opened bolls per plant	8.8	7.1	4.09*
Bad opened bolls per plant	0.05	3.0	23.6*

*Note: *Significance at 5 per cent*

Correlation analysis was used to determine the nature and degree of the association between different component properties and seed cotton production. The number of sympodia per plant, the number of bolls per plant, the average weight of a boll, the number of well-opened bolls per plant, and the amount of seed cotton produced were all positively correlated in both Bt and non-Bt plant groups. (Table 5, 6). Poorly opened bolls per plant showed a non-significant negative correlation with the seed cotton yield in both Bt and non-Bt plant groups. Studies by Majjiga *et al*. (2018), Tamilselvan *et al*. (2013), Salahuddin *et al*. (2010), Magadum *et al*. (2012) showed a favourable association between the yield of seed cotton and the number of bolls per plant and average boll weight. The recent research revealed that the number of bolls per plant, average boll weight, and lowest open boll damage are the traits most important to seed cotton yield. Choosing early maturing types will not boost seed cotton yield, since both Bt and non-Bt plant groups had a negative connection between seed cotton output and days to 50% flowering and days to boll opening. The trait % of incompletely opened bolls had a negative, insignificant connection with seed cotton output in both Bt (-0.08) and non-Bt plants (-0.10). The current study showed that the Bt gene has not considerably changed yield and traits related to yield since the population under study is a segregating generation.

Table 5: Phenotypic correlation co-efficient of yield and yield related traits on seed cotton yield in Bt plants of F_2 population of cross DHS-114 × Event No. 78

Characters	DFF	DOP	PH	NMP	NSP	NBP	ABW	GOB	BOB	SCY
DFF	1.0	1.0**	-0.008	-0.2	0.01	-0.51**	-0.28**	-0.49**	-0.08	-0.50
DOP		1.0	-0.007	-0.3	0.01	-0.45**	-0.25**	-0.41**	-0.07	-0.45
PH			1.0	1.0*	0.82**	0.01	0.14	0.01	0.13	0.08
NMP				1.0	0.11	0.23**	-0.04	0.23**	-0.02	0.19
NSP					1.0	0.005	0.21*	-0.01	0.13	0.09
NBP						1.0	0.31**	0.99**	-0.07	0.93**
ABW							1.0	0.30**	0.00	0.53**
GOB								1.0	-0.10	0.92**
BOB									1.0	-0.08

*Note: DFF= Days to 50% flowering, DBO= Days to first boll opening, PH= Plant height, NMP= Number of monopodia per plant, NSP= Number of sympodia per plant, NBP= Number of bolls per plant, ABW= Average boll weight, SCY= Seed cotton yield per plant, GOB= Good opened bolls per plant, BOB= Bad opened bolls per plant. *Significant at P = 0.05 **Significant at P = 0.01*

Table 6: Phenotypic correlation co-efficient of yield and yield related traits on seed cotton yield in non Bt plants of F_2 population of cross DHS-114 × Event No. 78

Characters	DFF	DOP	PH	NMP	NSP	NBP	ABW	GOB	BOB	SCY
DFF	1.0	0.99**	0.15	-0.01	0.12	-0.44**	-0.39**	-0.47**	0.20	-0.49
DOP		1.0	0.14	-0.003	0.10	-0.47**	-0.31**	-0.39**	0.19	-0.39
PH			1.0	0.25*	0.70**	-0.16	0.07	-0.17	0.11	-0.10
NMP				1.0	-0.11	0.25*	0.16	0.19	0.34	-0.26**
NSP					1.0	0.17	0.05	0.15	-0.10	0.22*
NBP						1.0	0.35**	0.97**	-0.06	0.84**
ABW							1.0	0.37**	-0.12	0.70**
GOB								1.0	-0.25	0.84**
BOB									1.0	-0.10

Independent path coefficient studies on Bt and non-Bt plant groups were performed on the F_2 population derived from the cross DHS-114 Event No. 78. Below is a discussion of the findings. The study's findings demonstrated that the number of bolls, average boll weight, and well-opened bolls per plant all had positive direct effects in the intended direction on the seed cotton output per plant (Table 7, 8). By concentrating on these characteristics, cotton selection tactics may be more effective. These characteristics also shown favorable indirect impacts via plant height and number of sympodia per plant in both Bt and non-Bt plant groups. The proportion of improperly opened bolls per plant had a direct detrimental impact on the production of seed cotton in Bt and non-Bt plants. Our results were in line with those of Dhillon *et al*. (2013), who discovered that Bt genotypes (non-Bt Mech 12) and non-Bt genotypes (40.2% and 12.8% boll damage, respectively) suffered from boll damage (Bt Mech 12). The F2 population descended from the cross DHS-114 Event No. 78 between Bt positive and negative plants showed in our analysis that both genotype groups recorded positive association with the of seed cotton yield with yield-

