Bul. Agron. 22 (1): 19-29 (1994)

SHORT CHAIN FATTY ACIDS, STRATIFICATION AND GIBBERELLIN RELATIONSHIPS IN APPLE EMBRYO GERMINATION

(HUBUNGAN ASAM LEMAK RANTAI PENDEK, STRATIFIKASI DAN GIBERELIN PADA PERKECAMBAHAN EMBRIO APEL)[1)]

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ABSTRACT

The objective of the present experiment was to determine the inhibitory properties of short chain fatty acids (SCFA) in apple embryo germination. The result showed that the SCFA inhibition on apple embryo germination was dependent on chain length and was in the millimolar range. No synergistic effect was observed when SCFA were applied simultaneously. The inhibition of SCFA was reversed by GA_{4+7}. A higher concentration of SCFA was needed to inhibit embryo germination as the stratification progressed.

RINGKASAN

Serangkaian percobaan dilakukan untuk mengetahui penghambatan perkecambahan embrio apel oleh asam lemak rantai pendek (ALRP), asam karboksilat normal (lurus) dengan jumlah karbon antara 7 sampai 10. Interaksi ALRP dengan lama stratifikasi juga dipelajari. Dari empat ALRP yang dicoba, penghambatan ALRP terhadap perkecambahan embrio apel tergantung pada jumlah karbon. Asam nonanoat dan dekanoat menghambat perkecambahan paling kuat. Konsentrasi yang efektif untuk penghambatan ada pada tingkat milimolar. Kombinasi ALRP yang diaplikasikan secara bersama tidak menunjukkan efek sinergis. Penghambatan perkecambahan embrio apel oleh ALRP ini dapat diatasi dengan aplikasi giberelin. Semakin lama embrio distratifikasi, konsentrasi ALRP yang diperlukan untuk menghambat perkecambahan semakin tinggi.

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INTRODUCTION

Apple seeds require cold stratification, a treatment at 5°C and moist condition, to break dormancy. Many investigators have assumed that dormancy is imposed or at least influenced by certain inhibitors. ABA has been implicated as a dormancy factor in seeds of woody plants. Early report on the release of seed dormancy of the genera *Rosa* showed that ABA decreased significantly during cold stratification (Jackson, 1968). A similar decrease of ABA during cold stratification has been reported in seeds of apple (Rudnicki, 1969). When warm controls (20°C) were included, ABA decreased in both temperatures at a similar rate but only the cold stratification break the dormancy (Balboa-Zavala and Dennis, 1977; Subbaiah and Powell, 1992).

Recently, short chain fatty acids (SCFA), straight chain saturated carboxylic acids with carbon numbers between 6 and 12, have been hypothesized to have a role in various physiological processes in plants, including seed dormancy (Berrie *et al.*, 1979), bud growth (Rogoyski, 1980), bulb dormancy (Ando and Tsukamoto, 1974), senescence (Whitehead and Halevy, 1989), and water stress (Wilmer *et al.*, 1978) among others. However, SCFA have received relatively little attention, especially concerning their role as inhibitory components in apple seed dormancy. The objective of the present experiments were to determine the inhibitory properties of SCFA in apple embryo germination and their relations to stratification and gibberellin.

MATERIALS AND METHODS

Seeds of apple (*Malus domestica*) c.v. Northern Spy 1991 were obtained from the Cornell Orchard, Ithaca, New York. Seeds were removed from mature, freshly harvested fruits and stored at room temperature until used. The seeds were stratified within 3 months after extraction from the fruit. Experiments were conducted in the Department of Fruit and Vegetable Science, Cornell University, Ithaca, New York, USA.

The Northern Spy apple seeds were surface sterilized with 10 % Clorox for 10 minutes, and rinsed with deionized water. The seeds were stratified at 5 ±1C in petri dishes containing six ml of deionized water and lined with two layers of Whatman No. 42 filter paper in the dark. The petri dishes were wrapped with Parafilm. After 40 days of stratification, except where otherwise noted, the intact seeds were removed from stratification. The embryos were excised and treated according to the respective treatments.

