

Study on Variation of non-polar metabolites in *Gossypium hirsutum* L. under water stress condition using gas chromatography-mass spectroscopy technique

Abstract

Present work was aimed at studying variation of non polar metabolites content in *Gossypium hirsutum* L. under water stress condition using a gas chromatography-mass spectrometry (GC-MS) technique. A total 17 non-polar metabolites were detected in control and water stressed *G. hirsutum* leaf. The major metabolites were quinoline derivative ($26.37 \pm 0.29\%$), 2-methylhexadecan-1-ol ($7.47 \pm 0.07\%$), phytol ($7.71 \pm 0.02\%$), myristic acid ($5.94 \pm 0.04\%$), hexadecanol ($14.30 \pm 0.94\%$), nonadecane ($1.67 \pm 0.05\%$) and palmitic acid ($3.20 \pm 1.39\%$). Fourteen metabolites were detected in control and water stressed *G. hirsutum* stem. The major metabolites were dodecene ($1.67 \pm 0.11\%$), L-lysine ($0.65 \pm 0.06\%$), dibutylphthalate ($5.06 \pm 1.88\%$), linoleic acid ($10.26 \pm 0.07\%$), campesterol ($0.87 \pm 0.04\%$) and stigmasterol ($1.13 \pm 0.55\%$). Statistical analysis of GC-MS data was carried out by Mann-Whitney U test without normal distribution using statistical software SYSTAT version 12.0. Significant variation in content of the most of the metabolites were observed between control and water stressed leaf or stem (Mann-Whitney U test, $P = .05$). It concludes that the major metabolites played an important role during water stress and can be consider as metabolites responsible for water stress tolerance in *G. hirsutum*.

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

Introduction

Cotton is one of the most important industrial crop comes under the genus “*Gossypium*” in the Malvaceae family and popularly known as “white gold” [1]. Globally, the *Gossypium* genus includes about 50 species [2]. Mainly four species in the genus *Gossypium*, namely *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L. were domesticated

independently as sources of textile fibre. *Gossypium hirsutum* L. was named due to its hairiness (hirsute), it is commonly known as upland cotton, American cotton or Mexican cotton [3]. Globally, about 90% of all cotton production is of cultivars derived from this species. It is native to Mexico, the West Indies, northern South America, Central America and possibly tropical Florida. *Gossypium hirsutum* includes a number of varieties or cultivars with varying quality. Cotton requires a minimum temperature of 16 °C during germination, 21 °C to 27 °C for proper crop growth and during the fruiting phase, the temperature ranging from 27 °C to 32 °C. It is cultivated largely under rain fed or dry land conditions and its harvesting period from mid-September to November [4]. It can successfully grow on all soils except sandy, saline or water logged types. It is moderately tolerant to salinity but sensitive to water logging as well as frost and extreme cold temperature [5]. Cotton has been utilized as fibre material since ancient times [6]. It is harvested as seed cotton which then ginned in order to separate the seed and linter. Processed cotton (linter) can be used in a variety of products including foods. The linters which have a longer fibre length can be used in the production of mattresses, furniture upholstery and mops. While the linters which have a much shorter fibre length are a major source of cellulose for both food and other applications. It is also used in a variety of products including edible vegetable oils and margarine, soap and plastics. Its seeds and flour or hulls are also used in food products for animal feed [7].

Water stress is one of the most important environmental factor which affects crop productivity and adversely affects fruit production, square and boll shedding and fiber quality in cotton [8]. Moreover, water stress is considered as the single most devastating environmental factor [9]. It severely affects plant development with substantial reductions in crop growth rate and biomass accumulation by reduction in the cell division, root proliferation, plant water and nutrient relations [10, 11]. 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 [12]. Thus, screening cotton varieties for resistance to water stress conditions and improving cotton tolerance to this stress conditions will mitigate negative consequences of this adversity. Cotton is normally not classified under water stress tolerant crop as some other plants species like sorghum [13]. Nevertheless, cotton has mechanisms that make it well adapted to semi-arid regions [14]. An understanding of the response of cultivars to water deficits is also important to model cotton growth and estimate irrigation needs [15]. The alteration of metabolites due to water stress

was previously reported for plant species and considered to be responsible for water stress tolerance [16, 17].

