Effect of Salinity on Fatty Acid and Fatty Alcohol Composition during the Germination of Jojoba (*Simmondsia chinensis*) Seeds

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The effects of salinity ($CaCl_2$ and NaCl (1:1, w/w)) on fatty acid and fatty alcohol composition in jojoba cotyledons were studied under greenhouse conditions. Seeds were germinated in an artificially salinized (electrical conductivity 0.0, 5.0, 10.0, 15.0 dS/m) sand medium. High-pressure liquid chromatography and gas chromatography/mass spectrometry were used to analyze wax ester and fatty acid composition. The fatty alcohols were determined by a difference method that assumes a molar material balance. The relative proportions of palmitic acid, eicosenoic acid, tetracosenoic acid, and tetracosenol did not vary significantly due to salinity treatments. However, increasing salt concentration led to a decrease in the percentage of oleyl alcohol and eicosenol to the advantage of docosenol and hexacosenol. There was also a slight decrease in stearic acid and oleic acid to the advantage of docosenoic acid. These changes were noted only in the last 10 days of the experiment.

Salinity is known to affect several aspects of plant metabolism, including lipid metabolism in many plants (Erdei et al., 1980; Muller and Santarius, 1978). In several cases, a change in lipid composition in response to salinity was correlated with adaptive properties of the species involved.

Jojoba oil is unique in the plant kingdom. Unlike other vegetable oils in which fatty acids are esterified with a glycerol molecule, in jojoba oil these fatty acids are connected directly to the fatty alcohols. It seems that jojoba possesses unique biosynthetic pathways to produce and store this unusual lipid (National Academy of Sciences, 1985), as well as to metabolize these esters during germination.

In a recent study of the effects of salinity on seed germination and lipid mobilization in jojoba (Kayani et al., 1989), it was observed that jojoba is somewhat salt sensitive at the germination stage. Germination and the quantities of lipid metabolized were affected by the salinity of the germinating medium. The main objective of the present work was to study the effects of salinity on fatty acid and fatty alcohol composition during the germination of jojoba seed. We are unaware of any similar studies reported in the literature.

MATERIALS AND METHODS

Germination of Jojoba. Seeds of jojoba (*Simmondsia chinensis* [Link] Schneider) were germinated in sand culture in perforated trays in a greenhouse kept at an average temperature of 17.5/32 °C (night/day). $CaCl_2$ and NaCl (1:1, w/w) salinity treatments having electrical conductivities of 0.0, 5.0, 10.0, and 15.0 dS/m were prepared in deionized water, and the solution for each respective treatment was used four times a day.

Five seeds and/or seedlings were sampled for their fatty acid and fatty alcohol contents at 3, 6, 10, 15, 20, 25, and 30 days after sowing.

Lipid Extraction and Wax Ester Analyses. Total lipids were extracted by the method of Moreau and Huang (1977). Wax esters were separated according to their carbon chain length by high-pressure liquid chromatography (HPLC) (Spencer et al., 1977) and identified by mass spectrometry. Four fractions of wax esters, C_{38}, C_{40}, C_{42}, and C_{44}, were separated, and their relative composition was determined by the ratio of peak areas.

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The details of the experimental setup, lipid extraction, and wax ester analyses were described earlier (Kayani et al., 1989).

Fatty Acid. The fatty acid component of each wax ester was converted to a fatty acid methyl ester derivative by boron trifluoride–methanol treatment (Morrison and Smith, 1964). A 10-mL portion of each HPLC fraction was evaporated to dryness under a nitrogen stream and redissolved in 0.4 mL of benzene in a capped, Teflon-lined centrifuge tube. The boron trifluoride–methanol reagent (4.0 mL) was added to the tube under nitrogen. The capped tube was heated in boiling water for 90 min and then cooled. Esters were extracted by adding 2 mL of isooctane and 1 mL of water and centrifuged for 5 min at 1500 rpm. The upper layer was collected and analyzed for fatty acid content by gas chromatography/mass spectrometry under the following specifications:

The capillary column used was a 60-m DB-5, 0.25-mm diameter, 25-μm thickness film. A sample (3 μL) was injected by splitless injection. Temperature conditions: initial temperature, 50 °C; programmed at 20 °C/min to 280 °C and held isothermally for 20 min; injection temperature, 240 °C.

