ANALYSIS OF BIOMOLECULAR COMPOUNDS IN TRANSGENIC AND NON-TRANSGENIC COTTON PLANTS

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Abstract— **The biodiversity studies carried out in transgenic and non-transgenic cotton plants, showed variation in the population of arthropods. The main reason behind this is the incorporation of Bt gene in transgenic cotton plants. This work has been designed to analyse whether the incorporation of Bt gene had altered the biomolecular compounds in transgenic plants. Based on this, phytochemical and biochemical analysis were carried out both in transgenic and non-transgenic cotton leaves. This work proved that there is alteration in both the phytochemicals and biochemical compounds in transgenic cotton plants.**

Keywords— **Phytochemical, GC-MS, Bt cotton, Arthropod, Insect resistance**

I. INTRODUCTION

Many crops have been genetically transformed to provide enhanced resistance to insect pests and diseases[1]. Among these, cotton has attracted much interest in the field of gene transfer with the aim of introducing agronomically interesting new traits. When genetically modified transgenic cotton (Bt cotton) was given commercial clearance, a gene coding for Bt, a protein in the bacterium B. thuringiensis, was introduced into cotton using genetic engineering methods[2]. Cotton plants are selected for analysis, as cotton is widely grown in Tamilnadu among other transgenic plants. Plant leaves are important organs with vital physiological functions such as photosynthesis and transpiration[3,4]. Physical and chemical factors on the leaf surface influence the performance of the herbivorous insects, predators and parasites[5]. Any changes in transgenic crops in physical and chemical characteristics of the leaf surface resulting from insertion of exotic genes can probably influence searching or acceptance of host plants by herbivore insects. Based on the above factors, the transgenic and non-transgenic cotton plant leaves were subjected to phytochemical analysis for protein, carbohydrate and phenol. The biochemical constituents of the leaves were analyzed by GC-MS analysis.

II. MATERIALS AND METHODS

Plant material

Bunny cotton seeds were collected from the Tamilnadu Cotton Research Centre, Srivilliputhur, Virudhunagar district, Tamilnadu for the study. Polymerase chain reaction (PCR) was carried out using specific primer pairs to amplify Cry1Ac trans genes from transgenic cotton leaves to confirm transgenecity. The extracts of leaves were prepared based on the work of Muhit *et al*[6] and used for further studies.

Estimation of phytochemical constituents

The total phenolic compounds from the leaves of both non-transgenic and transgenic plants were determined by Folin Ciocalteau Assay. The total protein from the leaves of both non-transgenic and transgenic plants were determined by Lowrey et al.[7] The total carbohydrate from the leaves of non-transgenic and transgenic cotton plants were determined using Anthrone method[8]. Student's't' test was done for carbohydrate, protein and phenol in transgenic and non-transgenic cotton leaves separately. For studying the different biochemical compounds present in both transgenic and non-transgenic leaf extracts, the samples were sent for GC-MS analysis to SITRA, Coimbatore.

III. RESULTS AND DISCUSSION

The genetic resistance is the most efficient method of protecting crops from pests. The transgenic cotton has in-built genetic resistance to bollworms which help in the protection of natural enemies of insect pests ie predators and parasites. As transgenic plants are considered to be efficient than non-transgenic plants, a comparison between them was analysed in this paper.

PCR

As the Cry protein is high in the leaves, the extract from the tender leaves of transgenic plants were subjected to molecular analysis (PCR) to confirm the transgenicity (Plate 1). In this PCR analysis, control reaction is done using plant specific primer designed to amplify a region around 700 bp length from plant chloroplast gene. PCR results confirmed the integration of Bt gene in the plants. Udikeri[9] and Yazdanpanah *et al*.[10] used PCR analysis for proving transgenicity.

Phytochemicals

When the transgenic and non-transgenic cotton plant leaves were subjected to phytochemical analysis for protein, carbohydrate and phenol, it was found that the total protein content, the carbohydrate content and the phenol content were high in transgenic cotton leaves than in non-transgenic cotton leaves. The phytochemical constituents (phenol, protein and carbohydrate) of transgenic and non-transgenic plant extract and their 't' test values were determined and recorded in table 1.

Protein

The total protein content from leaves was determined, because the extraction of Cry1Ac protein from other tissues for estimation purpose is a challenging exercise and this is not adequately described. Our results confirmed that the protein content in the transgenic plants (295.75 ± 11.74 mg/g) was higher than the non-transgenic plants (172.25 ± 14.52 mg/g). The't' test for protein content showed significant difference between transgenic and non-transgenic cotton leaves ($t = 38.16$, $p < 0.05$). The difference in the phytochemical analysis between transgenic and non-transgenic cotton plants were also confirmed by Momtaz *et al*.[11] who reported that there were some differences in mean content of some individual amino acids between transgenic and non-transgenic seeds, with some significant differences at higher level of salt stress. They confirmed that the total aminoacid content in transgenic was 264.30 and 224.50 for non-transgenics. Indeed transgenic seeds showed slightly higher concentration of amino acids compared with non-transgenic seeds.

