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PLANT GROWTH, METABOLISM AND ADAPTATION IN RELATION TO STRESS CONDITIONS V. EFFECTS OF SALINITY ON THE FATTY ACIDS OF GERMINATING FLAX, COTTON AND CASTOR BEAN SEEDS

By

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ABSTRACT

The same three fatty acids were detected in flax, cotton and castor bean seeds: myristic, plamitic and oleic. In addition, linoleic was found in flax and lauric and stearic in castor bean. The seeds were germinated over a period of 12 days and showed variable changes in the major fatty acids. In the three seeds, salinization with 0.5% and 1.0% NaCl led to decreases or increases in the contents of certain fatty acids. Also disappearance of certain fatty acids was accompanied by appearance of others.

INTRODUCTION

Fatty acid metabolism in a germinating oil-seed tissue is a complex process, the major energy reserve of the tissue is linoleic acid esterified to triacylglycerol and phospholipids. During germination of castor bean, the bulk of this lipid is broken down to free fatty acids which is then β-oxidized in the glyoxysomes and finally converted to sucrose *via* tricarboxylic acid cycle and gluconeogenic enzymes located in the mitochondria and cytosol (Nishimura and Beevers, 1979).

In a previous communication (Younis *et al.,* 1987), respiration, glycerol content and lipase activity were investigated in germinating seeds of flax, cotton and castor beans. There was an increase in all these parameters to maximum

and then a decrease in all controls with time. NaCl (0.5 % and 1.0 %) in general caused marked decreases in all parameters except for respiration in flax when the lower salt concentration caused a significant increase in respiration, and for lipase activity which showed a significant increase at the lower salt concentration in both cotton and in castor beans.

Individual fatty acids have been infrequently estimated under varied experimental conditions. Thus, Painter (1944), Huber and Zalik (1963) and Brockerhoff and Yukowski (1966) have analysed a number of vegetable fats including linseed and cotton seed oils. These authors have reported marked changes in the total amount and in the relative composition of fatty acids. Furthermore, in *Cuphea* mature seeds, $C_{10:0}$ constitutes over 80% of the fatty acids of the triacylglycerol fraction but is only a very minor constituent of the polar lipid fraction (Slabas *et al.,* 1982).

The fact that temperature can affect the oil composition of certain plants is substantiated by the work of Canvin (1965). Thus the fatty acid composition of the seed oils of rape, sunflower and flax depended on temperature when grown under lab conditions; sunflower and castor bean were not.

Thus it was thought of interest, in this work, to investigate the effects of salinity on the fatty acid composition of germinating seeds of flax, cotton and castor bean under the Egyptian climate.

MATERIALS AND METHODS

Homogeneous seeds of flax (*Linum usitatissimum* var. Giza 75), cotton (*Gossypium barbadense* var. Giza 5) and castor bean (*Ricinus communis* var. Baladi) were used in the present study. Pretreatments and germination of seeds were precisely carried out as described by Younis *et al.* (1987). After pretreatment, four samples of germinating seeds were taken for analyses of their initial saturated and unsaturated fatty acid contents. Sampling was also carried out every 4 days for an experimental period of 12 days germination. The oil samples were methylated and their methyl esters were prepared by using methyl alcohol and HCl–dry gas, whereafter, they were qualitatively and quantitatively analysed by gas–liquid chromatography (GLC).

Lipid analysis

The tissue was extracted as described by Meara (1955) and Younis *et al.* (1987) in hot isopropanol using a mortar and pestle, and the dried extract was further extracted by the method of Bligh and Dyer (1959).

An aliquot of the lipid was converted to fatty acid methyl esters by methanolysis in 2.5% methanolic-gaseous HCl (Kates, 1972). Fatty acid methyl esters were analyzed by gas -liquid chromatography on a column (2 m x 0.4 cm) of 20 % DEGS on 60/80 chromosorb WAW at 195 °C. Peaks were identified by comparison of retention times with those of authentic standards and quantified by integration of peak areas (Ferrante *et al.*, 1983). Each experiment was repeated twice in duplicate, so that the mean obtained was for four replicates.