Media for germination was prepared by adding SCFA stock into 7.5×10^{-3} M succinate buffer containing 0.3 % Tween 80. Captan was added at 0.01 % to impede microorganisms. The pH was adjusted to 4.9 with dilute KOH or HCl. Before the experiment was started, six ml of media was added in each of petri dish, except where otherwise noted.

The embryos were germinated in the dark at 20 ± 1C for 14 days. The germination was recorded when the radicle was more than 2 mm in length. The percent germination was determined after 14 days. Six replicates of ten seeds were used in all experiments described below. In most cases, the experiments were repeated twice.

The biological activity of SCFA were studied to determine the concentration range for each SCFA in the inhibition of apple embryo germination. Since several SCFA had been detected previously existing together in the same sample (Berrie *et al.*, 1975), interaction studies among SCFA were carried out.

SCFA tested were heptanoic acid (C7), octanoic acid (C8), nonanoic acid (C9), and decanoic acid (C10). Five concentrations were used: 0, 10^{-4}, $3x10^{-4}$, 10^{-3}, $3x10^{-3}$, and 10^{-2} M. Controls (no SCFA) were also included. This experiment was not repeated.

In interaction studies, a concentration of $3x10^{-3}$ M was used for C7, C8, C9, and C10 as single SCFA, while a concentration of $1.5x10^{-3}$ M of each acid was applied simultaneously in pairs of SCFA C7+C8, C7+C9, C7+C10, C8+C9, C8+C10, and C9+C10. Thus the combined concentration was $3x10^{-3}$ M. Controls (no SCFA) were also included.

Attempts were made to determine if different stratification times give different responses in the concentration needed to inhibit germination. Intact seeds were stratified for 25, 35, and 45 days. Excised embryos were then treated with nonanoic acid at the following concentrations: 0, 10^{-4}, $3x10^{-4}$, 10^{-3}, and $3x10^{-3}$ M. The embryos were germinated as previously described.

Gibberelin$_{4+7}$ have been identified in apple seeds. They are believed to be important hormone in alleviating the dormancy of apple seeds. The influence of SCFA and GA was studied to determine if the inhibitory action of SCFA could be reversed by GA. Treatments included in the SCFA and GA_{4+7} interaction studies were nonanoic (C9) acid at 0, 10^{-3}, and 5 $x10^{-3}$ M and GA $_{4+7}$ at 0, 10^{-5}, and 10^{-4} M. GA_{4+7} exist as a mixture, because they are difficult to separate. The ratio of GA_4 and GA_7 in the mixture was 46:54.

RESULTS

The data on effects of SCFA at different concentrations are summarized in Table 1. There was a strong correlation between the activity of SCFA and chain length (Table 2). Strong interaction between SCFA and concentration was contributed primarily by 10^{-2} M treatments.

Table 1. The effect of short chain fatty acids on germination of Northern Spy embryos (Pengaruh asam lemak rantai pendek pada perkecambahan embrio apel Northern Spy).

Molarity of SCFA	Short Chain Fatty Acid			
	C7	C8	C9	C10
		% germination ± SE[x]		
10^{-4}	93.3±2.1	93.3±3.3	90.0±2.5	93.3±2.1
3×10^{-4}	91.6±3.7	93.3±2.1	91.6±3.1	90.0±2.6
1×10^{-3}	86.7±2.1	86.7±2.1	85.0±2.2	83.3±3.3
3×10^{-3}	81.6±3.1	78.3±3.1	75.0±4.3	75.0±4.3
1×10^{-2}	60.0±10.3	50.0±7.3	0	0

[x] Percent germination of control was 95.5±1.9.

Table 2. Analysis of variance on the effect of short chain fatty acids on germination of Northern Spy embryos (Analisis ragam pengaruh asam lemak rantai pendek pada perkecam bahan embrio apel Northern Spy).

