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Fatty Acid and Lipid Composition of Germinated Seeds of *Citrullus colocynthis*

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The seeds of *Citrullus colocynthis* were germinated in the dark at 30°C and the variations in the lipid class and fatty acid composition of primary roots and the respective cotyledons at 5 mm to 30 mm root length were studied. During germination the relative amounts of triacylglycerols decreased while free fatty acids increased continuously in significant amounts. Polar lipids also increased with the increase of root length. Saturated fatty acids increased (except lauric acid) whereas the unsaturated fatty acids decreased gradually during the course of germination.

Key words. *Citrullus colocynthis,* Germinated seeds, Fatty acids, Lipid classes.

Introduction

Citrullus colocynthis (colocynth) belongs to an important plant family Cucurbitaceae, consisting of 100 genera and 850 species (Chopra 1977). It grows as a wild perennial in desert regions of the world and is also found in the sandy lands of North-West Frontier, Punjab and Sindh provinces of Pakistan. It is locally known as "Tumma" and has been used since time immemorial in the form of roots, dried pulp and seed oil for different ailments (Kirtikar and Basu 1957). The fatty acid composition of the oil has previously been reported (Alimchandani *et al* 1949; Abu Nasr 1955; Gupta and Chakrabarty 1957; Patel *et al* 1961), but the changes that take place during germination are reported for the first time in this paper. An effort is made to study the changes in each lipid class of primary roots and cotyledons at various stages of germination. It is hoped these studies will help to understand the biosynthesis of lipids and natural pathways for the formation and changes of fatty acids prior to the maturation of seeds.

Materials and Methods

Germination. Citrullus colocynthis seeds (50 g) were soaked in water for two hours and then incubated at 30 ± 0.5°C between the folds of moist sack in steel tray of 15"x 12"x 3" dimension in the dark. The watering process was carried out by spraying water (50 ml) on the sack after every 12 hours. The seedlings were picked up after 72 and 192 hours from the sowing time having root lengths of 5 mm and 30 mm, respectively. The above primary roots and the respective cotyledons were detached and pooled separately. These were dried in an oven at 105°C for moisture content.

Extraction of lipids. A known weight of fresh primary roots of 5 mm and 30 mm root length and the respective cotyledons were crushed and stirred for half an hour with the solvent mixture (40 ml) of chloroform and methanol (2:1 v/v) separately (Tiwana *et al* 1988). The supernatents were separated by centrifugation and the experiment was repeated three times with the solvent mixture (30 ml) to get maximum lipids. These lipids were washed to remove the non lipid impurities (Folch *et al* 1957). After removing the solvent, the weights of the lipids were determined and their percentages were thus calculated on dry weight basis in duplicate.

Thin layer chromatography. The lipids of cotyledons and primary roots were fractionated qualitatively and quantitatively on 0.25 and 0.5 mm thick silica gel chromato-plates, respectively, into neutral and polar lipids. The neutral lipids were separated with the solvent mixture of hexane, diethylether and acetic acid (40:10:1 v/v) while the polar lipids were developed in the solvent mixture chloroform, methanol, 30% ammonium hydroxide and water (24: 14: 2: 1 v/v). The specific locating reagents were used for the identification of neutral and polar lipids (Lowenstein 1969). The identified lipids were marked, scraped and extracted with chloroform methanol mixture (2:1 v/v) separately. The solvent was removed under reduced pressure and the respective lipids weighed for quantification in duplicate.

Identification of fatty acids. The fatty acid composition of neutral and polar lipids of primary roots and the respective

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cotyledons was determined after methylating each esterified lipid fraction with boron trifluoride/methanol reagent (Morrison and Smith 1964). The methyl esters after purification by thin layer chromatography were identified by using gas liquid chromatography. A Shimadzu gas chromatograph GC 14A with flame ionization detector and glass column 1.5 mm x 3 mm (i.d) packed with 15% diethylene glycol succinate was used. Column was coated on supporting media Shimalite AW 201 (60-80 mesh). Its temperature was programmed 150°C to 200°C with a rise of 5°C per minute. Injector and detector temperatures were kept at 250 and 300°C, respectively. Nitrogen was used as a carrier gas with a flow rate of 40 ml per minute. The fatty acids were identified by comparing their retention times with authentic methyl esters injected under the same conditions. The percentage of each fatty acid was determined by the Shimadzu chromatopac computing integrator C-R4A in duplicate.