RESULTS

The three seeds under study have the same three fatty acids (14:0, 16:0 and 18:1) but in varying amounts, while flax has an additional one (18:2) and castor bean two more (12:0 and 18:0). The seeds of flax contian 16:0 and 18:1 as their major fatty acids, followed by 18:2 and by 14:0 (Table 1). Cotton seeds on the other hand have large amounts of 14:0 followed by 16:0 and by 18:1 acid in decreasing order (Table 2). Castor bean seeds also have 14:0 as their major fatty acid followed very closely by 18:0 and with much smaller amounts of 18:1, 12:0 and 16:0 acids (Table 3).

Germination of flax seeds in water for 4 days induced the production of 2 new fatty acids namely: 12:0 and 18:0 and also the disappearance of 14:0. At 8 days germination, 16:0, 18:0, 18:1 and 18:2 were still present whereas 12:0 and 14:0 disappeared and reappeared respectively in relation to the initial and the 4-day values. At 12 days the acids still present were 14:0, 16:0, 18:0 and 18:1 (Table 1). Incubation of cotton seeds to germinate in water for 12 days induced the production of 3 more fatty acids, namely: 12:0, 18:0, and 18:2 after 8 and 12 days, in addition to those detected in the initial samples (Table 2). Castor bean seeds germinated for 4 days in distilled water showed the production of appreciable amounts of 18:2 and no 12:0 was observed until the completion of the present study. The 14:0 acid showed variable decreases whereas the 16:0 acid, which disappeared at 4 days, showed progressive sharp increases afterwards. 18:0 was found either to increase (after 4 days) or to decrease (after

8 and 12 days) above the initial levels. We can also observe that, first there is a shift from 14:0 production to stearic (18:0), followed by 18:1, then to 16:0 (Table 3).

Table 1

Effect of 0.5 % and of 1.0 % NaCl on the fatty acid composition of flax seeds over a 12–day germination period. Mean values of four replicates are given as percentage of total fatty acids ±SEM.

Days Germination	Saturated fatty acids				Unsaturated fatty acids		
	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Initial	0.0	7.7±0.34	35.5±1.42	0.0	34.3±1.50	22.4±0.52	0.0
Distilled water							
4	1.5±0.07	0.0	25.2±1.02	12.6±0.50	12.7±0.54	48.0±2.00	0.0
8	0.0	1.6±0.06	55.9±2.30	0.9±0.03	39.8±1.60	1.8±0.06	0.0
12	0.0	5.5±0.30	25.2±1.00	13.6±0.54	55.6±2.40	0.0	0.0
0.5 % NaCl							
4	18.0±0.70	7.2±0.32	1.2±0.06	0.0	73.5±3.10	0.0	0.0
8	0.0	2.2±0.09	35.4±1.55	4.6±0.28	57.8±2.50	0.0	0.0
12	0.0	0.0	27.1±1.10	2.3±0.09	12.1±0.52	58.5±2.50	0.0
1.0 % NaCl							
4	6.5±0.34	9.7±0.40	28.4±1.20	1.3±0.05	51.5±2.20	2.6±0.10	0.0
8	0.0	0.1±0.01	25.3±1.02	11.7±0.50	0.0	13.8±0.56	49.3±2.00
12	0.0	5.4±0.32	75.4±3.12	2.2±0.08	16.1±0.66	1.0±0.04	0.0

Incubation of flax seeds in 0.5% NaCl induced variable changes in the percentage composition of fatty acids as compared with the initial fatty acid values. After the first 4 days of germination, there was a sharp accumulation of 12:0 and 18:1 acids, whereas a sharp drop in 16:0 and 18:2 acids was observed. At the end of 12 days germination, the following sequence of fatty acids (18:2 > 16:0 > 18:1 > 18:0) was displayed with regard to their percentage occurrence in the oil of germinating flax seeds (Table 1). Treatment of cotton germinating seeds with 0.5% NaCl induced variable changes in the relative composition of the different fatty acids. 12:0 acid appeared after 4 days of germination and afterwards complete disappearance of this acid was observed. At the end of the germination period, 16:0 and 18:1 acids increased, while 14:0, 18:0 and 18:2

showed, in general, a marked decrease (Table 2). Salinization of germinating castor bean seeds with 0.5 % NaCl induced the seeds to produce variable amounts of 14:0, 16:0, 18:0, 18:1 and 18:2 (after 4 days), but after 8 days, the germinating seeds contained high amounts of 18:1 (48.6 %) and 16:0 (51.0 %) in addition to traces of 12:0 (0.4 %); Table 3.

Table 2

Effect of 0.5 % and of 1.0 % NaCl on the fatty acid composition of cotton seeds over a 12-day germination period. Mean values of four replicates are given as percentage of total fatty acids ±SEM.