Lv *et al.* evaluated five homozygous transgenic *G. hirsutum* L. plants under water stress condition and the result suggested that glycine betaine may be involved in osmotic adjustment in the plant [18]. Rodriguez-Urbe *et al.* used microarray analysis to identify water deficit-responsive genes in the *G. hirsutum* under water stress conditions [19]. Yoo and Wendel, conducted comparative transcriptome profiling of developing *G. hirsutum* fibres using RNA-Seq by Illumina sequencing [20]. Although some other aspect of the changes in *G. hirsutum* under water stresses conditions have been reported. But there were no reports on a thorough study on non polar metabolites content and their variation in *G. hirsutum* under water stress condition. This can be an important study for identifying the metabolites responsible for water stress tolerance in *G. hirsutum* under water stress condition. Therefore, it was imperative to study the variation of non-polar metabolites in *G. hirsutum* plants under water stressed condition. Furthermore, identifying the metabolites responsible for water stress tolerance may be helpful for agriculture researchers in understanding metabolic pathways during water stress. To the best of our knowledge, this was the first study which deals with the variations of non-polar metabolites content in *G. hirsutum* L. plants under water stressed condition by gas chromatography-mass spectrometry (GC-MS) method.

Material and Methods

Cotton seeds were purchased from Central Institute for Cotton research, Regional station, Coimbatore, Tamil Nadu, India. These seeds were sown in trays (52 cm x 27 cm) placed in a cultivation chamber. The seedlings were transplanted into pots. After four months, the best plants of approximately the same height and with the same number of leaves were selected for the study (Figure.1). Further, these selected plants were divided into two groups. First group of plants were irrigated in every 12 hour interval at room temperature and considered as control plant. While second group plants were maintained in the same environment as the control plants but without addition of water to the container for 4 days. This would allow the pots to dry out and plants were then considered as water stressed. Finally leaf and stem samples were collected from each group of plants for further study.



Figure 1. Selected plant of *G. hirsutum*

Dried samples of 3g of 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 by using evaporator. The resulting sticky mass was stored at -5 °C. 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). Thereafter, the mixture was further continuously shaken for 30 min at the same condition. After completion of TMS derivatization, 1µl of derivatized mixture was taken for GCMS analysis. 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 split was 1:90, with helium as the carrier gas at a flow rate of 1 ml/min, while the damping gas flow was 0.3 ml/min. The GC oven temperature program was as follows: 40 °C-220 °C, by ramping at 3 °C, and held at 220 °C for 20 min. The injector temperature was maintained at 220 °C and the transfer line was held at 220 °C.

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.

The Mann-Whitney U test, a nonparametric test of the null hypothesis was used to compare differences in metabolites content between two independent groups i.e., control and water stressed leaf or stem. Statistical analysis of metabolites 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

Different class of non-polar metabolites were identified from non-polar extracts of leaf and stem of *G. hirsutum* (Table 1). Plotted GCMS chromatogram of the control and water stressed leaf of *G. hirsutum* are shown in figure 2 and 3.