influencing traits, indicating that efforts are required in identifying potential plants carrying the Cry1Ac gene to produce Bt varieties. Tracing the gene's transmission across crop improvement projects will be made easier with the help of the current research on the inheritance of the Cry1Ac gene in recently created transgenic events. When choosing high yielding Bt cotton genotypes, breeders might benefit from research on variability and connections among several character categories. Choosing one of two connected likeable characters will invariably favor the other. Therefore, efforts must be made to select the genotypes for all desirable features in addition to the Cry1Ac gene in order to acquire suitable Bt genotypes.

Table 7: Direct and indirect effects of yield related and entomological traits on seed cotton yield in Bt plants of F_2 population of cross DHS-114 × Event No. 78

Characters	DFF	DOP	PH	NMP	NSP	NBP	ABW	GOB	BOB	SCY
DFF	**0.0016**	0.0011	0.0000	0.0000	0.0000	-0.0008	-0.0005	-0.0008	-0.0007	-0.5056
DOP	0.0000	**0.0013**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-0.4043
PH	-0.0001	-0.0002	**0.0111**	0.0022	0.0091	0.0002	0.0016	0.0001	0.0003	0.0838
NMP	0.0000	0.0000	-0.0001	**-0.0007**	-0.0001	-0.0002	0.0000	-0.0002	-0.0001	0.1952
NSP	0.0004	0.0005	0.0223	0.0031	**0.0271**	0.0001	0.0058	0.0003	0.0023	0.0943
NBP	-0.2839	-0.2452	0.0085	0.1324	0.0030	**0.5542**	0.1747	0.5486	-0.3910	0.9315
ABW	-0.0736	-0.0856	0.0378	-0.0123	0.0555	0.0820	**0.2600**	0.0792	-0.0677	0.5341
GOB	-0.1359	-0.6734	0.0034	0.0649	-0.0030	0.2734	0.0841	**0.2762**	-0.1652	0.9220
BOB	-0.0140	-0.0213	0.0008	0.0057	0.0027	-0.0226	-0.0084	-0.0192	**-0.0006**	-0.08

Table 8: Direct and indirect effects of yield related and entomological traits on seed cotton yield in non Bt plants of F_2 population of cross DHS-114 × Event No. 78

Characters	DFF	DOP	PH	NMP	NSP	NBP	ABW	GOB	BOB	SCY
DFF	**1.3033**	1.3013	0.2008	-0.0152	0.1593	-0.5823	-0.5084	-0.6132	-0.3884	-0.4951
DOP	-1.3116	**-1.3137**	-0.1862	0.0040	-0.1379	0.5844	0.5177	0.6138	0.3934	-0.4979
PH	0.0013	0.0012	**0.0086**	0.0022	0.0060	-0.0014	0.0007	-0.0015	-0.0008	-0.1094
NMP	-0.0003	-0.0001	0.0071	**-0.0281**	-0.0031	0.0071	0.0045	0.0054	0.0097	0.2616
NSP	-0.0110	-0.0094	-0.0631	0.0100	**0.0899**	0.0154	0.0046	0.0140	0.0158	0.2207
NBP	-0.2139	-0.2342	0.0084	0.1434	0.0040	**0.5421**	0.1543	0.5385	0.3515	0.8345
ABW	-0.1744	-0.1761	0.0352	0.0723	-0.0227	0.1567	**0.4470**	0.1687	-0.0957	0.7046
GOB	-0.3719	-0.6261	-0.2470	0.6171	-0.5235	0.2461	0.0043	**0.9161**	-0.2924	0.8436
BOB	0.0018	0.0017	0.0010	0.0030	-0.0009	-0.0006	-0.0011	-0.0822	**-0.2075**	-0.1047

