Mechanism of Sucrose Fatty Acid Ester Promoting Invertase Biosynthesis in Soybean Leaves*

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Abstract Results showed that 2mmol/L of sucrose fatty acid ester (SFE) could increase the activity of invertase in soybean leaves, but no effects on the permeability of cell membrane including the leakage of K^+ and reducing glucose. The analysis of scanning on SDS-PAGE gels showed that the increase of invertase activity after treated with 2mmol/L of SFE was caused by SFE enhancing the content of increase. Experiments also indicated that 8mg/L of actinomycin D (AMD, a transcription inhibitor) and 1.5mg/L of cycloheximede (CHI, a translation inhibitor) could decline the activity of invertase promoted by SFE in very short period obviously. It was suggested that SFE could increase invertase de novo biosynthesis on the level of the transcription mainly.

Key words Soybean; Invertase; SFE; AMD; CHI
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Sucrose fatty acid ester (SFE), a nonionic surfactant, could adjust the opening or closing of stomata in soybean[1], promote the absorption of metal ion, such as Mn^{2+}. SFE could also increase the content of chlorophyll in leaf and nitrogen in seed[2]. Within 12 hours after being sprayed SFE, the activities of invertase[3] and SOD[2] would be increase 160% and 40%. It was caused by that SFE could delay the senescence of leaf, improve the absorption to nutrient.

Invertase (EC 3.2.1.26) is widespread in the higher plants[4, 5]. It catalyzes the irreversible cleavage of sucrose to glucose and fructose, which is the main pathway through which a plant cell obtains energy and carbon from sucrose[6]. It was reported that the activity of invertase could be affected by many factors, such as phytohormones, stress-related stimuli, and glucose- or mannitol-treatment[5, 7]. Further results showed SFE could elevate the activity of invertase in vivo distinctly, which is not caused by the induction of sucrose, but by an increase in invertase content[3].

Up to now, there is no report on how surfactants could increase the enzyme activity or enzyme content. Therefore, the objective of the present study was to study how SFE increases the invertase content in soybean in a short time. Transcript inhibitor (Actinomycin D, AMD) or translation inhibitor (Cycloheximede, CHI) would be added into SFE solution, respectively, and be sprayed on the soybean leaves during both periods of flowering and pod-filling, to survey the changes in invertase in vivo. Subsequently, which level in the biosynthesis of invertase that SFE could increase invertase content would be determined.

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1 Material and Method

1.1 Materials

Sucrose fatty acid ester ($C_{18}H_{37}COOC_{12}H_{21}O_{10}$, HLB= 15, SFE) was purchased from Chongqing Chemical Co. and used directly. Actinomycin D (AMD) was purchased from Fluka Company and Cycloheximide (CHI) was purchased from Sigma Company in U.S. AMD and CHI was used as transcript and translation inhibitor respectively.

Soybean (Xidou No. 3, *Glycine max*) was planted in an open field and irrigated properly. During both periods of flowering and pod filling, soybean plants were sprayed with the mixture of SFE and inhibitors at 6:00 pm on a clear day and samples were taken in the next morning. To keep the uniformity of plant materials throughout the experiments, fully expanded leaves (third to sixth leaves from the apex) were chosen and used for the extraction of invertase.

1.2 Extraction and determination of invertase[3]

1.3 SDS-PAGE and scanning of invertase gel

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE) analyses of invertase treated with SFE, SFE+AMD and SFE+CHI were carried out. SDS-PAGE gels were scanned with a dual-wavelength TLC CS-930 scanner at a wavelength of 540nm.

1.4 Determination of permeability of cell membrane

1.4.1 Measurement of the relative conductivity: The relative conductivity was expressed by the ratio of conductivity to total conductivity that was measured according to Li JS[8].

1.4.2 Measurement of the leakage of K^+: Soybean leaves during flowering was taken and soaked in distilled water for about 10 min and washed. And leaves were cut to small round slice (diameter 8mm). 60 pieces were chosen and soaked into distilled water, 2mmol/L of SFE, 2mmol/L of SFE+8mg/LAMD and 2mmol/L SFE+1.5mg/LCHI, respectively. The K^+ electric potentials of solutions above were measured by K^+ electrode (PXS-5) in 1h, 6h, 12h and 24h. And the leakage concentration of K^+ was calculated by Nernst equation.

