Original Research Article

Comparative metabolite profiling of drought stressed leaf and stem of *Gossypium hirsutum* L. using a gas chromatography-mass spectroscopy technique

Abstract

In the present study, the variation of non-polar metabolites in leaf and stem of water stressed *Gossypium hirsutum* L. plants was observed by gas chromatography-mass spectrometry (GC-MS) method. Total 17 non-polar metabolites were detected in control and water stressed *G. hirsutum* leaf. The major metabolites were quinoline derivative (26.37±0.29%), 2-methylhexadecan-1-ol (7.47±0.07%), phytol (7.71±0.02%), myristic acid (5.94±0.04%), hexadecanol (14.30±0.94%), nonadecane (1.67±0.05%) and palmitic acid (3.20±1.39%). Total 14 metabolites were detected in stem and the major metabolites were dodecene (1.67±0.11%), L-lysine (0.65±0.06%), dibutylphthalate (5.06±1.88%), linoleic acid (10.26±0.07%), campesterol (0.87±0.04%) and stigmasterol (1.13±0.55%). Significant variation (P = .05) in content of some of the metabolites was observed under water stress condition. It includes that; these metabolites might have played an important role in drought stress tolerance. This study indicates that drought stress treated leaves and stems of *G. hirsutum* have distinct mechanisms of metabolite accumulation and regulation, which is valuable for the better understanding of overall abiotic stress tolerance mechanism.

Keywords: *Gossypium hirsutum*, water stressed, metabolites, gas chromatography-mass spectrometry.

Introduction

Abiotic stress (water stress) is the most important factor which affects crop productivity and adversely affects fruit production, square and boll shedding and fiber quality properties in cotton [1]. As the water shortage and drought have become an increasingly serious constraint and considered the single most devastating environmental stress, which decreases crop productivity more than any other environmental stress [2]. It severely affects plant development with substantial reductions in crop growth rate and biomass accumulation. The

main consequences of drought in crop plants are: reduces the cell division and expansion, root proliferation and disturbed stomatal oscillations, plant water and nutrient relations with diminished crop productivity, and water use efficiency (WUE) [3,4].

Previous studies revealed that 2 to 4 °C increase in temperature and the expected 30% decrease in precipitation may adversely affect crop productivity and water availability by the year 2050 [5]. Thus, screening cotton varieties for resistance to drought stress conditions and improving cotton tolerance to this stress conditions will mitigate negative consequences of this adversity. Cotton is normally not classified as a drought tolerant crop as some other plants species such as sorghum which is cultivated in areas normally too hot and dry to grow other crops [6]. Nevertheless, cotton has mechanisms that make it well adapted to semi-arid regions [7]. An understanding of the response of cultivars to water deficits is also important to model cotton growth and estimate irrigation needs [8]. The alteration of metabolites due to drought was previously reported for other plant species and considered to be responsible for drought stress tolerance [9,10]. Similarly, it was imperative to understand the metabolic changes in *G. hirsutum* under water stress condition, so that the drought stress tolerance metabolite can be investigated. Further, the finding of this study will helpful for agriculture researchers in better understanding of metabolic pathways during abiotic stress.

Material and Methods

Cotton seeds were purchased from Central Institute for Cotton research, Regional station, Coimbatore, Tamil Nadu, India. Seeds were sown in trays (52 cm x 27 cm) placed in a cultivation chamber, the seedlings were transplanted into pots. On fully matured cotton plants (after four month), water stress was done for 48 hours in few pots. For metabolic analysis, five replicates of each sample were taken from each group i.e. healthy and drought stressed plants.

Dried samples of 3g each leaves and stems were taken for extraction with hexane (1:10 w/v). The solvent portion was collected by filtration and repeated five times until the hexane layer became almost colourless. The separated solvent layer was concentrated under reduced pressure. The resulting sticky mass was stored at -5 ºC. Volatile trimethylsilyl (TMS) derivatives of the samples were prepared by using 3.6 mg of the sample, 40 µl of methoxylamine hydrochloride in GC grade pyridine (20 mg/ml). The mixture was shaken for 2 h at 37 °C in a temperature controlled vortex, followed by the addition of 70 µl of the N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and followed by continuous shaking

for 30 min. The GC-MS analysis was performed using a GCs-Agilent 7890 A coupled with a 5975 C MS: MS detector and Electron Impact Ionization to generate mass spectra. The scan mass range was 30m/z-600m/z and the total run time in minutes was 54 min.

The resulting GC-MS profile was analyzed using the NIST mass spectral library and by matching the chromatogram with appropriate standards. The estimation of the metabolites was done using the percentage peak area that appeared at the total ion chromatogram in the GC-MS analysis. The molecular weights and fragmentation patterns were ascertained by use of the NIST library and the Duke phytochemical data base.