Results and Discussion

Moisture and lipid contents. Moisture and total lipid contents in the primary roots and the respective cotyledons of *Citrullus colocynthis* at different stages of germination are given in Table 1. The table shows increase of moisture from 78.6 to 82.0% in primary roots and 47.5 to 74.0% in cotyledons as the root length increased from 5 mm to 30 mm.

Table 1
Lipid and moisture contents of primary roots and cotyledons at different root lengths

Root length (mm)	Cotyledons		Primary Roots	
	Moisture %	Lipid %	Moisture %	Lipid %
5	47.5	20.2	78.6	15.5
10	51.3	20.5	79.3	14.3
15	59.5	20.6	80.0	12.5
20	67.3	20.8	80.5	11.9
25	71.2	20.9	81.1	11.0
30	74.0	21.1	82.0	10.7

The moisture content was more significant in primary roots as compared to cotyledons during the seed germination. The lipid content on dry weight basis decreased from 15.5 to 10.7% in primary roots as the length increased from 5 mm to 30 mm showing that there was a rapid utilization of lipids as energy source during the processes of germination. But slight increase in lipid content (20.2 to 21.1%) of cotyledon could be due to their biosynthesis and the preferential utilization of non-lipid components. These observations are well supported by the similar results obtained in other such studies regarding germination of the seeds of cereal grain, corn, cotton, sunflower (Bolling and Elbaya 1983; Belozerova

Table 2
Percentage and R_f values of lipid fractions of primary roots and cotyledons at various root length

Lipid classes	Primary roots			Cotyledons	
	R_f values	5mm	30mm	5mm	30mm
Neutral lipids					
HC	0.96	1.2	0.8	1.2	1.0
WE	0.90	2.6	1.9	2.5	1.7
TG	0.5	67.5	54.0	70.2	56.4
FFA	0.40	4.5	12.1	2.9	13.0
1,3-DG	0.33	3.6	2.9	3.5	2.8
1, 2-DG	0.30	3.8	3.5	3.7	3.1
GL	0.27	0.9	1.6	0.7	1.3
FAl	0.24	0.6	1.4	0.6	1.1
S	0.21	1.5	2.3	1.4	2.2
2-MG	0.18	3.6	5.2	3.5	4.0
1-MG	0.13	3.7	4.5	3.6	4.3
Polar lipids					
PE	0.65	1.3	1.7	1.3	2.2
PC	0.53	1.1	1.8	1.0	1.4
LPE	0.46	1.2	1.9	1.1	1.5
LPC	0.42	1.2	2.0	1.2	1.7
PI	0.17	1.7	2.4	1.6	2.3

Table 3
Fatty acid (%) in cotyledon lipid of 5 mm root length

Lipids	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Neutral lipids											
WE	4.5	6.8	8.8	10.0	15.2	6.6	5.9	8.9	28.3	1.8	3.2
TG	0.2	0.1	0.6	-	12.6	8.1	11.6	23.7	49.8	1.3	-
FFA	4.1	3.7	6.8	9.8	15.2	7.1	7.8	12.7	22.6	4.8	5.4
1,3-DG	4.9	4.1	8.9	6.4	18.2	4.7	7.3	11.8	27.8	5.9	-
1,2-DG	3.0	6.5	8.6	6.1	17.1	5.2	6.4	11.7	26.9	8.5	-
GL	5.1	4.7	7.9	5.6	24.1	6.7	5.6	10.2	28.3	1.8	-
2-MG	4.7	4.9	8.2	7.0	16.8	6.1	9.3	8.7	28.8	5.5	-
1-MG	3.2	3.1	8.9	6.8	18.6	5.3	6.9	12.8	30.7	3.7	-
Polar lipids											
PE	4.7	4.9	9.7	11.1	24.3	7.4	9.7	5.8	18.6	3.8	-
PC	5.0	6.9	11.8	11.9	17.8	11.8	9.9	6.5	13.9	4.5	-
LPE	5.8	5.4	7.4	6.8	26.6	12.0	8.2	6.5	16.5	4.8	-
LPC	3.4	6.9	12.8	11.6	18.6	10.3	11.5	7.0	13.8	4.1	-
PI	7.1	7.6	9.3	8.5	21.1	9.4	11.7	4.5	16.8	4.0	-