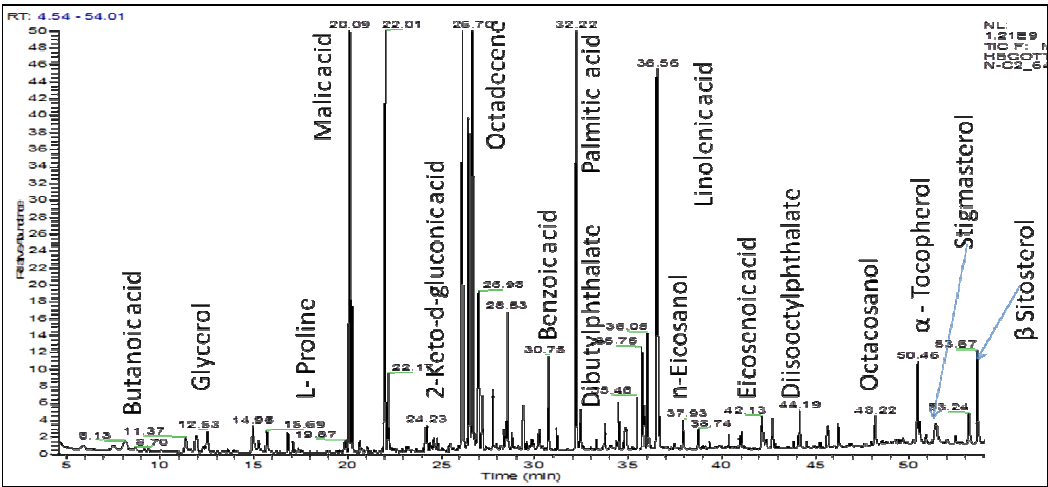


Figure 2. Major non polar metabolites in control *G. hirsutum* leaf

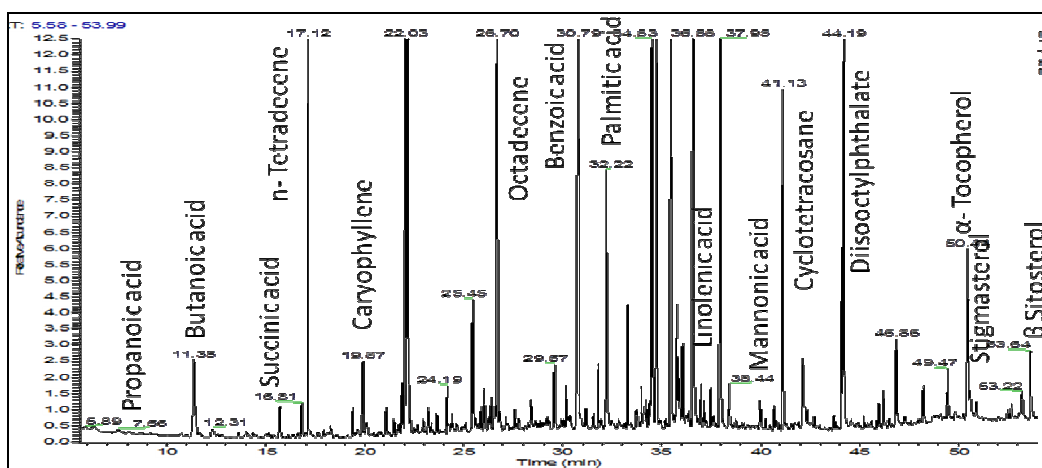


Figure 3. Major non polar metabolites in water stressed *G. hirsutum* leaf

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

Serial Number	tR (min)	Compound	Molecular Weight	Mass Data (m/z)
1.	11.66	Dodecene	168	m/z 168 (M^+) (6%), 97 (24%), 84 (28%), 83 (30%), 70 (48%), 56 (62%), 55 (72%), 43 (100%)
2.	17.12	Tetradecene	196	m/z 196 (M^+) (2%), 111 (34%), 97 (70%), 70 (82%), 69 (100%), 55 (78%),
3.	17.45	Nonanoic acid	230	m/z 230 (M^+) (2%), 215 (70%), 129 (22%), 117 (52%), 97 (62%), 73 (100%), 75 (80%)
4.	19.75	L-Lysine	450	m/z 450 (M^+) (2%), 258 (12%), 232 (34%), 172 (30%), 102 (88%), 77 (48%), 73 (100%)
5.	19.87	Caryophyllene	204	m/z 204 (M^+) (2%), 189 (24%), 147 (34%), 133 (84%), 105 (58%), 93 (74%), 69 (100%)
6.	22.36	Quinoline derivative	278	m/z 278 (M^+) (16%), 264 (20%), 263 (100%), 73 (26%)
7.	24.23	2-Keto-d-gluconic acid	554	m/z 554 (M^+) (2%), 437 (22%), 217 (30%), 204 (72%), 73 (100%)
8.	24.56	Cinnamic acid	220	m/z 220 (M^+), (98%), 215 (72%), 132 (26%), 75 (94%), 73 (100%)
9.	25.86	Maleic acid dibutylester	228	m/z 228 (M^+) (2%), 173 (10%), 155 (16%), 117 (42%), 57 (48%), 41 (38%), 99 (100%)
10.	26.15	Butanal	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	256	<i>m/z</i> 256 (M^+) (2%), 111 (22%), 97 (38%), 71 (52%), 69 (58%), 57 (100%)
12.	26.72	Octadecene	252	<i>m/z</i> 252 (M^+) (2%), 111 (44%), 97 (89%), 83 (92%), 69 (76%), 57 (100%),
13.	27.78	Phytol	296	<i>m/z</i> 296 (M^+) (2%), 123 (28%), 95(32%), 82 (38%), 81 (46%), 71 (100%), 57 (64%)
14.	28.53	Myristic acid	300	<i>m/z</i> 300 (M^+) (4%), 285 (86%), 75 (100%), 73 (80%)