1.4.3 Measurement of the soluble glucose[3]

1.5 Survey of scanning electron microscope

Soybean leaves sprayed by distilled water, 2mmol/L of SFE, 2mmol/L of SFE+8mg/LAMD and 2mmol/L SFE+1.5mg/LCHI, respectively, were taken within 12h and treated by the methods of Ye XL to carry out the survey of scanning electron microscope[1].

2 Results

2.1 Determination of effective concentration

2.1.1 Determination of effective concentration of SFE

SFE at different concentrations was sprayed on soybean leaves and invertase activity was measured in different time to study the effects of SFE on invertase. The results were shown in Fig. 1. The total tendency was as follows: within 12h after being sprayed SFE, the activity of invertase was increased obviously. Especially, 2mmol/L of SFE could make the invertase activity increase by 1.6 times of control within 12h. However, the invertase activity would be declined along with time elongating. Therefore, 2mmol/L was chosen to the best concentration of application.

2.1.2 Determination to optimum concentration of AMD

AMD was added into SFE solution and mixed evenly. The final concentration of SFE was2 mmol/L, and the final concentration of AMD were 1mg/L, 4mg/L, 8mg/L, 12mg/L, and 20mg/L respectively. These solutions were sprayed on soybean leaves. After 12h, leaves were taken to measure the activity of invertase. Fig. 2 showed that AMD would inhibit the invertase activity, and the higher the concentration of AMD, the more the invertase activity was declined. When the concentration of AMD was exceeded to 8mg/L, the invertase activity was hardly decreased with AMD concentration increase. So, the optimum concentration

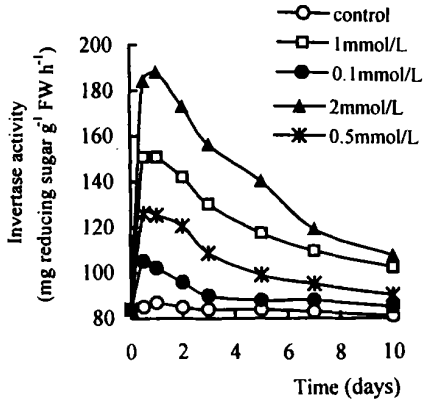

Fig. 1 Invertase activity after treated with SFE

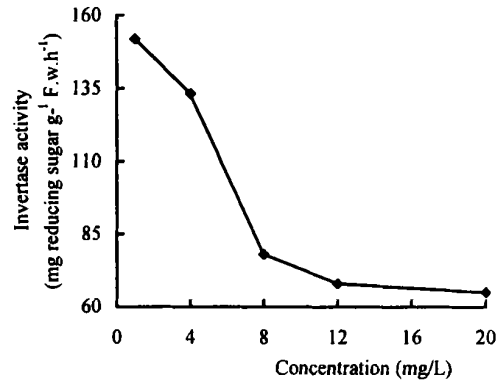

Fig. 2 Optimum conecntration of AMD

of AMD was 8mg/L.

2.1.3 Determination to the optimum concentration of CHI

From Fig. 3, the invertase activity was declined rapidly along with the rise of CHI concentration. If the concentration of CHI was higher than 1.5mg/L, there was no decrease of invertase activity. According to Fig. 3, the effective concentration of CHI was 1.5mg/L.

2.2 Effects of SFE on the permeability of cell membrane in soybean leaves

Soybean leaves were sprayed by following solutions: A(2mmol/L SFE), B(2mmol/L SFE+8mg/LAMD) and C(2mmol/L SFE+1.5mg/LCHI). After 1h, 6h, 12h and 24h respectively, the relative conductivity of membrane, leakage K^+ concentration and leakage soluble glucose were measured, and the size of stomata was surveyed by scanning electron microscope to evaluate the effects of AMD and CHI on membrane permeability.