Table 4
Fatty acid (%) in cotyledon lipid of 30 mm root length

Lipids	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Neutral lipids											
WE	3.7	5.7	12.9	6.4	20.7	4.3	10.8	6.4	24.7	0.9	3.5
TG	-	-	3.1	-	17.8	-	16.9	17.5	44.1	0.6	-
FFA	3.1	2.5	11.3	6.1	18.2	4.3	13.7	9.5	20.5	4.3	6.5
1,3-DG	3.7	2.6	14.9	4.2	22.2	2.8	13.4	7.8	24.0	4.4	-
1,2-DG	2.5	4.7	12.5	4.5	22.1	3.1	13.3	8.0	23.1	6.2	-
GL	3.9	3.5	13.4	3.4	26.5	4.6	11.3	6.0	26.9	0.5	-
2-MG	3.4	3.1	13.6	4.2	22.9	3.7	13.8	6.0	25.0	4.3	-
1-MG	2.0	1.9	13.7	4.4	24.8	3.2	13.8	8.9	25.1	2.2	-
Polar lipids											
PE	3.2	3.1	13.5	7.6	32.2	5.7	13.9	3.6	14.7	2.5	-
PC	3.5	5.3	13.3	9.1	26.6	7.0	13.4	5.2	13.5	3.1	-
LPE	5.4	5.0	9.3	5.7	33.5	10.7	8.7	4.4	13.3	4.0	-
LPC	2.5	5.5	13.2	8.0	26.0	8.6	13.0	6.7	13.4	3.1	-
PI	6.0	6.2	13.9	5.0	26.7	6.1	15.1	2.8	15.5	2.7	-

and Karpova 1983; Rahman and Hamid 1983; Bose *et al* 1987).

Lipid composition in primary roots and cotyledons. Germination of *Citrullus colocynthis* seeds has not been studied previously as revealed by the literature survey. However, in the present study, efforts were made for the fractionation of neutral and polar lipids in the germinated seeds to fill the knowledge gap. It was observed that the neutral lipids increased or decreased whereas the polar lipids only increased during the course of germination. In view of the biological transformation of neutral lipids, it has been observed that triacylglycerols are reduced to partial acylglycerols which leater on are converted into free fatty acids. The lipolytic enzyme plays a vital role in the hydrolysis of triacylglycerols and thus changes them to mono and diacylglycerols and eventually fatty acids are produced. These lipids in different forms are also used as a source of energy for the nourishment of the plant (Abdel Al and Rahma 1986).

Table 5
Fatty acid (%) in lipids of primary roots of 5 mm root length

Lipids	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Neutral lipids											
WE	4.3	6.8	8.5	8.2	15.1	5.6	6.7	8.6	32.5	0.7	3.0
TG	0.1	-	2.3	-	13.6	-	12.7	23.6	46.9	0.8	-
FFA	4.2	3.7	8.1	9.2	16.5	6.4	7.7	11.9	22.3	4.6	5.4
1,3-DG	5.4	4.3	8.5	6.9	17.7	4.8	6.2	11.4	29.1	5.7	-
1,2-DG	4.2	6.4	7.5	5.7	18.9	5.8	3.6	12.1	27.3	8.5	-
GL	5.4	3.6	7.5	5.7	24.6	6.9	4.0	11.1	29.4	1.8	-
2-MG	5.6	5.0	7.5	7.2	17.4	6.7	8.6	8.9	27.8	5.3	-
1-MG	3.8	3.1	8.7	7.2	18.8	4.3	7.1	12.0	31.4	3.6	-
Polar lipids											
PE	5.2	5.3	9.5	11.7	25.3	7.9	9.8	5.7	15.6	4.0	-
PC	5.3	6.9	12.2	12.8	17.9	9.3	9.4	7.4	13.7	5.1	
LPE	5.7	5.3	6.6	6.2	26.5	12.7	9.6	6.0	16.9	4.5	
LPC	5.7	7.4	12.7	12.6	17.9	10.3	10.5	7.1	13.8	4.0	
PI	8.3	8.0	6.9	9.4	18.7	10.5	10.9	5.8	17.2	4.3	