15.	29.61	Tridecanedial	212	<i>m/z</i> 212 (M^+) (2%), 150 (18%), 109 (42%), 95 (96%), 81 (78%), 67 (84%), 55 (100%)
16.	29.94	Hexadecanol	314	<i>m/z</i> 314 (M^+) (2%), 300 (22%), 299 (100%), 103 (18%), 75 (50%), 73 (22%)
17.	31.12	Nonadecane	266	<i>m/z</i> 266 (M^+) (2%), 111 (32%), 97 (62%) 83 (64%), 57 (80%), 55 (92%), 43 (98%), 41 (100%)
18.	32.16	Quinoline Acetamide derivative	366	<i>m/z</i> 366 (M^+) (28%), 351 (26%), 235 (68%), 219 (58%), 75 (38%), 73 (100%)
19.	32.22	Palmitic acid	328	<i>m/z</i> 328 (M^+) (4%), 314 (6%), 313 (34%), 145 (26%), 132 (38%), 117 (72%), 75 (82%)
20.	35.87	Dibutylphthalate	278	<i>m/z</i> 278 (M^+) (2%), 149 (100%), 150 (10%), 104 (6%), 41 (8%)
21.	36.05	Linoleic acid	352	<i>m/z</i> 352 (M^+) (6%), 337 (70%), 129 (44%), 95 (40%), 73 (100%), 54 (52%)
22.	36.14	Stearic acid	284	<i>m/z</i> 284 (M^+) (4%), 145 (24%), 132 (38%), 129 (64%), 117 (72%), 75 (72%), 73 (100%)
23.	38.32	Docosene	308	<i>m/z</i> 308 (M^+) (2%), 125 (12%), 111 (28%), 97 (62%) ,69 (68%), 55 (100%)
24.	41.50	n-Eicosanol	298	<i>m/z</i> 298 (M^+) (2%), 125 (12%), 111 (30%), 97 (52%) 53 (60%)
25.	44.60	Diethylphthalate	390	<i>m/z</i> 390 (M^+) (2%), 279 (20%), 167 (40%), 149 (100%), 113 (14%), 71 (26%), 57 (38%)
26.	47.25	Nonacosanol	424	<i>m/z</i> 424 (M^+) (2%), 139 (10%), 125 (22%), 111 (38%), 97 (90%) ,69 (68%), 57 (100%)
27.	48.22	Octacosanol	482	<i>m/z</i> 482 (M^+) (2%), 467 (76%), 111 (18%), 103 (44%), 83 (34%), 75 (100%), 57 (58%)

28.	52.56	Campesterol	472	m/z 472 (M^+) (4%), 343 (28%), 257 (20%), 147 (24%), 137 (44%), 69 (74%), 73 (100%), 57 (72%)
29.	53.77	Stigmasterol	486	m/z 486 (M^+) (38%), 255 (98%), 147 (36%), 129 (18%), 95 (34%),