Source	df	MS (full data)	df	MS (no 10^{-2} M)[x]
Control vs Treated	1	0.2305**	1	0.0382**
SCFA	3	0.1761**	3	0.0059^{ns}
C7&C8 vs C9&C10	1	0.5201**	1	0.0176^{+}
C7 vs C8	1	0.0082^{ns}	1	0.0002ns
C9 vs C10	1	0.0000^{ns}	1	0.0000^{ns}
Concentration	4	1.7730**	3	0.1157**
Linear	1	4.9882**	1	0.3152**
Quadratic	1	1.7143**	1	0.0301*
LOF	2	0.1948	1	0.0017^{ns}
SCFA*Concentration	12	0.1123**	9	0.0015^{ns}
7&8vs9&10*Linear	1	0.7260**	1	0.0035^{ns}
7&8vs9&10*Quadratic	1	0.4430**	1	0.0009^{ns}
Other Interactions	10	0.0179*	7	0.0013^{ns}
Error	105	0.0086	85	0.0050

Note for tables: $^{+}$, *, **, significant at P = 0.10, 0.05, 0.01 respectively; ^{ns}: not significant.
[x] Since there was abrupt effect of C9 and C10 at 10^{-2} M, the data were analyzed without 10^{-2} M treatment. It is shown that interaction (SCFA*concentration) in the full data analysis is contributed by the 10^{-2} M treatments.

The inhibitory activity of SCFA started at 10^{-3} M. There was abrupt separation of the inhibitory properties of C9 and C10 at 10^{-2} M. At the highest concentration applied, C7 inhibited embryo germination up to 60 % and C8 up to 50 % (Table 1).

Table 3. Interaction of short chain fatty acids applied simulta- neously on germination of Northern Spy embryos (Pengaruh asam lemak rantai pendek secara simultan pada perkecambahan embrio apel Northern Spy).

SCFA	Germination
	% germination ± SE
C7	82.5±2.7
C8	80.0±2.1
C9	70.8±3.1
C10	73.3±1.9
C7+C8	87.5±2.5
C7+C9	84.1±2.3
C7+C10	77.5±4.5
C8+C9	78.3±3.8
C8+C10	75.0±4.0
C9+C10	72.5±4.1
Control	95.8±1.5

Table 4. Analysis of variance on the interaction of short chain fatty acids applied simultaneously on germination of Northern Spy embryos (Analisa ragam pengaruh aplikasi asam lemak rantai pendek secara simultan pada perkecambahan embrio apel Northern Spy).

Source	df	Mean Square
Control vs Treated	1	0.3400**
SCFA	10	0.0658**
C7 and C8 vs C7+C8	1	0.0313^{+}
C7 and C9 vs C7+C9	1	0.0450*
C7 and C10 vs C7+C10	1	0.0001^{ns}
C8 and C9 vs C8+C9	1	0.0068^{ns}
C8 and C10 vs C8+C10	1	0.0022^{ns}
C9 and C10 vs C9+C10	1	0.0001^{ns}
C7 and C8 vs C9 and C10	1	0.1008**
C7 vs C8	1	0.0038^{ns}
C9 vs C10	1	0.0038^{ns}
Block	1	0.0473*
Error	120	0.0114

The simultaneous application of SCFA was studied to determine if there was interaction among SCFA (Table 3). There were no significant differences except between C7 and C9 vs C7+C9 and between C7 and C8 vs C7+C8 (Table 4). Though significant, the ef- fect was less inhibitory in the combined application than in the single application.

Table 5. The effect of stratification time and nonanoic acid on germination of Northern Spy embryos (Pengaruh lama stratifikasi dan asam nonanoat pada perkecambahan embrio apel Northern Spy).

Molarity of C9	Days at 5C 25	35	45
	% germination ± SE		
0	67.5±2.1	91.6±2.4	95.8±1.9
$1x10^{-4}$	65.8±1.9	88.3±2.9	95.8±1.4
$3x10^{-4}$	60.8±3.3	84.1±2.8	97.5±1.3
$1x10^{-3}$	57.5±3.0	80.0±2.4	95.8±1.9
$3x10^{-3}$	43.3±4.4	68.3±3.8	80.8±3.7

Table 6. Analysis of variance on the effect of stratification time and nonanoic acid on germination of Northern Spy embryos (Analisa ragam pengaruh lama stratifikasi dan asam nonanoat pada perkecambahan embrio apel Northern Spy) .