Days Germination	Saturated fatty acids				Unsaturated fatty acids		
	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Initial	0.0	66.1±2.80	21.5±0.88	0.0	12.4±0.52	0.0	0.0
Distilled water							
4	0.0	3.2±0.14	59.5±2.40	0.0	37.3±1.50	0.0	0.0
8	1.9±0.08	0.0	34.1±1.16	0.1±0.01	36.6±1.60	27.5±1.12	0.0
12	0.7±0.03	0.4±0.02	59.5±2.20	0.8±0.04	36.2±1.40	2.4±0.01	0.0
0.5 % NaCl							
4	3.6±0.16	0.1±0.01	8.8±0.40	0.0	34.6±1.50	52.9±2.20	0.0
8	0.0	0.0	28.6±1.20	0.0	45.5±1.80	25.9±1.10	0.0
12	0.0	2.0±0.08	52.3±2.20	0.5±0.03	43.8±1.82	1.5±0.06	0.0
1.0 % NaCl							
4	6.8±0.34	7.5±0.34	36.5±1.14	0.0	49.2±2.00	0.0	0.0
8	0.0	1.2±0.01	22.6±0.10	1.9±0.08	41.8±1.80	32.5±1.40	0.0
12	0.0	26.3±1.08	23.9±0.98	0.0	48.7±2.00	1.2±0.06	0.0

Treatment of germinating flax seeds with 1.0 % NaCl induced an abrupt change in the distribution of the different fatty acids after 4 days of germination as compared with those in seeds treated with 0.5 % NaCl. At the second period of germination (after 8 days), a sharp decrease in 12:0, 14:0 and 18:1 and an increase in 18:0 and 18:2 acids were apparent. At 12 days, the following sequence of fatty acids (16:0 >18:1 > 14:0 > 18:0 >18:2) was displayed with respect to their percentage occurrence in germinating flax seeds (Table 1).

Table 3

Effect of 0.5 % and of 1.0 % NaCl on the fatty acid composition of castor beans over a 12–day germination period. Mean values of four replicates are given as percentage of total fatty acids ± SEM.

Days Germination	Saturated fatty acids				Unsaturated fatty acids		
	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Initial	2.1±0.10	49.3±2.20	0.8±0.04	41.9±1.80	6.02±0.30	0.0	0.0
Distilled water							
4	0.0	6.2±0.34	0.0	70.3±2.10	0.0	23.6±0.98	0.0
8	10.5±0.50	23.4±1.00	1.2±0.05	57.6±2.50	7.3±0.48	0.0	
12	0.0	0.9±0.04	51.7±2.20	7.1±0.30	28.3±1.20	12.1±0.54	0.0
0.5 % NaCl							
4	0.0	0.9±0.05	56.2±2.50	0.8±0.04	19.1±1.00	22.9±1.00	0.0
8	0.4±0.03	0.0	51.0±2.20	0.0	48.6±2.02	0.0	0.0
12	1.2±0.01	1.5±0.08	49.3±2.00	2.5±0.10	9.6±0.52	36.0±1.80	0.0
1.0 % NaCl							
4	0.0	1.0±0.07	10.1±0.40	0.8±0.05	88.1±3.62	0.0	0.0
8	7.9±0.40	7.7±0.40	9.5±0.50	66.6±2.80	8.3±0.48	0.0	0.0
12	0.0	24.1±0.96	0.0	44.0±1.80	32.2±1.40	0.0	0.0

Treatment of germinating cotton seeds with high NaCl concentration (1.0 %) elicited variable reductions in 14:0 acid. On the other hand, a remarkable increase in 16:0 and 18:1 acids were, in general, apparent at the end of the germination period, as compared with those values detected in dry seeds. It is of interest to mention that 12:0 and 18:0 acids were apparent only at days 4 and 8 respectively, otherwise these acids were absent (Table 2). Treatment of germinating castor bean seeds with 1.0 % NaCl induced remarkable changes in the acid percentages when compared with those detected in either control or in 0.5 % saline treated seeds. After 4 days , the main bulk of the fatty acid pool was represented by 18:1 acid (88.0 %), whereas at the second and third periods of germination (after 8 and 12 days), 18:0 was the main constituent fatty acid in the germinating castor bean seeds (Table 3).