140

141 Metabolites in leaf

142 Total 17 non-polar metabolites were detected from leaves of water stressed *G. hirsutum*. The
 143 higher amount of quinoline derivative (26.37%), 2- methylhexadecan-1-ol (7.47%), phytol
 144 (7.71%), myristic acid (5.94%), hexadecanol (14.30%), nonadecane(1.67%) and palmitic
 145 acid (3.20%) were detected in water stressed leaves in compare to control. Moreover two
 146 metabolites i.e. caryophyllene and phytol were detected only in stressed leaves.

147 **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

148 Mean values ± SD (standard deviation) values of mg/gm of fresh weight. ND = Not Detected;

149 ^a denotes statistical significance $P = .05$ between groups (control vs stress).

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

155 such as 2-keto-d-gluconic acid (7.13%), maleic acid dibutylester (1.16%), butanal (2.92%)
 156 and tridecanedial (1.63%) were detected only in control leaf. The caryophyllene (0.58%) and
 157 phytol (7.71%) were present only in stressed leaf (Table 2 and Figure 4).

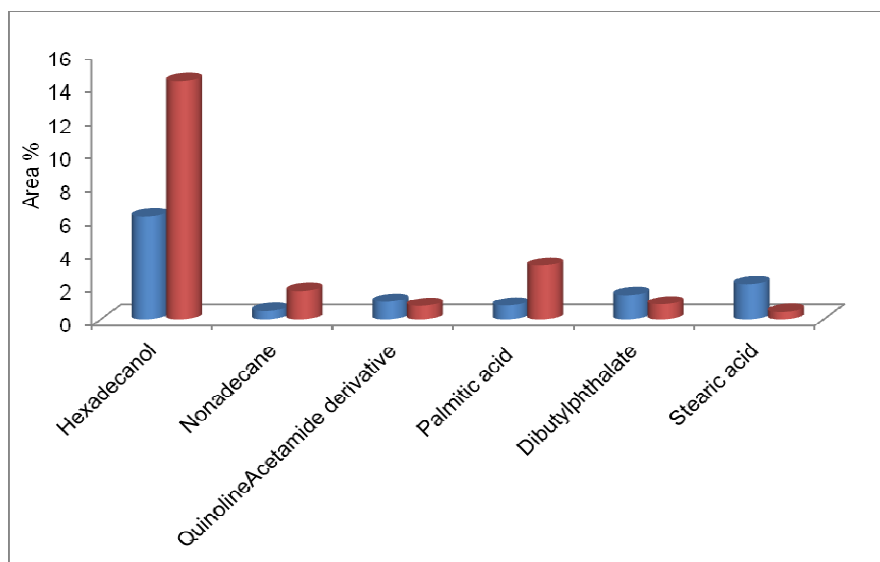


Figure 4. Variation of major non polar metabolites in control vs water stressed *G. hirsutum* leaf

161 Metabolites in stem

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

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.	Diocetylphthalate	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).

The higher average amount of maleic acid dibutylester (0.72%) and dioctylphthalate (4.56%) were detected in control stem compare to stress stem. 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 few metabolites content was found between control and water stressed *G. hirsutum* stem (Table 3, Figure 5).

