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FATTY ACID CHANGES IN *HIBISCUS ESCULENTUS* TISSUES DURING GROWTH

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Abstract—Lipids were isolated from roots, stems, cotyledons, leaves, buds, flowers, pods and seeds of okra (*Hibiscus esculentus*) at different stages of plant growth from germination to seed formation and their fatty acid compositions analysed. The lipid contents of roots and stems were 1–3%, cotyledons 3.7–9%, leaves 2.5–5.1% and seeds 2.2–20.2%. Palmitic, linoleic and linolenic were the main fatty acids present in all tissues at all stages, but their relative proportions varied. Cyclopropene fatty acids (CFA) were present at some stages in roots and seeds. In the roots their formation coincided with bud formation (35 days after sowing) and their content reached a maximum (12.8%) seven days after flowering. CFA were present in maturing seeds from 31 days after flowering and occurred as dihydro derivatives throughout. Dihydro derivatives of the CFA were absent in all other tissues. Heptadecenoic acid was present (0.4–1.3%) in root lipids at all stages and in the stem lipids (0.4–1.2%) in the initial stages and after flowering.

INTRODUCTION

Numerous studies have been reported on the changes in fatty acid composition of storage lipids of seeds during their maturation [1, 2]. Similar studies on structural lipids of various plant tissues during plant growth may elucidate their formation and function. Reported here are the fatty acid changes in tissues of *Hibiscus esculentus* during its growth from germination to seed maturation. Okra seed is a potential source of oil and protein and the oil is reported to contain linoleic, oleic and palmitic as major acids and malvalic and vernolic as very minor acids [3].

RESULTS

Roots

The lipid content was maximum (3.2%) at the end of germination, i.e. 7 days after sowing (DAS) and minimum (1.7%) at the bud formation stage (Table 1). The major acids were 18:2, 16:0 and 18:3, respectively. The formation of CFA, viz. malvalic and sterculic, coincided with bud formation (35 DAS) and their contents continuously increased till the beginning of seed formation (49 DAS). The content of malvalic acid was higher than that of sterculic acid except in the initial stage. Also present were 16:1 and 17:1 (trace–4.7% and 0.4–1.3%, respectively) at all stages.

Stems

The lipid content was maximum (3.6%) at the end of germination (7 DAS) and minimum (1%) at the beginning of seed maturation (Table 2). The major acids were 18:2, 16:0 and 18:3, respectively. Small quantities (0.3–1.2%) of 17:1 were found until leaf formation (14 DAS) and after flowering (42 DAS).

Cotyledons

The lipid content decreased from a maximum of 9% at the end of germination (7 DAS) to a minimum of 3.7% at the leaf formation stage (Table 3). The predominant acid was 18:3; its content was maximum (59.7%) at the leaf formation stage and continuously decreased thereafter. The other major acids were 18:2 and 16:0. The 16:0 content increased until bud formation (28 DAS) and decreased during tissue senescence accompanied by a colour change from green to yellow (35 DAS). During tissue senescence 20:0 and 22:0 were also found.

Leaves

The lipid content varied from 2.6 to 5.1% (Table 4). The predominant fatty acid was 18:3 which increased continuously. A reverse pattern was observed for 18:2.

Buds, flowers and pods

The major acids were 16:0, 18:2 and 18:3 (Table 5). During bud growth the 16:0 content decreased while the 18:1 and 18:3 contents increased. The pod lipids contained the highest content of 16:0 among the tissues studied.

Seeds

The lipid content was maximum (20.2%) by 67 DAS, i.e. 25 days after flowering (DAF) (Table 6). The lipid contents and the fatty acid compositions of the sown seeds (0 DAS) and of the mature seeds (81 DAS, 40

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DAF) were almost the same. The major fatty acids were 18:2, 16:0 and 18:1, respectively. The 16:0 content increased from a minimum of 25.7% (51 DAS, 9 DAF) to a maximum of 39.8% in the fully mature seeds. The 18:3 content decreased continuously throughout. Until the formation of CFA in the seeds on the 31 DAF, 16:1 and 17:1 were present. An epoxy acid was present from 25 DAF. Dihydromalvalic and dihydrosterculic acids were present in all the tissues and were maximum by 16 DAF (0.6 and 2.8%, respectively).