Statistical analysis of GC-MS data was carried out by Mann-Whitney U test without normal distribution using statistical software SYSTAT version 12.0 (Microsoft Corp. SYSTAT Software, Inc., USA).

Results and Discussion

Metabolic profile analysis upon control and drought stress treatment-

Different class of non-polar metabolites were identified from non-polar extracts of leaf and stem of *G. hirsutum* (Table 1).

Table1: Mass data of GC-MS identified metabolites from control and water-stressed *G. hirsutum* leaf and stem**.**

Serial Number	tR (min)	Compound Name	Molecular Formula	Molecular Weight	Mass Data *(m/z)*
1.	11.66	Dodecene	$C_{12}H_{24}$	168	*m/z* 168 (M^+) (6%), 97 (24%), 84 (28%), 83 (30%), 70 (48%), 56 (62%), 55 (72%), 43 (100%)
2.	17.12	Tetradecene	$C_{14}H_{28}$	196	*m/z* 196 (M^+) (2%), 125 (8%), 111 (34%), 97 (70%), 70 (82%), 69 (100%), 55 (78%),
3.	17.45	Nonanoic acid	$C_{12}H_{26}O_2Si$	230	*m/z* 230 (M^+) (2%), 215 (70%), 129 (22%), 117 (52%), 97 (62%), 73 (100%), 75 (80%)
4.	19.75	L-Lysine	$C_{18}H_{46}N_2O_3Si_4$	450	*m/z* 450 (M^+) (2%), 360 (4%), 258 (12%), 232 (34%), 172 (30%),102 (88%), 77 (48%), 73 (100%)
5.	19.87	Caryophyllene	$C_{15}H_{24}$	204	*m/z* 204 (M^+) (2%), 189 (24%), 147 (34%), 133 (84%), 105 (58%), 93 (74%), 69 (100%)
6.	22.36	Quinoline derivative	$C_{18}H_{18}N_2O$	278	*m/z* 278 (M^+) (16%), 264 (20%), 263 (100%), 73 (26%)
7.	24.23	2-Keto-d-gluconic acid	$C_{21}H_{50}O_7Si_5$	554	*m/z* 554 (M^+) (2%), 437 (22%), 292 (10%), 217 (30%), 204 (72%), 73 (100%) (Me_3Si)
8.	24.56	Cinnamic acid	$C_{12}H_6O_2Si$	220	*m/z* 220 (M^+), (98%), 215 (72%), 132 (26%), 75 (94%), 73 (100%)
9.	25.86	Maleic acid dibutylester	$C_{12}H_{20}O_4$	228	*m/z* 228 (M^+) (2%), 173 (10%), 155 (16%), 117 (42%), 57 (48%), 41 (38%), 99 (100%)