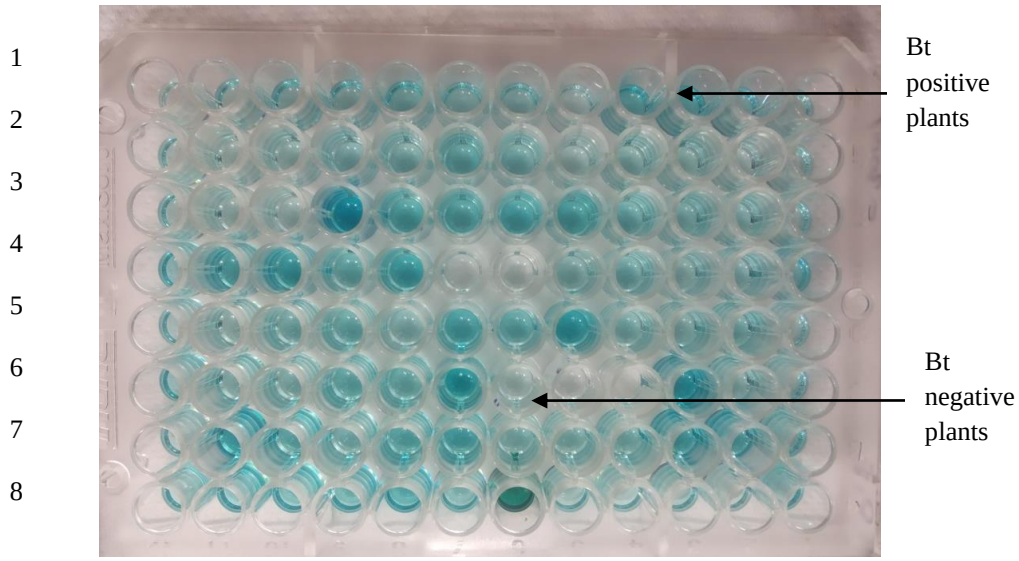


Fig. 1: ELISA test in the F_2 population of cross DHS-114 × Event No.78

ACKNOWLEDMENT

I thank my parents for their constant support and my gratitude towards my chairmen and committee members. I sincerely thank UAS, Dharwad for providing a platform for conducting the study.

DISCLOSURE STATEMENT

"The author(s) declare(s) no known conflict of interests that could have appeared to influence the work reported in this paper."

REFERENCES

Canming, T., Jing, S., Xiefei, Z., Vangzhen, G., Tianzhen, Z., Jinliang, S., Congfen, G., Weijun, Z., Zhixian, C. and Sandui, G. 2000. Inheritance of resistance to *Helicoverpa armigera* of 3 kinds of transgenic *Bt* strains available in upland cotton in China. *Chinese Sci. Bull*., **45**(4): 363-67.

Dhillon, M.K. and Sharma, H.C. 2013. Comparative studies on the effects of Bt transgenic and non-transgenic cotton on arthropod diversity, seed cotton yield and bollworms control. *J. Environ. Biol*., **34**: 45-51.

Duan, X.X., Li, Q., Xue, M., Abo, S.D., Xu, D. and Wu, R. 1996. Transgenic rice plants harbouring an introduced potato proteinase inhibitor II gene are insect resistant. *Nature Biotechnol*., **14**: 494-98.

Fearing, P.L., Brown, D., Vlachos, D., Meghji, M. and Privalle, L. 1997. Quantitative analysis of Cy1Ab protein expression in Bt maize plants, tissues and silage and stability of expression over successive generations. *Mol. Breed.*, **3**: 169-76.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*. A Wiley-Interscience Publication, New York.

Hussain, T., Bakhsh, A., Munir, B. and Hassan, S. 2014. Mendelian segregation pattern and expression studies of insecticidal gene (cry1Ac) in insect resistant cotton progeny. *Emir. J. Food Agric*. 26(8): 706-71.

Magadum, S., Banerjee, U., Ravikesavan, R., Gangapur, D. and Boopati, N.M. 2012. Variability and heritability analysis of yield and quality traits in interspecific population of cotton (*Gossypium hirsutum* L.). *Seed*, **1**: 1.

Majjiga, K., Meenakshi, N.G. and Kumar, M. 2018. Genetic variability, heritability and correction analysis in F_2 populations of ratoon upland cotton hybrids. *Int. J. Agri. Environ. Biotechnol.,* **11**(6): 815-27.

Rashid, B., Saleem, Z., Husanain, T. and Riazuddin, S. 2008. Transformation and Inheritance of Bt genes in *Gossypium hirsutum. J. Plant. Bio*., **51**(4): 248-54.

Salahuddin, S., Abro, S., Rehman, A. and Iqbal, K. 2010. Correlation analysis of seed cotton yield with come quantitative traits in upland cotton (*Gossypium hirsutum* L.). *Pakistan J. Bot*., **42**(6): 3799-05.

Tamilselvan, G., Rajendran, R. and Anbarasan, K. 2013. Association and path analysis in cotton (*Gossypium hirsutum* L.). *Int. J. Plant Sci*., **3**(2): 36-38.

Weber, C. R. and Moorthy, B.R. 1952. Heritable and non-heritable relationship and variabilites of oil content and organic character in F_2 generation of soyabean crosses. *Agron. J*., **44**: 202-09.

Wright, S. 1921. Correlation and causation. *J. Agric. Res*., **20**: 557-85.