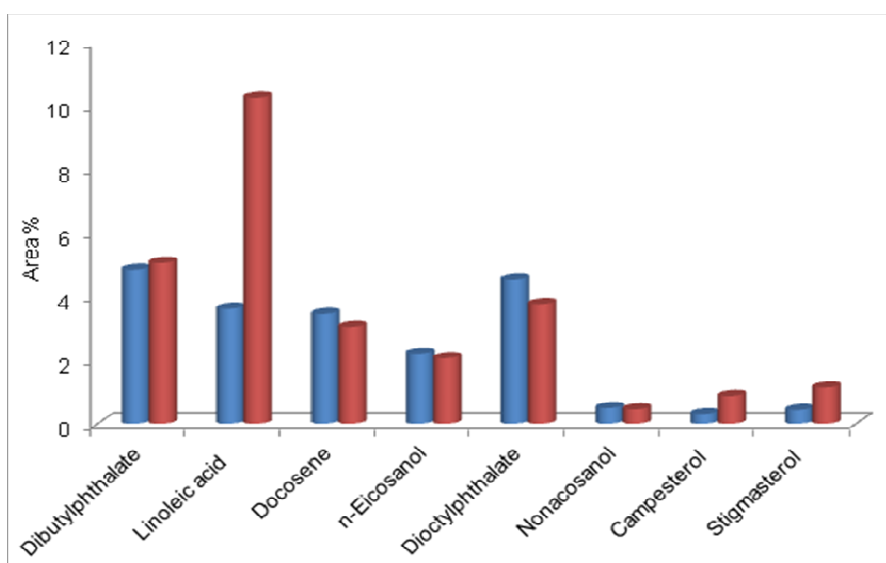


Figure 5. Variation of major non-polar metabolites in control vs water stressed *G. hirsutum* stem

Mainly 2- methylhexadecan-1-ol, hexadecanol and palmitic acid in leaf while linoleic acid in stem was found to be accumulating under water stress condition. The accumulation of these metabolites was previously reported for other plant species. These metabolites were observed to be responsible for water stress tolerance [16, 17]. Moreover, plant sterol i.e. campesterol was found in high amount in stress stem. Plant sterols regulate fluidity and permeability of phospholipid bilayer [21], cell division and plant growth [22]. Sterols are also essential for synthesis of prostaglandins and leukotrienes, important component for immune system [23]. In order to see affect of chemical variation within its species, further study of variation of non-polar metabolites in *G. hirsutum* L. varieties under water stress can be carried out.

188 Conclusion

189 Accumulations of metabolites such as quinoline derivative, 2- methylhexadecan-1-ol, phytol,
190 myristic acid, hexadecanol and palmitic acid were observed in the stressed leaf compare to
191 control leaf. Metabolites like L-lysine, linoleic acid, campesterol and stigmasterol were also
192 accumulated in stressed stem as compare to control stem. While consumption of the
193 metabolites i.e., cinnamic acid, octadecene, stearic acid in leaf and quinoline derivative,
194 docosene, dioctylphthalate in stem was observed. These observations indicate that the
195 selective accumulation and consumption of the metabolites were occurred during the water
196 stress in *G. hirsutum* leaf and stem. It concludes that above metabolites played a crucial role
197 during the water stress and can be considered as metabolites responsible for water stress
198 tolerance in *G. hirsutum*.

199 References

- 200 1. Smith CW. Cotton (*Gossypium hirsutum* L.). Crop Production: Evolution, History,
201 and Technology. John Wiley and Sons, Inc., New York. 1995; 287-349.
202
- 203 2. Fryxell PA. A revised taxonomic interpretation of *Gossypium* L.
204 (*Malvaceae*.) *Rheede*, 1992; 2: 108-165.
205
- 206 3. Smith CW and Cothren JT. Cotton: origin, history, technology and production. John
207 Wiley and Sons, Inc., New York. 1999; 33-64.
208
- 209 4. Oosterhuis DM. Development of a Cotton Plant. In: Seagull, R. and P. Alspaugh (eds)
210 Cotton Fiber Development and Processing, an illustrated overview. 2001;
211 International Textile Center, Texas Tech University, Lubbock, TX.
212
- 213 5. Singh C, Singh P and Singh R. Modern Techniques of Raising Field Crops. Second
214 edition. Oxford and IBH publishing. 2009; 393-399.
- 215 6. Fryxell PA. A revised taxonomic interpretation of *Gossypium* L. (*Malvaceae*.)
216 *Rheede*, 1992; 2: 108-165.
217
- 218 7. Proto M, Supino S. and Malandrino O. Cotton: a flow cycle to exploit. Industrial
219 Crops and Products. 2000; 11: 173-178.
220
- 221 8. El-Zik KM and Thaxton PM. Genetic improvement for resistance to pests and stresses
222 in cotton. In Frisbie, RE, El-Zik, KM and Wilson, LT. (Eds) Integrated pest
223 management systems and cotton production. John Wiley and Sons, New York,
224 N.Y.1989; 191-224.
225
- 226 9. Lambers H, Chapin F, Pons T. Plant physiological ecology. Springer, New York.
227 2008; 540.
228