Source	df	Mean Square
Stratification	2	1.8334**
Linear	1	3.5020**
Quadratic	1	0.1647**
Concentration	4	0.2490**
Control vs C9 Treated	1	0.2067**
Linear	1	0.6600**
Quadratic	1	0.1111**
LOF	1	0.0180^{ns}
Stratif*Concentration	8	0.0090^{ns}
Block	1	0.0020^{ns}
Error	164	0.0096

The inhibition by C9 was less when the embryos received more stratification (Tables 5 and 6). At 25 and 35 days of stratification, nonanoic acid started to inhibit embryo germination at $3x10^{-4}$ M. A higher concentration ($3x10^{-3}$ M) was needed to inhibit germination of embryos stratified for 45 days (Table 5).

Table 7. Influence of nonanoic acid and gibberellins $_{4+7}$ on germination of Northern Spy embryos (Pengaruh asam nonanoat dan giberelin $_{4+7}$ pada perkecambahan embrio apel Northern Spy).

$GA_{4}+7^{x}$	Nonanoic Acid^{x}		
	0	10^{-3}	5×10^{-3}
		% germination ± SE	
0	95.8±1.4	84.1±1.4	20.8±2.8
$1x10^{-5}$	99.1±0.8	100.0	51.6±5.1
$1x10^{-4}$	99.1±0.8	99.1±0.8	75.0±3.1

x) Concentration in Molarity

Inhibition of germination by C9 at 10^{-3} M could be reversed completely by GA_{4+7} at 10^{-5} M. However, the inhibition by C9 at $5x10^{-3}$ M could not be reversed completely by 10^{-4} M of GA_{4+7} (Table 7). Interaction between C9 and GA_{4+7} was significant (Table 8). Treatment of a certain level of C9 gave different res- ponses at various concentrations of GA_{4+7} applied.

Table 8. Analysis of variance on the interaction of nonanoic acid and gibberellins $_{4+7}$ on germination of Northern Spy embryos (Analisis ragam pengaruh asam nonanoat dan giberelin $_{4+7}$ pada perkecambahan embrio apel Northern Spy).

Source	df	Mean Square
GA	2	0.5508**
Linear	1	1.0513**
Quadratic	1	0.0504**
C9	2	2.6719**
Control vs C9 Treated	1	1.6538**
C9 High vs C9 Low	1	3.6901**
GA*C9	4	0.2173**
GAlin*Ctrl vs C9 treated	1	0.3906**
GAlin*C9 high vs C9 low	1	0.4602**
GAquad*Ctrl vs C9 treated	1	0.0102^{ns}
GAquad*C9 high vs C9 low	1	0.0084^{ns}
Block	1	0.0533**
Error	98	0.0064

DISCUSSION

Short chain fatty acids and their derivatives have been shown to inhibit growth of different plant organs, including bud growth and seed germination. The biological activity of SCFA in apple embryo germination was studied and found to be dependent on chain length (Tabel 1). Of the SCFA homologous series C7 to C10, C9 and C10 were the most inhibitory. The inhibition by C7 were similar to C8.

Tso (1964) reported that methyl esters of C8 to C14 were effective in inhibiting axillary buds of tobacco. Methyl caprate (C10) resulted in almost complete inhibition. In an effort to find chemicals to delay budbreak, Rogoyski and Powell (1981) found that apple bud growth *in vitro* was inhibited by SCFA, C8- C10 being the most inhibitory in the C6 to C12 series. However, field application of SCFA was not effective in delaying budbreak in the spring.

The dependency of SCFA activity on chain length has been documented in seed germination. Those included seeds of mustard (LePoidevin, 1965), common and wild oats (Berrie *et al.*, 1975), chickpea (Babiano *et al.*, 1985), rice (Majumder *et al.*, 1989) and lettuce (Berrie *et al.*, 1975; Ulbright *et al.*, 1982). However, the inhibition by SCFA on root elongation of lettuce was not dependent on chain length (Ulbright *et al.*, 1982).