10.	26.15	Butanal	$C_{18}H_{45}NO_5Si_4$	467	*m/z* 467 (M^+) (2%), 307 (28%), 217(20%), 160(10%), 147 (18%), 103 (64%), 73 (100%),
11.	26.39	2-Methylhexadecan-1-ol	$C_{17}H_{36}O$	256	*m/z* 256 (M^+) (2%), 125 (10%), 111 (22%), 97 (38%), 71 (52%), 69 (58%), 57 (100%)
12.	26.72	Octadecene	$C_{18}H_{36}$	252	*m/z* 252 (M^+) (2%), 139 (10%), 111 (44%), 97 (89%), 83 (92%), 69 (76%), 57 (100%),
13.	27.78	Phytol	$C_{20}H_{40}O$	296	*m/z* 296 (M^+) (2%), 123 (28%), 95(32%), 82 (38%), 81 (46%), 71 (100%), 57 (64%)
14.	28.53	Myristic acid	$C_{14}H_{28}O_2$	300	*m/z* 300 (M^+) (4%), 285 (86%), 145 (3 132 (18%), 75 (100%), 73 (80%)
15.	29.61	Tridecanedial	$C_{13}H_{24}O_2$	212	*m/z* 212 (M^+) (2%), 150 (18%), 109 (42%), 95 (96%), 81 (78%), 67 (84%), 55 (100%)
16.	29.94	Hexadecanol	$C_{19}H_{42}OSi$	314	*m/z* 314 (M^+) (2%), 300 (22%), 299 (100%), 103 (18%), 75 (50%), 73 (22%)
17.	31.12	Nonadecane	$C_{18}H_{38}$	266	*m/z* 266 (M^+) (2%), 111 (32%), 97 (62%) 83 (64%), 57 (80%), 55 (92%), 43 (98%), 41 (100%)
18.	32.16	QuinolineAcetamide derivative	$C_{20}H_{18}N_2O_5$	366	*m/z* 366 (M^+) (28%), 351 (26%), 235 (68%), 219 (58%), 75 (38%), 73 (100%)
19.	32.22	Palmitic acid	$C_{19}H_{40}O_2Si$	328	*m/z* 328 (M^+) (4%), 314 (6%), 313 (34%), 201 (2%), 145 (26%), 132 (38%), 117 (72%), 75 (82%)
20.	35.87	Dibutylphthalate	$C_{16}H_{22}O_4$	278	*m/z* 278 (M^+) (2%), 149 (100%), 150 (10%), 104 (6%), 41 (8%)
21.	36.05	Linoleic acid	$C_{21}H_{40}O_2Si$	352	*m/z* 352 (M^+) (6%), 337 (70%), 129 (44%), 95 (40%), 73 (100%), 54 (52%)
22.	36.14	Stearic acid	$C_{18}H_{36}O_4$	284	*m/z* 284 (M^+) (4%), 145 (24%), 132 (38%), 129 (64%), 117 (72%), 75 (72%), 73 (100%)
23.	38.32	Docosene	$C_{22}H_{44}$	308	*m/z* 308 (M^+) (2%), 139 (6%), 125 (12%), 111 (28%), 97 (62%) ,69 (68%), 55 (100%)
24.	41.50	n-Eicosanol	$C_{20}H_{42}O$	298	*m/z* 298 (M^+) (2%), 153 (4%), 139 (6%), 125 (12%), 111 (30%), 97 (52%) 53 (60%)
25.	44.60	Dioctylphthalate	$C_{24}H_{38}O_4$	390	*m/z* 390 (M^+) (2%), 280 (4%), 279 (20%), 167 (40%), 149 (100%), 113 (14%), 71 (26%), 57 (38%)
26.	47.25	Nonacosanol	$C_{29}H_{60}O$	424	*m/z* 424 (M^+) (2%), 139 (10%), 125 (22%), 111 (38%), 97 (90%) ,69 (68%), 57 (100%)
27.	48.22	Octacosanol	$C_{31}H_{66}OSi$	482	*m/z* 482 (M^+) (2%), 468 (12%), 467 (76%), 111 (18%), 103 (44%), 83 (34%), 75 (100%), 57 (58%)
28.	52.56	Campesterol	$C_{31}H_{56}OSi$	472	*m/z* 472 (M^+) (4%), 343 (28%), 257 (20%), 147 (24%), 137 (44%), 69 (74%), 73 (100%), 57 (72%)
29.	53.77	Stigmasterol	$C_{32}H_{58}OSi$	486	*m/z* 486 (M^+) (38%), 398 (6%), 255 ((34%), 147 (36%), 129 (18%), 95 (34%), 73 (

Major metabolites in leaf:

Total 17 non-polar metabolites were detected from leaves of water stressed *G. hirsutum*. The higher amount of quinoline derivative (26.37%), 2- methylhexadecan-1-ol (7.47%), phytol (7.71%), myristic acid (5.94%), hexadecanol (14.30%), nonadecane(1.67%) and palmitic acid (3.20%) were detected in water stressed leaves in compare to control. Moreover two metabolites i.e. caryophyllene and phytol were detected only in stressed leaves.

The higher amount of metabolites cinnamic acid (23.93%), octadecene (6.74%), quninoline acetamide derivative (1.03%) and stearic acid (2.06%) were present in control leaf in compare to stressed leaf. While the higher amount of quinoline derivative (26.37%), myristic acid (5.94%), hexadecanol (14.30%), nonadecane(1.67%) and palmitic acid (3.20%) were detected in stressed leaf in compare to control leaf.

The other non-polar metabolites such as 2-Keto-d-gluconic acid (7.13%), maleic acid dibutylester (1.16%), butanal (2.92%) and tridecanedial (1.63%) were detected only in control leaf while caryophyllene (0.58%) and phytol (7.71%) were present only in stressed leaf (Table 2).

Table 2: Variation of non-polar metabolites in control and water stressed *G. hirsutum* leaf.