Carbohydrate

The carbohydrate content in transgenic plants was 33.75±2.89 mg/g and 24.43±1.04 mg/g in non-transgenic plants. The carbohydrate content also showed significant difference between transgenic and non-transgenic cotton leaves through 't' test ($t= 6.93$, $p < 0.05$). Veramendi *et al*.[12] ; Dai *et al*.[13] concluded that there was over accumulation of starch in leaves of potato, inhibition of growth, photosynthesis, induced rapid senescence, decreased fruit setting, sucrose unloading capacity and delayed softening of fruit in tomato that were transformed with the antisense genes.

Phenol

Phenolic compounds are much essential for the plants for defence mechanism. It is clear that the phenol content play a major role in transgenic plants in defence mechanism against Lepidopterans[14]. From our findings it was clear that the transgenic plants have a higher concentration of phenols (55.75 ± 3.21mg/g) than in non-transgenic plants (40 ± 2.63 mg/g). In the present study, the 't' test showed significant difference between the phenolic content of transgenic and non-transgenic cotton leaves ($t= 10.69$, $p < 0.05$). Among secondary metabolites, phenolic compounds have been repeatedly shown to play a vital role in plant resistance and protect fruits and vegetables against pests[15]. This may be the reason transgenic plants show a slight variation in phenol content as it is resistant to Lepidopterans. The main precursors for phenol synthesis in plant tissue are carbohydrates, especially soluble carbohydrate which lead to the formation of the essential substances required for simple and poly phenols synthesis. Our results showed increase in the total content of both carbohydrate and phenols. The reduction in phenolic compounds may be due to the reduction in soluble carbohydrate[16].

Biochemical

The leaf extracts of both the transgenic and non-transgenic cotton plants were subjected to GC-MS analysis and it was found that there were some variation in the compounds present in both of them. No work was cited for the GC-MS analysis of transgenic plants. But Essien *et al*.[17] subjected the non-transgenic Gossypium plants for GC/MS analysis.

GC-MS

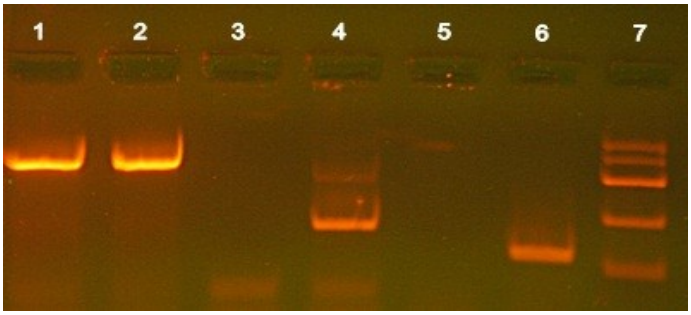
The leaf extract of non-transgenic plants were subjected to GC-MS analysis. About seven peaks were obtained with retention times 7.85, 12.23, 18.16, 22.07, 26.23, 29.11 and 31.45 in the GC analysis (Fig1.). MS analysis was done for five retention times, 12.23, 18.16, 22.07, 26.23 and 31.45. The possible compounds for the various retention times for transgenic cotton leaf extract were recorded in table 2.

The leaf extract of transgenic plants were subjected to GC-MS analysis. About seven peaks were obtained with retention times 9.38, 12.27, 18.20, 20.99, 24.39, 31.50 and 35.75 in the GC analysis (Fig 2). MS analysis was done for six retention times, 12.27, 18.20, 20.99, 24.39, 31.50 and 35.75. The possible compounds for the various retention times for non-transgenic cotton leaf extract were recorded in table 3.

In the present study, some biochemical compounds are specific to transgenic cotton and some are specific to non-transgenic cotton plants as as highlighted in tables 2 and 3. Our findings were supported by some of the authors. Hedin *et al*.[18] showed the presence of d-cadinene, caryophyllene oxide and copaene as major compounds of G. barbadense var. L. Both humulene and caryophyllene, though could be identified, were present in insignificant quantity. According to Minyard *et al*.[19]; Minyard *et al*.[20]; Hedin *et al*.[21,22]; Hedin *et al*.[23]; aliphatic alcohols as well as the terpenoids, terpineol, and bisabolol and bisabolene oxide are present as characteristics compounds of G.hirsutum var Deltaphine. According to Essien *et al*.[17] the compounds present in the various species of Gossypium were not the same.