DISCUSSION

The lipid contents decreased from their maximum contents in roots (3.2%), stems (3.6%) and cotyledons (9%) at the end of germination to their minimum contents in roots (1.7%) at the bud formation stage, in stems (1%) at the beginning of seed formation and in cotyledons (3.7%) at the leaf formation stage, perhaps due to their utilization in tissue growth. After reaching a minimum the lipid content increased in roots and cotyledons perhaps due to a decreased rate of mobilization and increased rate of lipid synthesis.

Table 1. Changes in *H. esculentus* root fatty acids during plant growth

Days after sowing	Plant growth stage	Lipid % (dry basis)	Fatty acids* (wt%)									
			16:0	16:1	17:1	18:0	18:1	18:2	18:3	22:0	18:1† (CFA)	19:1† (CFA)
7	Germination	3.2	23.6	Trace	1.3	1.8	7.9	52.2	13.5	0.0	0.0	0.0
14	Leaf formation	2.0	30.0	4.7	1.0	2.4	7.0	41.4	13.2	Trace	0.0	0.0
21		2.2	30.0	2.6	0.4	2.9	12.3	37.1	14.4	Trace	0.0	0.0
28	Bud formation	1.7	22.7	2.5	0.8	1.8	11.7	43.3	15.6	1.0	0.0	0.0
35		2.3	18.6	1.9	0.8	1.2	10.9	42.7	18.7	0.8	1.8	2.6
42	Flower formation	2.4	20.1	1.3	0.8	1.6	11.9	39.6	16.0	1.0	5.1	2.4
49	Seed maturation	2.7	26.8	2.5	0.8	1.9	9.8	31.2	11.3	2.7	9.2	3.6
56		2.5	26.5	1.6	1.0	2.6	9.6	33.1	11.4	3.4	7.0	3.4

*12:0, 14:0 and 20:0 were also present at all the stages (each $< 0.5\%$).
†Cyclopropene fatty acids.

Table 2. Changes in *H. esculentus* stem fatty acids during plant growth

Days after sowing	Plant growth stage	Lipid % (dry basis)	Fatty acids* (wt%)						
			16:0	16:1	17:1	18:0	18:1	18:2	18:3
7	Germination	3.6	26.1	Trace	1.2	3.3	6.8	50.6	12.1
14	Leaf formation	1.5	33.2	Trace	Trace	2.6	3.6	43.7	16.3
21		1.8	29.2	Trace	0.0	1.8	4.9	45.4	18.1
28	Bud formation	1.6	34.3	Trace	0.0	4.7	6.2	37.9	14.6
35		1.7	32.4	Trace	0.0	3.5	5.1	39.7	18.7
42	Flower formation	1.0	25.7	Trace	0.3	1.1	9.4	47.9	15.8
49	Seed maturation	1.1	28.5	1.0	0.7	1.8	8.2	39.7	18.9
56		1.0	16.7	0.9	0.4	0.7	6.5	40.7	25.7

*12:0, 14:0 and 22:0 were also present (each $< 0.5\%$). The sample at 56 DAS contained 7% of 20:0 and other samples traces.

Table 3. Changes in *H. esculentus* cotyledon fatty acids during plant growth

Days after sowing	Plant growth stage	Lipid % (dry basis)	Fatty acids* (wt%)						
			16:0	16:1	18:0	18:1	18:2	18:3	22:0
7	Germination	9.0	16.7	3.4	1.5	3.0	24.0	51.3	0.0
14	Leaf formation	3.7	17.5	2.3	1.3	2.0	17.1	59.7	Trace
21		8.1	19.8	3.2	1.7	1.5	17.2	55.8	0.0
28	Bud formation	7.3	26.1	3.9	1.6	2.3	14.8	52.8	0.0
35		6.9	21.7	2.7	2.1	3.2	16.3	49.6	2.5
41	Flower formation	5.3	17.0	1.5	4.1	6.8	18.7	48.8	1.0

*12:0, 14:0 and 20:0 were also present (each $< 0.6\%$).