Spectra were obtained with a VG-ZAB mass spectrometer. The mass scan was 50–500/1.25 s scan, with resolution 2000, electron impact 70 V, source temperature 200 °C, and transfer line temperature 250 °C. The individual methyl esters were identified by comparison with the NBS Library of Spectra in the Mass Spectroscopy Facility.

Fatty Alcohol. Alcohols were determined by the difference of the identified fatty acid from each particular ester by assuming the remainder to be alcohol. For example, if a C_{40} wax ester fraction contains fatty acids $C_{18:1}$ (5.0%), $C_{20:1}$ (89.5%), and $C_{22:1}$ (5.5%), then values for alcohols $C_{22:1}$ (5.9%), $C_{20:1}$ (89.47%), and $C_{18:1}$ (4.56%) were calculated by converting the mole ratio of fatty acid to the respective weight of the complementary alcohol.

Total Composition. The amount of each constituent fatty acid and alcohol in original jojoba wax was determined by assuming that the ratio of peak area from HPLC can be used to calculate each respective weight. The final values in percentage are reported in Tables I and II.

RESULTS AND DISCUSSION

The results pertaining to the composition of lipid extracted from the cotyledons of untreated seeds (0 day) are shown in Tables I and II. They are in agreement with earlier reports on jojoba oil analyses (Duncan et al., 1974; Spencer et al., 1977; Yermanos, 1975; Yermanos

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Table I. Effects of Salinity on Fatty Acid Composition in Jojoba Cotyledons (Values Based on Three Replicates of Five Seeds Each)

days	EC, dS/m	fatty acid,[a] %						ratio between 20:1 from C_{40} and C_{42}
		16:0	18:0	18:1	20:1	22:1	24:1	
0	0.0	0.92	0.98	6.1	78.9	12.76	0.43	0.60
20	0.0	0.74	0.78x	8.8	79.53	10.23x	-	1.04x
	5.0	0.75	0.8x	6.66	81.0	10.5x	0.28	0.9x
	10.0	0.81	0.87y	4.68y	82.08	11.25y	-	0.94x
	15.0	1.02	1.09z	2.94z	81.64	12.84y	-	0.58y
25	0.0	0.64	0.68x	10.16x	79.33	9.15x	-	1.2x
	5.0	0.72	0.77x	6.85y	81.26	10.40x	-	1.13x
	10.0	0.85	0.91x	4.98z	81.78	12.3y	-	0.95y
	15.0	0.84	1.0y	3.52	82.3	12.7y	-	0.75z
30	0.0	0.71	0.78x	9.61x	80.77	8.8x	-	1.05x
	5.0	0.71	0.76x	5.66y	82.6	10.34x	-	1.19x
	10.0	0.82	0.91x	4.14y	82.6	11.50y	-	0.85y
	15.0	0.86	0.96y	2.9y	82.83	12.35y	-	0.76z

[a] Key: 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 20:1, eicosenoic acid; 22:1, docosenoic acid; 24:1, tetracosenoic acid. Means with the same letter in the same column do not differ significantly at $p < 0.05$ according to Duncan's multiple-range test of mean differences. - = amount not detectable.

Table II. Effects of Salinity on Fatty Alcohol Composition in Jojoba Cotyledons (Values Based on Three Replicates of Five Seeds Each)

days	EC, dS/m	fatty alcohol,[a] %					ratio between C20:1 and C22:1
		18:1	20:1	22:1	24:1	26:1	
0	0.0	4.77x	40.20	48.54	5.55	0.98	0.82x
20	0.0	8.87x	50.58y	36.4y	3.73	0.66x	1.34y
	5.0	6.65x	49.19y	39.44y	3.75	0.73x	1.13y
	10.0	5.72y	49.46y	43.25y	0.88	0.83y	1.14y
	15.0	2.173z	41.90z	53.20x	1.08	1.02z	0.78z
25	0.0	10.14x	52.46y	36.04x	0.68	0.64	1.45x
	5.0	7.08y	53.44y	38.55x	0.79	0.74	1.38x
	10.0	4.29y	47.59y	45.65y	0.89	0.84	1.04x
	15.0	4.29y	41.07z	52.17z	0.89	0.84	0.78y
30	0.0	9.92x	53.79y	35.36x	0.68	0.64x	1.52x
	5.0	6.86y	53.80y	37.65x	0.75	0.71x	1.42x
	10.0	5.59y	50.34y	42.37x	0.87	0.82yx	1.20y
	15.0	2.99z	46.48z	48.16y	0.98	0.97y	0.92z

