academicJournals

Vol. 8(41), pp. 5093-5095, 24 October, 2013
DOI: 10.5897/AJAR2013.0760
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Short Communication

Comparative analysis of volatile constituents in *Bacillus thuringiensis* (Bt) cotton (RCH$_2$ Bt) and non-Bt cotton by gas chromatography-mass spectrometry

Palanisamy Parimala, Subbiah Ramalakshmi and Krishnaswamy Muthuchelian*

Department of Bioenergy, School of Energy, Environment and Natural resources, Madurai Kamaraj University, Madurai, India.

Accepted 15 October, 2013

The differences in composition and concentrations of volatile allelochemicals between transgenic *Bacillus thuringiensis* (Bt) cotton and non Bt cotton leaves were compared and analyzed by using gas chromatography-mass spectrometry (GC-MS). The dominant compounds present in both non Bt and Bt cotton leaves were α-caryophyllene (4.62, 1.42), β-myrcene (3.67, 3.68), β-carene (0.31, 0.80), benzene acetaldehyde (0.88, 0.48), heptanoic acid (2.0, 0.67), 1-methoxy, 2-methyl benzene (0.67, 0.30) and acetic acid, phenyl methyl ester (4.32, 2.48). The differences in main components and ratios of volatile allelochemics between Bt cotton and non Bt cotton suggested that, the expression of Bt toxin protein in cotton was found to have an impact on their volatile profiles.

Key words: Bt cotton (RCH$_2$ Bt), non-Bt cotton, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

Plants under herbivore attack release volatile compounds that help in the attraction of parasitoids in finding the prey (Carroll et al., 2006; Raguso, 2008). Release of volatile compounds not only benefits the parasitoids but also the host plant by attracting the natural enemies of the herbivores that feeds on its foliage. For example, corn and cotton plants release volatiles that attract parasitic hymenoptera that feeds on the larvae of many lepidopteron insects (Tumlinson et al., 1993). Main volatile components in cotton plants are monoterpenes and sesquiterpenes (Loughrin et al., 1994).

Cotton (*Gossypium hirsutum*) is an important cash crop in India and it plays a significant role in world economy (representing 20 to 25% of the world economy). While India has the largest area under cotton in the world, rank third in terms of production after China and USA. The major factors that cause low yield in the production of cotton is due to insect pest. To overcome this, a number of plant species, particularly crops, such as cotton, corn, potatoes, tobacco, tomato, and sugarcane have been genetically modified to produce δ-endotoxin proteins from Bt (Mendelsohn et al., 2003).

In addition to the release of volatiles at the site of herbivore feeding, analysis of volatile in the undamaged leaves also established that there is a systemic release (Turlings and Tumlinson, 1992; Rose et al., 1996). Due to integration of foreign gene, some metabolic changes might take place in Bt cotton. So, in order to find whether there is any variation in the volatile components and its concentration between non Bt and Bt cotton, we have investigated it using gas chromatography-mass spectrometry (GC-MS) analysis.

Previous study in our lab investigated the *in vitro* cytotoxicity and genotoxicity of *Cry1Ac* toxin isolated from

---

*Corresponding author. E-mail: drchelian1960@yahoo.co.in. Tel: +91 452 2458020. Fax: +91 452-2459181.

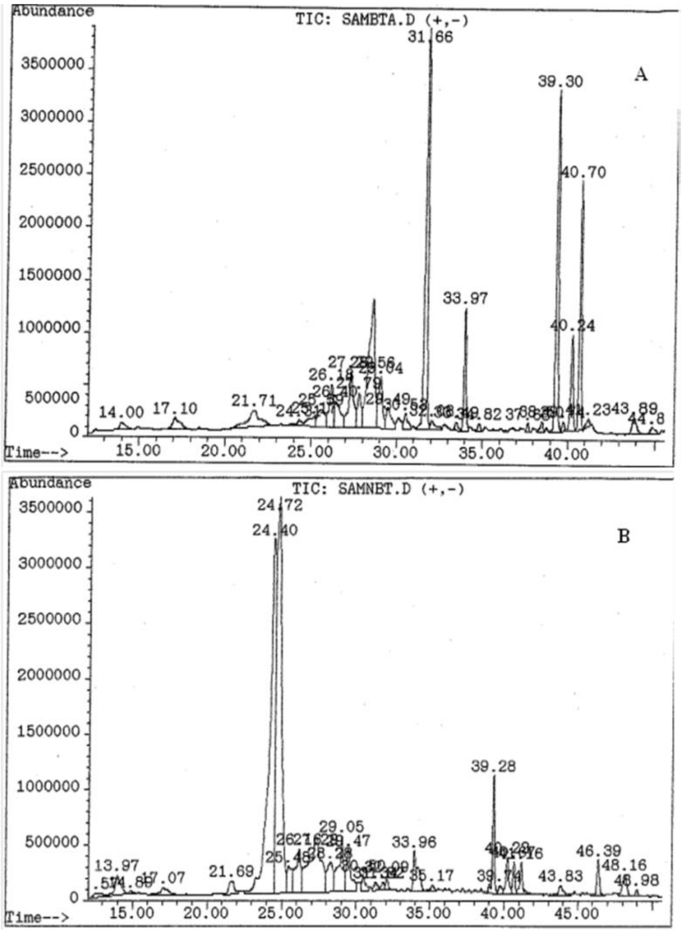

Figure 1. GC-MS volatile profile of Bt cotton extract (A) and non Bt cotton (B) extract.

Bt Cotton (RCH2 Bt) on human lymphocytes. It was concluded through the study that, crude *Cry1Ac* toxin had possible cytotoxic potential on human lymphocytes with increased dosage of toxin and time of exposure (Bhat et al., 2011). Thus, the objective of the study is to compare the volatile constituents of non Bt and Bt cotton.