The concentration range effective for embryo germination was narrow, i.e. in millimolar range (Tabel 1). C7 and C8 had broader concentration ranges for inhibition of germination. The inhibition by C9 and C10 did not show the log linear concentration relationship usually shown with hormone responses. A similar dose response relationship has been reported for seeds of other species (Stewart and Berrie, 1979; Ulbright *et al.*, 1982; Metzger and Sebesta, 1982; and Majumder *et al.*, 1989). A more gradual effect was reported for coleoptile growth of wild oat (Metzger and Sebesta, 1982) and root growth of lettuce (Ulbright *et al.*, 1982).

The mode of action of SCFA is not well understood. They may change membrane permeability when incorporated into the membrane system. Thus the membrane may lose its normal organization, causing perhaps, differential ion exchange (Stewart and Berrie, 1979; Willmer *et al.*, 1978; Metzger and Sebesta, 1979; Babiano *et al.*, 1984), or affecting enzyme activity/binding (Buller and Parker, 1976; Hyodo and Tanaka, 1982).

Rogoyski (1980) suggested that SCFA may inhibit lipid meta- bolism Using Nuclear Magnetic Resonance (NMR), he found that there was a major decrease in lipid signal during apple seed germination. He also found that octanoic acid inhibited the disappearance of the lipid signal. Further evidence showed that potassium nonanoate inhibited the hydrolysis rate of triglycerides *in vitro* in an olive oil emulsion system.

In interaction studies among SCFA, combination applications were either equal to or less inhibitory than single applications (Tabel 3 and 4). No synergism were observed. Berrie *et al.*(1975) reported that there was some synergistic effect of SCFA in lettuce seed germination when two SCFA were applied simultaneously. A similar synergism were also observed in studies of SCFA on germination of rice seeds (Majumder *et al.*, 1989).

As stratification progressed, the concentration of nonanoic acid needed to inhibit germination of apple embryos was higher. In the embryos isolated from seeds stratified for 25 and 35 days, C9 started to inhibit germination at a concentration of $3x10^{-4}$ M, whereas in those stratified for 45 days, C9 started to inhibit germination at $3x10^{-3}$ M (Tabel 5). These data indicate that there may be a change in sensitivity in embryos toward nonanoic acid during stratification, or there may be the emergence of promotive substances that counteract the effect of nonanoic acid. Rudnicki (1969) reported that the effect of ABA was less when the seeds received more stratification. With respect to hormonal action, it has been shown that GA_4 appeared as a sharp peak on the 30^{th} day of apple seed stratification and acid lipase on the 50^{th} day (Zarska-Maciezewska *et al.*, 1980). They concluded from this and other evidence that GA activated acid lipase. In these studies, it is possible that SCFA in the embryos isolated from seeds stratified for 45 days was more readily metabolized than in the case of embryos stratified for shorter periods.

The inhibition of C9 was reversed by GA_{4+7} in these experiments. Berrie *et al.* (1975) showed that GA_3 could reverse the inhibition of lettuce germination by SCFA (C6-C10). However, GA_3 did not have any effect on subsequent root growth (Ulbright *et al.*, 1982). Metzger and Sebesta (1982) could not find a GA-reversed effect on decanoic acid inhibition of wild oat seed germination. Gibberellic acid has been shown to partially alleviate the dormancy in seeds of apple (Bulard, 1985), peach (Diaz and Martin, 1972), and cherry (Lin and Boe, 1972). SCFA inhibited GA-induced amylolysis in barley aleurone layers (Buller and Parker, 1976).

CONCLUSION

In summary, SCFA inhibition of apple embryo germination was dependent on chain length. The concentration effective for the inhibition was in the millimolar range. SCFA added in combination did not have any synergistic effect. The inhibition of SCFA was reversed by GA_{4+7}. As the stratification progressed, a higher concentration of SCFA was needed to inhibit embryo germination.

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