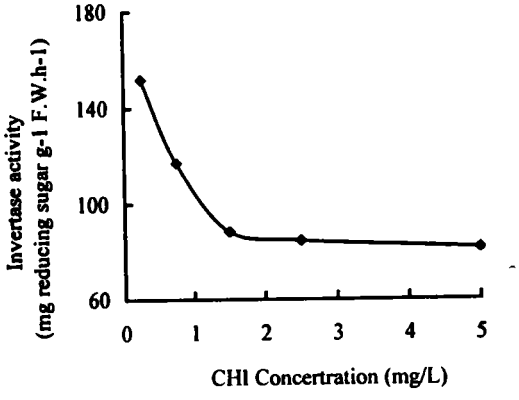

Fig. 3 Optimum concentration of CHI

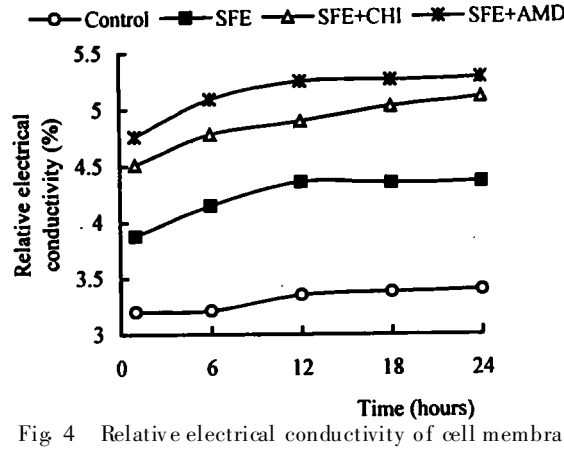

Fig. 4 Relative electrical conductivity of cell membrane

2.2.1 Relative conductivity

Fig. 4 showed the effects of 8mg/L of AMD and 1.5mg/L of CHI on the relative conductivity. Within 1h after application, three kinds of treatment solution would make the relative conductivity increase in different degree. With the elongation of application time, the relative conductivity would be increased slowly and gradually. After addition of AMD and CHI, the relative conductivity increased obviously. It was suggested that if the application time was too long, SFE, AMD and CHI could change the membrane permeability.

2.2.2 Effects of AMD and CHI on the leakage of K^+ and soluble glucose

The data in Table 1 indicated that there was no effect of SFE on the leakage of K^+ and soluble glucose. After addition of 8mg/L of AMD and 1.5mg/L of CHI, the concentration of K^+ and soluble glucose would be increased obviously. The longer treatment time was, the higher the concentration of leakage substances. It was indicated that the longer treatment time would make the cell injured severely.

Table 1 The effects of inhibitors on the leakage of K^+ and glucose of soybean leaves

Inhibitor	Time (hour)	K^+ leakage (μmol · 60discs^{-1} · h^{-1})	Glucose leakage (mg · 60discs^{-1} · h^{-1})
Control		0.04	0.591
SFE(2mmol/L)	1	0.04	0.6
	6	0.048	0.64
	12	0.051	0.68
	24	0.053	0.67
SFE+AMD	1	0.058	0.67
	6	0.073	0.74
	12	0.086	0.88
	24	0.092	0.96
SFE+CHI	1	0.054	0.69
	6	0.077	0.78
	12	0.085	0.82
	24	0.088	0.84

2.2.3 Effects of AMD and CHI on the aperture of stomata in soybean leaf

Fig. 5 indicated that 2mmol/L of SFE would make the stomata open to help leaves uptake nutrients. However, after the addition of AMD or CHI the stomata were closed gradually. Especially, 8mg/L of AMD would make stomata closed totally, and cause epicuticular was dissolved partially.