Serial Number	Compound Name	Control Leaf (Area %)	Stress Leaf (Area %)
1.	Caryophyllene	ND	0.58 ± 0.02[a]
2.	Quinoline derivative	7.70±0.11[a]	26.37±0.29[a]
3.	2-Keto-d-gluconic acid	7.13± 0.17[a]	ND
4.	Cinnamic acid	23.93± 0.49[a]	9.18 ± 0.11[a]
5.	Maleic acid dibutylester	1.16± 0.07[a]	ND
6.	Butanal	2.92± 0.24[a]	ND
7.	2- Methylhexadecan-1-ol	1.05± 0.01[a]	7.47 ±0.07[a]
8.	Octadecene	6.74± 0.38[a]	1.64 ± 0.17[a]
9.	Phytol	ND	7.71 ± 0.02[a]
10.	Myristic acid	0.63± 0.01[a]	5.94 ±0.04[a]
11.	Tridecanedial	1.63± 0.03[a]	ND
12.	Hexadecanol	6.14± 0.24[a]	14.30±0.94[a]
13.	Nonadecane	0.49± 0.05[a]	1.67 ± 0.05[a]
14.	QuinolineAcetamide derivative	1.03± 0.06[a]	0.79 ± 0.12[a]
15.	Palmitic acid	0.81± 0.21[a]	3.20 ± 1.39[a]
16.	Dibutylphthalate	1.43± 1.05	0.88 ± 0.57
17.	Stearic acid	2.06± 0.03[a]	0.43 ± 0.21[a]

Mean values ± SD (standard deviation) values of mg/gm of fresh weight. ND = Not Detected;
a denotes statistical significance $P = .05$ between groups (control vs stress).

Major metabolites in stem:

Total 14 non-polar metabolites were detected from water stressed *G. hirsutum* stem (Table 3). The higher amount of L-lysine (0.65%), linoleic acid (10.26%) and campesterol (0.87%) were detected in water stressed stem in compare to control, while the other metabolites were slightly decreased than control.

The higher average amount of maleic acid dibutylester (0.72%) and dioctylphthalate (4.56%) were detected in control stem compare to stress. The higher average amount of dodecene (1.67%), L-lysine (0.65%), linoleic acid (10.26%) and campesterol (0.87%) were found in stress stem compare to control. 2- methylhexadecan-1-ol (0.73%) was present only in control stem. Statistically significant variation (P = .05) in above metabolites content was found between control and stressed *G. hirsutum* stem (Table 3).

Table 3: Variation of non-polar metabolites in control and water stressed *G. hirsutum* stem.

Serial Number	Compound Name	Control Stem (Area %)	Stress Stem (Area %)
1.	Dodecene	1.04 ± 0.04[a]	1.67 ± 0.11[a]
2.	Nonanoic acid	5.36 ± 0.24	5.24 ± 0.05
3.	L-Lysine	0.43± 0.11[a]	0.65 ± 0.06[a]
4.	Quinoline derivative	28.01 ±0.17	25.87± 1.16
5.	Maleic acid dibutylester	0.72± 0.11[a]	0.51 ± 0.03[sa]
6.	2- Methylhexadecan-1-ol	0.73± 0.03[a]	ND
7.	Dibutylphthalate	4.85± 0.21	5.06 ± 1.88
8.	Linoleic acid	3.63± 0.49[a]	10.26±0.07[a]
9.	Docosene	3.47± 0.23	3.05 ± 0.28
10.	n-Eicosanol	2.20± 0.08	2.06 ± 0.25
11.	Dioctylphthalate	4.56± 0.07[a]	3.77 ± 0.09[a]
12.	Nonacosanol	0.50± 0.06	0.46 ± 0.05
13.	Campesterol	0.31± 0.04[a]	0.87 ± 0.04[a]
14.	Stigmasterol	0.44± 0.26	1.13 ± 0.55

Mean values ± SD (standard deviation) values of mg/gm of fresh weight. ND= Not Detected;
a denotes statistical significance P = .05 between groups (control vs stress).

Mainly 2- methylhexadecan-1-ol, hexadecanol and palmitic acid in leaf while linoleic acid in stem was found to be accumulating upon drought stress treatment. The accumulation of these metabolites was previously reported for other plant species and these metabolites were observed to be responsible for drought stress tolerance [9,10]. Moreover, plant sterol i.e. campesterol was found in high amount in stress stem. Plant sterols regulate fluidity and permeability of phospholipid bilayer [11], cell division and plant growth [12]. Sterols are also essential for synthesis of prostaglandins and leukotrienes, important component for immune system [13].

Conclusion

Metabolic analysis of *Gossypium hirsutum* leaves and stem revealed an alteration in metabolites in response to water stress. This study provides information on different class of metabolites that include major fatty acids, aldehydes, phytosterols etc. In general, it was observed that the amount of major metabolites such as 2- methylhexadecan-1-ol, phytol, myristic acid, hexadecanol, palmitic acid, linoleic acid and campesterol increases, while the amount of cinnamic acid, octadecene and stearic acid decreases during stress. These metabolites might have played an important role in drought stress tolerance. Moreover, this finding can be used for the better understanding of various metabolic pathways during abiotic stress in *G. hirsutum.*

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