The fatty acid compositions are expressed as wt percentages (Tables 1–6). The same conclusions are drawn when the fatty acid contents are calculated as mg/100 g dry tissue. The major fatty acids were 18:2, 16:0 and 18:1 in the seeds and 16:0, 18:2 and 18:3 in the other tissues, the predominant one being 18:2 in roots, stems, buds, flowers and seeds, 18:3 in cotyledons and leaves and 16:0 in pods. Cotyledons and leaves resembled each other in their fatty acid changes which is not surprising since the cotyledon is a photosynthetic tissue like the leaf. The CFA were absent from leaves and stems, although they were reported to be present in the same tissues of a few plants of Malvaceae [4]. The fatty acid composition of buds just before flowering resembled that of flowers. As expected the fully mature seeds did not differ in fatty acid composition from the sown seeds. An epoxy acid, which was present in traces both in the sown and mature seeds, was first observed in maturing seeds only after lipid accumulation (25 DAF) was complete.

Malvalic (18:1 CFA) and sterculic (19:1 CFA) acids were found in the roots after bud formation and their contents continuously increased. While 17:1 and 16:1 were also present, no dihydroderivatives of CFA were found in roots. In maturing seeds, dihydro derivatives of CFA were found throughout while the CFA were observed only in traces at the final stage of maturity. The 17:1 and 16:1 were also found at all stages except in fully mature seeds. The occurrence of CFA was reported [5] in the root lipids of *Ceiba pentandra* (Malvaceae) and *Sterculia foetida*

Table 4. Changes in *H. esculentus* leaf fatty acids during plant growth

Days after sowing	Plant growth stage	Lipid % (dry basis)	Fatty acids* (wt%)					
			16:0	16:1	18:0	18:1	18:2	18:3
14	Leaf formation	4.0	21.1	Trace	1.1	2.8	31.7	43.2
21		2.6	16.4	3.8	0.8	2.4	23.3	51.3
28	Bud formation	4.2	21.4	3.7	2.0	2.2	19.6	53.7
35		3.8	20.1	3.7	1.2	1.8	16.3	56.5
42	Flower formation	4.6	12.0	3.2	0.9	1.8	15.8	57.1
49	Seed maturation	4.7	14.5	1.3	0.7	1.6	21.2	60.3
56		5.1	21.0	2.8	1.8	2.5	23.8	46.5

*12:0, 14:0 and 22:0 were present (each $< 0.8\%$). The 42 DAS sample contained 8% of 20:0 and other samples traces.

Table 5. Fatty acid composition (wt%) of *H. esculentus* bud, flower and pod

Plant tissue	Lipid % (dry basis)	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0
Bud (initial)	2.3	0.2	0.3	35.7	Trace	2.1	5.9	39.8	16.2	0.0	0.0
Bud (final)	2.6	0.1	0.2	20.9	1.6	2.0	13.0	35.5	27.6	Trace	0.5
Flower	3.8	Trace	0.2	23.6	Trace	2.3	11.6	36.9	24.4	Trace	1.0
Pod (51 DAS, 9 DAF)	2.8	0.2	0.3	45.8	0.0	4.9	11.1	30.2	6.9	0.6	0.0