Plate. 1 Molecular Analysis (PCR)

35S viral promoter	BT gene	NOS terminator



Lane 1: Control Leaf sample with control rbcL gene
Lane 2: BT cotton leaf sample with control rbcL gene
Lane 3: Control leaf sample with 35S specific primer
Lane 4: BT cotton leaf sample with 35S specific primer
Lane 5: Control leaf sample with NOS specific primer
Lane 6: BT leaf sample with NOS specific primer
Lane 7: HELINI Quickref DNA ladder [100bp, 250bp, 500bp, 750bp, 1000bp]

Table 1 Phytochemical constituents of cotton plants

S.No	Phytochemical constituents	Transgenic (mg/g)	Non-transgenic (mg/g)	t-test
1	Phenol	55.75±3.21	40 ± 2.63	**10.69***
2	Protein	295.75±11.74	172.25 ± 14.52	**38.16***
3	Carbohydrate	33.75±2.89	24.43 ± 1.04	**6.93***

* $p < 0.05$

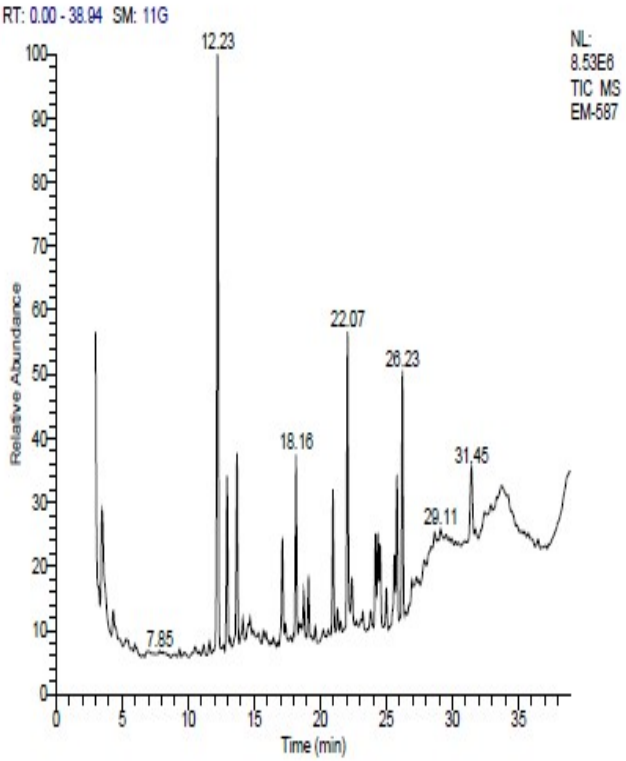


Figure 1 GC pattern of Non transgenic cotton leaf extract

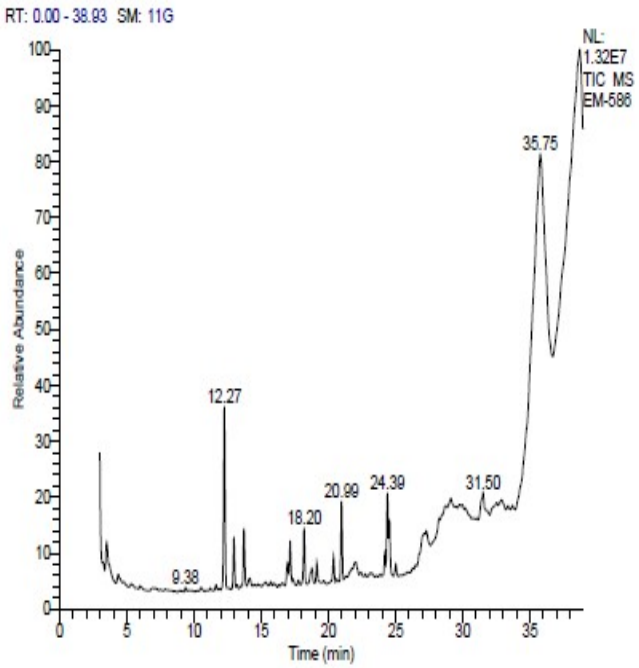


Figure 2 GC pattern of transgenic cotton leaf extract

Table 2 Possible compounds recorded for the GC-MS analysis of non-transgenic cotton leaves