[a] Key: 18:1, oleyl alcohol; 20:1, eicosenol; 22:1, docosenol; 24:1, tetracosenol; 26:1, hexacosenol. Means with same letter in the same column do not differ significantly at $p < 0.05$ according to Duncan's multiple-range test of mean differences.

and Duncan, 1976). This supports the assumption that the fatty alcohol can be determined by our difference method following the preparation of fatty acid methyl ester by the boron trifluoride–methanol reagent for gas chromatography/mass spectrometry. This indirect method is easier to use and does not require prior saponification as in earlier gas chromatography methods (Miwa, 1971; Duncan et al., 1974) or prior hydrogenation as used by Spencer et al. (1977). We assume that, for the comparative values needed in this study, any systematic differences will affect all the samples equally.

Effects of Salinity. The data presented in Tables I and II also show that the relative proportions of palmitic acid, eicosenoic acid, tetracosenoic acid, and tetracosenol among the treatments did not differ significantly, while those of stearic acid, oleic acid, docosenoic acid, oleyl alcohol, eicosenol, docosenol, and hexacosenol did show some changes. There were significant decreases in the relative proportions of oleic acid, oleyl alcohol, and eicosenol with an increase in the salinity level of the germinating media. The reverse was true for stearic acid, docosenoic acid, docosenol, and hexacosenol. These changes were not observed in the first 20 days of the experiment (data not shown) and were noted only in the last 10 days of the experiment. The said changes seems to be due to the smaller utilization of the lipid in the saline treatment (Kayani et al., 1989). As in moderate (EC = 10 dS/m) and strong (EC = 15 dS/m) saline treatments, values for acids and alcohols are close to the corresponding values for ungerminated (0 day) seeds. Lees and Thompson (1980) reported an increase in the relative proportion of oleic acid extracted from plasma membrane fractions of germinating cotyledons of *Phaseolus vulgaris* with advancing senescence. They also reported that relative proportions of eicosenoic acid and docosenoic acid remained unaffected during the senescence, and in our results the relative proportions of eicosenoic acid remained unchanged (Table I).

It does not seem possible that a change in fatty acid and fatty alcohol composition is a direct effect of salinity. Yermanos et al. (1964) and Irving et al. (1988) reported that salinity stress reduced oil content without affecting fatty acid composition of high-linoleate cultivars of safflower. However, a change in fatty acid composition, particularly in the degree of fatty acid unsaturation in response to salinity, was observed in the case of the chloroplastic lipid of olive (*Olea europea* L.) leaves (Zarrouk and Cherif, 1984) and sunflower (*Helianthus annuus* L.) leaves (Gharsalli and Cherif, 1984), which can be associated with an inhibition of [^{14}C]oleate desaturation (Davies and Harwood, 1984). In our results, no such decrease was observed (Table I).

A decrease in the ratio between eicosenoic acid derived from wax esters C_{40} and C_{42} with an increase in salinity, particularly in the last 10 days of the experiment, might have been the result of less utilization of the total lipid and consequently less preferential utilization of C_{42} in the saline treatments (Kayani et al., 1989). The decrease in utilization of C_{42} may also be responsible for the decrease in the ratio between eiosenol and docosenol in the moderate and strong salinity treatments (Table II).

From the results of the present work, it can be stated

that the salinity of the germinating media induced some changes in the composition of lipids in jojoba seeds and these changes are most likely from an indirect effect of salinity on lipid utilization. While we cannot be certain that individual metabolic pathways are being affected differently by increased salinity, if the same general pathway suffers a slower rate of metabolism because of an increase in the free energy of activation, we would expect the pathway to exhibit a greater degree of discrimination among the different substrates.

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Registry No. stearic acid, 57-11-4; oleic acid, 112-80-1; docosenoic acid, 28929-01-3; oleyl alcohol, 143-28-2; eicosenol, 115218-60-5; docosenol, 81736-43-8; hexacosenol, 81724-40-5.

Design of Enzyme-Targeted Agrochemicals

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The deliberate and considered design of chemical pesticides to inhibit specific enzyme (protein) sites represents the next likely step in the pursuit of novel agrochemicals. The transition from the current procedure of random screening, or analogue synthesis, to enzyme-targeted design will occur through positive cooperativity rather than competition. Possible strategies through which the design of enzyme-targeted agrochemicals can occur are discussed.

The pursuit of novel pesticidal compounds for application in agriculture is now at a critical point that will determine the future of this industry. In almost every case where a pesticide acts through specific enzyme inhi-

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