Table 6. Changes in *H. esculentus* seed fatty acids during plant growth

Days after sowing	Days after flowering	Lipid % (dry basis)	Fatty acids* (wt%)								
			16:0	16:1	17:1	18:0	18:1	18:2	18:3	18:0 (cyclopropane)	19:0 (cyclopropane)
0		18.0	39.3	Trace	0.0	2.6	16.5	41.4	Trace	0.2	1.0
51	9	2.2	25.7	0.6	1.1	1.4	8.6	53.8	7.2	Trace	0.5
58	16	8.6	29.6	Trace	Trace	4.4	16.9	43.8	1.6	0.6	2.8
67	25	20.2	32.0	1.7	0.2	3.8	15.4	46.1	0.3	0.2	1.3
73	31	20.0	27.6	1.1	Trace	3.8	17.3	47.4	0.3	0.4	1.2
81	40 (mature)	18.3	39.8	0.0	0.0	2.6	17.7	40.6	Trace	0.2	0.8

*12:0 and 14:0 were present in all the samples (each $< 0.5\%$). Epoxy acid was present from 25 DAF and CFA from 31 DAF in traces.

(Sterculaceae). The CFA were found in roots of *S. foetida* both at the mature and immature stages of seed maturation [6]. Dihydro derivatives of the CFA were also found in appreciable concentrations in roots [5] and in traces in seeds ([5], unpublished observations) of *C. pentandra* and *S. foetida*.

CFA are postulated to be synthesized in the seeds by the addition of a methylene group across the double bond of a monoenoic acid, followed by desaturation [7, 8]. Oleic acid is thus the precursor for sterculic acid and 17:1 is the precursor of malvalic acid. Malvalic acid can also be synthesized by α-oxidation of dihydrosterculic acid followed by desaturation [8]. Acetylenic acids are also postulated to be precursors for CFA in which pathway dihydroderivatives are not expected as intermediates [9]. The occurrence of CFA, dihydro derivatives of CFA and 17:1 and the absence of acetylenic acid (as seen by Ag^+ TLC, GC and IR) in seed lipids lend support to the operations of methylation, α-oxidation and desaturation. The same pathway appears to operate for synthesis of CFA in root lipids although the intermediates, namely, dihydro derivatives of CFA were not detected. It appears that the formation of CFA from their dihydro derivatives is slower in seeds compared to that in roots of *H. esculentus*. The biosynthesis of CFA and their function in root lipids deserve further study.

EXPERIMENTAL

Okra seeds (var. Pusa savani) were purchased from National Seeds Corporation, Hyderabad. Seeds were sown in prepared plots (% germination, 94). Roots, stems, cotyledons, leaves and seeds were collected at intervals. Buds, flowers, and pods were also analysed. Moisture content was determined by heating in an air-oven at 110° to constant wt.

Extraction of lipid. The plant tissue was ground in a mortar and extracted $\times 3$ with $CHCl_3$–MeOH (2:1) at room temp. The extract was concd at 40° in a rotary vacuum evaporator, diluted with H_2O and extracted $\times 3$ with petrol. The petrol extract was washed with 0.9 N NaCl soln, dried, concd, and transferred quantitatively into a 50 ml standard flask. An aliquot was used for estimating lipid content.

Fatty acid composition. The lipid sample was treated with CH_2N_2 and then NaOMe in MeOH in the dark overnight at room temp. Methyl esters were extracted with petrol, washed with H_2O, dried and concd. The esters were examined for CFA by the Halphen test [10] and for epoxy acids by Si gel TLC [11] and purified by prep. TLC on Si gel using petrol–Et_2O (47:3). The esters of roots and seeds were treated with methanolic $AgNO_3$ [12]. GC of the methyl esters was carried out on Silar-10 C at 210°. The detector and injection port were maintained at 240°. N_2 was the carrier gas (40 ml/min). The presence of cyclopropane fatty acids was examined by fractionating the purified esters by Ag^+ TLC using petrol–Et_2O (9:1) and analysing the saturated esters by GC. CFA were isolated from *S. foetida* oil [13], esterified, hydrogenated over Pd/C, and used as ref. The presence of 17:1 was confirmed by analysis of the monoenes on a SE-30 column.

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