DISCUSSION

Under the climate of Egypt, the fatty acid composition of oils of the 3 plant varieties used could be different from those of plants grown in other parts of the world. Thus it has long been kown that the composition of the oil obtained from some plants varies according to the temperature at which they grow. Of interest in this connection, we should mention that Canvin (1965) observed that the fatty acid composition of the seed oils of rape, sunflower and flax depended on temperature when grown under laboratory conditions; safflower and castor oils were not affected.

Flax seeds appeared to be rich in 14:0, 16:0, 18:1 and 18:2 acids; cotton seeds are rich in 14:0, 16:0 and 18:1 and castor beans are rich in 12:0, 14:0, 18:0 and 18:1 acids. On germination of these seeds in water, new fatty acids appeared with the concomitant disappearance of others (see Tables 1, 2 and 3). In this context, it is of interest to mention that linseed was analysed at various stages of its development, but no trace of the $C_{12} - C_{16}$ trienoic fatty acids was detected. It appears unlikely that the formation of 18:3 acid in linseed occurs to any extent by desaturation of a shorter chain acid to the trienoate followed by elongation to C_{18} (Oulaghan and Wills, 1976).

Nyman (1966) found linoleic, oleic, palmitic and stearic acids to be the main fatty acids in germinated Scots pine seeds. After the onset of imbibition, linoleic acid appeared in the largest proportion. The total content of free fatty acids increased with increasing germination period.

As compared with the control values, at the same period, salinity induced the formation of 12:0 at day 8, 14:0 at day 4, 18:2 at day 12 and 18:3 acid at day 8 in germinating flax seeds; 12:0 and 18:2 acids at day 4 in the germinating cotton seeds and 16:0 and 18:1 at day 4, 12:0 at days 8 and 12 in the germinating castor beans.

Our results presented in Tables 1, 2 and 3 appeared to reveal a pattern of changes similar to that of Nyman (1966), and salinity appears to increase 12:0 and 14:0 in the 3 seeds under study , and in addition 18:1 in castor beans only as compared with controls, especially on the first day of germination. This may be considered as evidence of highly active lipase enzyme in these respective seeds in response to salinity (Younis *et al.*, 1987); while 18:0, 18:1 and 18:2

which apear to decrease in germinating flax seeds, can be considered as evidence of saline–inhibited lipase activity in flax seeds. However, the role of simultaneous consumption cannot be ruled out, because the content of free fatty acids is the result of lipolysis and simultaneous consumption (Ching, 1963; Angelo and Altschul, 1964) and lipolysis is affected to a certain extent by salinization; hence, fluctuating results were obtained.

In conclusion, our results show marked changes in fatty acids of the 3 different oily seeds, throughout the germination period, as influenced by salinization. These changes could be explained on the basis that the fatty acids involved in the composition of body fats arise from 3 sources: (1) by lipolytic cleavage; (2) by synthesis from carbohydrates or non–lipids ; and (3) by alternation of fatty acids derived from the other two sources.

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نمو وأيض وملاءمة النباتات لظروف الاجهاد المختلفة
٥ - تأثير الملوحة على الأحماض الدهنية في بذور الكتان والقطن والخـروع النابتـــة

محمود الباز يونس - محمد نجيب عبد الغني حسانين و ممدوح محمد نعمة الله

يهدف هذا البحث إلى دراسة أثر الملوحة على محتوى الأحماض الدهنية في زيوت بذور الكتان والقطن والخروع أثناء انباتها لمدة اثنى عشر يوماً.

ففي البذور الثلاثة المستنبتة في الماء ، وجدت أحماض الميرستيك والبالمتيك والأوليبك ، وبالاضافة إلى ذلك فقد أمكن فصل حمض اللينولييك من بذور الكتان ، وحمضي اللوريك والاستياريك من بذور الخروع النابتة .

أدت المعـاملة بمحـاليل كلوريد الصـوديوم (٠,٥٪ ، ١٪) إلى تغـيرات ملحوظة ومختلفة في محتوى الأحماض الدهنية الأساسية وذلك بالمقارنة إلى محتوى هذه الأحمـاض في البـذور المستنبتـة في المـاء . فعلى حين نقص محتوى بعض الأحماض ، وجدت زيادة في محتوى البعض الآخر ، وبالاضافة إلى ذلك فقد كان إختفاء بعض الأحماض الدهنية مقروناً بظهور أحماض أخرى جديدة.