- 229 10. Li LC, Grimshaw JM, Nielsen CP, PCM Judd Coyte, Graham ID. Evolution of
230 Wenger's concept of community of practice. *Implementation Science*. 2009; 4:11.
231
- 232 11. Farooq M, Wahid A, Kobayashi N, Fujita D and Basra SMA. Plant drought stress:
233 effects, mechanisms and management. *Agronomy for Sustainable Development*.
234 2009; 29: 185-212.
235
- 236 12. Ben-Asher JI, Tsuyuki BA, Bravdo and Sagih M. Irrigation of grapevines with saline
237 water, I. leaf area index, stomatal conductance, transpiration and photosynthesis.
238 *Agriculture Water Management*. 2006; 83: 13-21.
- 239 13. Poehlman ET, Tremblay A, Perusse L et al. Heredity and changes in hormones and
240 metabolic rates with short-term training. *American Journal of Physiology*. 1986; 350:
241 E711-717.
242
- 243 14. Malik MFA, Qureshi AS, Ashraf M and Ghafoor A. Genetic variability of the main
244 yield related characters in soybean. *International Journal of Agriculture and Biology*.
245 2006; 8(6): 815-619.
246
- 247 15. Pace ML, Cole JJ, Carpenter SR, and Kitchell JF. Trophic cascades revealed in
248 diverse ecosystems. *Trends in Ecology and Evolution*. 1999; 14: 483-488.
249
- 250 16. Witt S, Galicia L, Lisec J, Cairns J, Tiessen A, Araus JL et al. Metabolic and
251 phenotypic responses of greenhouse-grown maize hybrids to experimentally
252 controlled drought stress. *Molecular Plant*. 2012; 5: 401-417.
253
- 254 17. Charlton AJ, Donarski JA, Harrison M, Jones SA, Godward J, Oehlschlager S et al.
255 Responses of the pea (*Pisum sativum* L.) leaf metabolome to drought stress assessed
256 by nuclear magnetic resonance spectroscopy. *Metabolomics*. 2008; 4: 312-27.
257
- 258 18. Lv S, Yang A, Zhang K, Wang L and Zhang J. Increase of glycinebetaine synthesis
259 improves drought tolerance in cotton. *Molecular Breeding*. 2007; 20(3): 233-248.
260
- 261 19. Rodriguez-Urbe L, Abdelraheem A, Tiwari R, Sengupta-Gopalan C, Hughs S.E. and
262 Zhang J. Identification of drought-responsive genes in a drought-tolerant cotton
263 (*Gossypium hirsutum* L.) cultivar under reduced irrigation field conditions and
264 development of candidate gene markers for drought tolerance. *Molecular Breeding*.
265 2014; 34(4): 1777-1796.
266
- 267 20. Yoo M and Wendel J. Comparative Evolutionary and Developmental Dynamics of
268 the Cotton (*Gossypium hirsutum*) Fiber Transcriptome. *PLoS Genetics*. 2014; 10(1):
269 e1004073.
270
- 271 21. Hartmann MA. Plant sterols and the membrane environment. *Trends Plant Science*.
272 1998; 3(5): 170-5.
273
- 274 22. Clouse SD, Sasse JM. Brassinosteroids: essential regulators of plant growth and
275 development. *Annual Review Plant Physiology. Plant Molecular Biology*. 1998; 49:
276 427- 51.
277

278 **23.** Horrocks AL, Farooqui AA. Docosahexaenoic acid in the diet: its importance in
279 maintenance and restoration of neural membrane function. Prostaglandins,
280 Leukotrienes and Essential Fatty Acids. 2004; 70(4): 361-372.