Table 6
Fatty acid (%) of lipids in primary roots of 30 mm root length

Lipids	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Neutral lipids											
WE	2.8	5.3	13.7	5.1	20.6	4.0	11.4	5.9	27.5	-	3.7
TG	-	-	3.5	-	18.1	-	17.7	19.4	41.3	-	-
FFA	2.3	2.8	11.9	6.0	20.2	5.0	14.9	7.6	20.4	3.1	5.8
1,3-DG	4.0	2.5	15.3	4.6	22.9	3.4	11.2	9.0	23.5	3.5	-
1,2-DG	3.0	4.7	13.4	4.0	22.9	4.2	11.7	9.6	23.0	3.5	-
GL	4.2	1.5	14.4	4.3	26.3	4.8	8.7	9.0	26.5	0.3	-
2-MG	4.0	3.8	15.7	4.9	22.1	4.7	12.4	6.9	22.5	3.0	-
1-MG	2.3	1.9	13.3	5.1	23.8	2.9	13.8	7.2	27.3	2.4	-
Polar lipids											
PE	4.0	3.7	14.2	9.1	28.8	5.7	11.5	4.3	14.4	2.3	-
PC	4.9	5.3	13.1	9.8	23.9	6.5	13.1	6.4	13.3	3.7	-
LPE	4.5	4.0	9.7	4.7	31.8	11.4	13.0	4.2	13.5	3.2	-
LPC	3.0	6.5	13.1	8.9	25.2	8.3	13.1	5.6	13.5	2.8	-
PI	6.8	6.2	11.7	7.3	26.1	7.5	14.2	3.7	14.4	2.1	-

The percentages of these lipids in primary roots and the cotyledons are shown in Table 2. Hydrocarbons and waxs esters decreased with the increase of root length. Glycolipids, fatty alcohols and sterols increased to some extent during the course of germination as observed on the germination of cotton seed. (El Nokrashy *et al* 1974) and *Sterculia foetida* seeds (Lakshaminarayana *et al* 1985). These changes might be playing their role for new cell building in the plants. All polar lipid fractions increased with an increase of root length. Such type of results were also reported in germinating studies on caster bean (Bowden and Lords 1975) and watermelon seeds (Hardman and Crombie 1958). This suggests that the polar lipids function as the component of membrane system as in the resting seeds.

Fatty acid composition. The fatty acid composition of different neutral and polar lipids of primary roots and cotyledons are shown in Table 3 to 4. The fatty acids ranged from $C_{12:0}$ to $C_{20:0}$ and the graph of saturated fatty acids in-

creased (except lauric acid) where the percentage of unsaturated fatty acids decreased during germination. The overall percentage of unsaturated fatty acids as compared to saturated fatty acids was higher in both the cases either in cotyledons or primary roots at 5 mm root length. At 30 mm root length, the percentage of unsaturated fatty acids was lower as compared to saturated fatty acids in all lipid classes except triacylglycerols. The increase of saturated fatty acids and decrease of unsaturated fatty acids is also supported by similar previous works in the germination of corn germ, (Weber 1984) and ground nuts (Ramakrishnan 1954). This suggests a preferential utilization of unsaturated fatty acids as compared to saturated fatty acid during germination. The metabolic system of the plant shows the inter-conversion of unsaturated fatty acids to saturated fatty acids which is also supported by similar findings reported earlier (El Sebaiy *et al* 1983; Lakshminarayana *et al* 1985).

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