MATERIALS AND METHODS

Leaves of transgenic Bt cotton (RCH2 Bt) and non-Bt cotton were separately shaded dried, powdered and extracted with ethanol for 6 to 8 h using Soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The ethanolic extracts of the plant was used for GC-MS analysis. *2* µl of the ethanolic extract of transgenic Bt cotton (RCH2 Bt) and non-Bt cotton was subjected to GC-MS analysis. GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising of a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 to 200°C/min, then 5 to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns.

RESULTS AND DISCUSSION

The GC-MS chromatogram of Bt and Non-Bt cotton are presented in Figure 1A and B. Most of the volatile components released from both non Bt and Bt cotton were similar but they differ in percentages (Table 1). The most important components such as β - myrcene, α - caryophyllene and 3-carene were found both in non Bt and Bt cotton at ratio of 3.68, 1.42, 0.80, and 3.67, 4.62, 0.31% respectively (Table 1). Some of the components present in non Bt cotton were absent in Bt cotton and vice versa. α-pinene, styrene, and d-allose were absent in non Bt cotton but Bt cotton showed a ratio of 2.23,

Table 1. Comparative volatile profile of Bt cotton and non Bt cotton.

Volatile compound	Bt cotton	Non Bt cotton
α-caryophyllene	4.62	1.42
β-myrcene	3.67	3.68
B-carene	0.31	0.80
Styrene	4.84	-
ϵ-pinane	-	2.76
Limonene oxide	-	9.24
P-xylene	-	3.11
Acetic acid, phenyl methyl ester	4.32	2.48
Octen-1-ol, 3,7-dimethyl acetate	0.39	0.24
Benzene acetaldehyde	0.88	0.48
Benzene 1-methoxy, 2-methyl	0.67	0.30
Heptanoic acid	2.00	0.67
1,3-bis ((2-cyclo, propyl, 2-methyl cyclopropyl(-but-2-en-1-one)	0.48	3.29

4.84, and 14.97%, respectively. ε-pinane and limonene oxide were absent in Bt cotton but non Bt cotton showed 2.76 and 9.24%, respectively (Table 1).

The volatile blend released from a plant may consist of more than hundreds of different components (Raguso, 2004). Earlier studies have also suggested that, certain volatiles emitted by corn and cotton (that is, nerolidol and caryophyllene) could detrimentally and directly target the herbivores (Ted et al., 1995). The insertion of foreign gene in the Bt cotton, results in the changes in physiological activities and also the production of volatile components.

In the present study, some volatile components that are present in non Bt cotton are absent in Bt cotton and vice versa. The changes in the volatile components may be due to the metabolic changes resulted by the insertion of foreign gene. The compounds mostly present in the extracts of both varieties of cotton are said to be terpenoids, which are naturally occurring in plants as secondary metabolites. The terpenoids are basically made of isoprene units. Terpenoids possess various biological as well as pharmacological activities. From this study, we suggest that, the variation of volatile compounds in Bt cotton might have impact on the ecological relationship.

Conclusion

The acceptance or rejection of genetically modified crops mainly depends upon farmers and consumers. Many studies concerning this question have been conducted in developed nations. In addition to the difference between non Bt and Bt cotton in the production of volatile components, many researchers have found significant difference between the two under abiotic stress conditions (Chen et al., 2005; Parimala and Muthuchelian, 2010). This indicates that, some modification has to be done in the new technology, to make it useful even under the future environmental conditions.

REFERENCES

Bhat MS, Parimala P, Ramalakshmi S, Muthuchelian K (2011). *In-Vitro* Cytotoxic and Genotoxicity Studies of *Cry1Ac* Toxin Isolated from Bt Cotton (RCH2 Bt) on Human Lymphocytes. Acad. J. Plant. Sci. 4:64-68.

Carroll MJ, Schmelz EA, Meagher RL (2006). Attraction of Spodoptera frugiperda larvae to volatiles from herbivore-damaged maize seedlings. J. Chem. Ecol. 32:1911-1924.

Chen FJ, Wu G, Ge F, Parajulee MN, Shrestha RB (2005). Effects of elevated CO_2 and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. Entomol. Exp. Appl. 115:341-346.

Loughrin JH, Manukian A, Heath RR, Turlings TC, Tumlinson JH (1994). Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. Proc. Natl. Acad. Sci. USA 91:11836-11840.

Mendelsohn M, Kough J, Vaituzis Z, Mathews K (2003). Are Bt crops safe? Nat. Biotechnol. 21:1003-1009.

Parimala P, Muthuchelian K (2010). Physiological response of non Bt and Bt cotton to short term drought stress. Photosynthetica 48:630-634.

Raguso RA (2008). The “invisible hand” of floral chemistry. Science 321:1163-1164.

Raguso RA (2004). Why do flowers smell? The chemical ecology of fragrance-driven pollination. - Advances in Insect Chemical Ecology (Eds. R. T. Cardé and J. G. Millar). University Press Cambridge, pp. 151-178.

Rose US, Manukian A, Heath RR, Tumlinson JH (1996). Volatile semiochemicals released from undamaged cotton leaves: a systemic response of living plants to caterpillar damage. Plant Physiol. 111:487-495.

Ted CJ, Turlings, Loughrin JH, Mccall PJ, Rose USR, Lewis WJ, Tumlinson JH (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. 92:4169-4174.

Tumlinson JH, Lewis WJ, Vet LEM (1993). How parasitic wasps find their hosts. Sci. Am. 268:100-106.

Turlings TC, Tumlinson JH (1992). Systemic release of chemical signals by herbivore-injured corn. Proc. Natl. Acad. Sci. USA 89:8399-8402.