S.No	RT	SI	RSI	Name of the compound	Molecular formula	Molecular weight	Area %
1	12.23	901 723 278	908 932 968	Trans- Caryophyllene Cis- Caryophyllene a-Elemene	$C_{15}H_{24}$ $C_{15}H_{24}$ $C_{15}H_{24}$	204 204 204	22.60 22.60 22.60
2	18.16	595 577 492 429	938 947 955 952	**(+)-2-endo,3-endo-dimethylbornate** Phytolacetate **Lavandulyl acetate** Neophytadiene	$C_{12}H_{22}$ $C_{22}H_{42}O_2$ $C_{12}H_{20}O_2$ $C_{20}H_{38}$	166 338 196 278	5.99 5.99 5.99 5.99
3	22.07	540 468 415 406	943 995 994 967	**Hexadecanoic acid, ethyl ester (CAS)** **Decanoic acid,2,8-dimethyl-,methyl ester (CAS)** **Tetradecanoic acid, ethyl ester (CAS)** **Nananoic acid,2,4,6-trimethyl-,methyl ester,(R,R,R)-(-)-(CAS)**	$C_{18}H_{36}O_2$ $C_{13}H_{26}O_2$ $C_{16}H_{32}O_2$ $C_{13}H_{26}O_2$	284 214 256 214	9.88 9.88 9.88 9.88
4	26.23	799 391 316	912 931 953	**9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-(CAS)** **Endo/exo-2-methyl-2-(propen-2-yl)-7,8-diazobicyclo[2,2,2]oct-7-ene** **Bicyclo[10,1,0]tridec-1(12)-ene-13-one**	$C_{19}H_{32}O_2$ $C_{10}H_{16}N_2$ $C_{13}H_{20}O$	292 164 192	8.45 8.45 8.45
5	31.45	262 61 61 55	879 960 879 902	**5-Diazo-1-(2'-methyl-5'-nitrophenylazo)-1,3-cyclopentadiene** **Cis-1-(triisopropylsilyl)propene** **2-Bromo-3-chloropropyl Methacrylate** **®-5,5,5-trifluor-4-hydroxyvaleriansaure-ethylester**	$C_{12}H_9N_5O_2$ $C_{12}H_{26}Si$ $C_7H_{10}BrClO_2$ $C_7H_{11}F_3O_3$	255 198 240 200	3.71 3.71 3.71 3.71

RT- RETENTION TIME, SI- STRENGTH INDEX, RSI- RELATIVE STRENGTH INDEX

Table 3 Possible compounds recorded for the GC-MS analysis of transgenic cotton leaves

S.NO	RT	SI	RSI	Name of the Compound	Molecular formula	Molecular weight	Area%
1	12.27	900 771 343	904 930 972	Trans- Caryophyllene Cis- Caryophyllene a-elemene	$C_{15}H_{24}$ $C_{15}H_{24}$ $C_{15}H_{24}$	204 204 204	14.69 14.69 14.69
2	18.20	598 478 358	969 959 959	Phytolacetate **6Nonen-1-ol,acetate,(z)-** Neophytadiene	$C_{22}H_{42}O_2$ $C_{11}H_{20}O_2$ $C_{20}H_{38}$	338 184 278	4.24 4.24 4.24
3	20.99	395 309 309 236	834 880 842 819	**1,1-Dianysyl-2,2-dimethoxyethane** **2-Iodotetradecanoic acid** **®-(-)-nonane-1,3-diol** **2-Pentanone, 1,3-dimethoxy-3-methyl-**	$C_{18}H_{22}O_4$ $C_{14}H_{27}IO_2$ $C_9H_{20}O_2$ $C_8H_{16}O_3$	302 354 160 160	6.26 6.26 6.26 6.26
4	24.39	635 225 212	945 985 994	**9-Octadecanoic acid, methyl ester,(E)-(CAS)** **13- Octadecanoic acid, methyl ester (CAS)** **6- Octadecanoic acid, methyl ester (CAS)**	$C_{19}H_{36}O_2$ $C_{19}H_{36}O_2$ $C_{19}H_{36}O_2$	296 296 296	5.49 5.49 5.49
5	31.50	435 61	992 896	**22-(Benzyloxy)-3a-[(t-butyldimethylsilyl)oxy]-5a-23,24-bisnorcholan-16a-ol** **2,4-Dimethyl-6-(tetrahydropyran-2-yloxy)heptan-1-ol**	$C_{35}H_{58}O_3Si$ $C_{14}H_{28}O_3$	554 244	3.10 3.10
6	35.75	125 87 57	999 872 938	**18-Iodo-17a-Acetamido-5 a-Androstane** **Methyl2-(1,3-difluorophenanthren-2-yl)-2-hydroxypropionate** **Methyl2-(6,8-difluorophenanthren-1-yl)-2-hydroxypropionate**	$C_{21}H_{34}INO$ $C_{18}H_{14}F_2O_3$ $C_{18}H_{14}F_2O_3$	443 316 316	31.25 31.25 31.25

RT- RETENTION TIME, SI- STRENGTH INDEX, RSI- RELATIVE STRENGTH INDEX

IV. CONCLUSION

From the above findings it was clear that both transgenic and non-transgenic Bunny cotton plants showed moderate variation in the phytochemical constituents which may be due to the incorporation of Cry1Ac protein. The GC-MS analysis of transgenic and non-transgenic leaf extract showed variation in some of the compounds. So it could be concluded that the incorporation of Bt gene in plants alters the phytochemical and biochemical constituents and consequently alters the biodiversity.

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