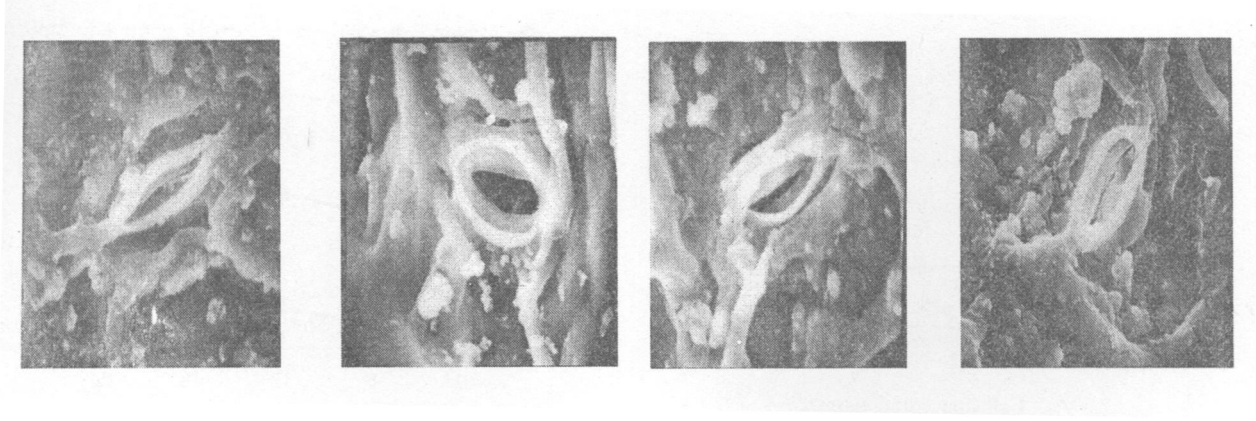

Fig. 5 Effects of AMD and CHI on the aperture of stomata in soybean leaf

According to the above results, once soybean leaves contact with SFE, AMD and CHI in a longer time, the cuticle cell would be injured severely and furthermore stimulate some stress-related proteins occurring. Under this condition, the stress invertase would emerge which would be mistaken as the influence of SFE or inhibitors. So, samples should be taken within 12h after application to avoid the produce of stress invertase, and in the other hand, the effects of SFE enhancing the invertase activity were the most obvious within 12h.

2.3 Studies on SFE promoting invertase synthesis

2.3.1 Mechanism of SFE promoting invertase synthesis during flowering

During flowering and pod-filling, the solutions of A, B and C were sprayed on the leaves of soybean, respectively. After 12h, samples were taken to survey the changes in invertase activity and the content.

After invertase extracted from above samples during flowering was purified by fractional ammonium sulfate precipitation, SDS/PAGE analyses of invertase were carried. The gels were scanned using the dual-wavelength TLC scanner CS-930.

The results were shown in Fig. 6.

The location of invertase on the lane of SDS/PAGE gels was determined according to relative migration rate by comparison with those of standard proteins. The results of comparison indicated that the location of invertase was the peak under the symbol of "↓". The area of peak represents the invertase content.

During flowering, the invertase content of the control was about 5.28 according to the peak area, and that treated with SFE was about 14.56, which was 2.75 times of the control. It was showed that 2mmol/L of SFE would make invertase content increased by 175%. Correspondingly, the content of invertase treated with CHI was about 8.4, and that treated with AMD was about 5.5. According to the experimental results, AMD and CHI could inhibit the transcript process and the translation process obviously during the invertase biosynthesis, respectively. They would hinder the function of SFE on invertase. Or rather, SFE could increase the invertase content in vivo by promoting the transcript and translation process of invertase in which acceleration of the transcript process was the main factor.

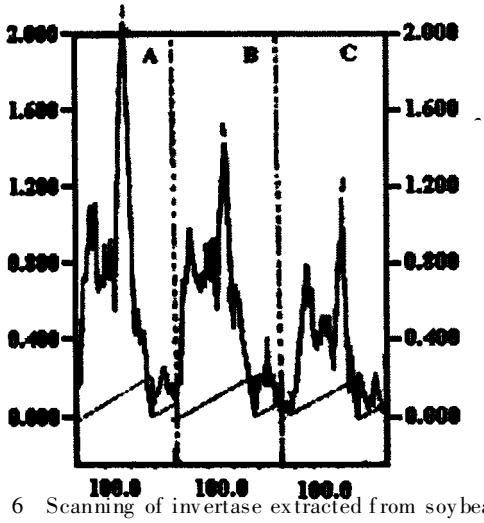

Fig. 6 Scanning of invertase extracted from soybean leaf after application of SFE, AMD and CHI on the SDS-PAGE gel A - treatment with 2mmol/L SFE, B - treatment with SFE and CHI, C - treatment with SFE and AMD.

2.3.2 Mechanism of SFE promoting invertase synthesis during pod-filling

Fig. 7 showed the results as follows. The invertase activity of the control was maintained at about 75mg reducing sugar · g^{-1} fresh weight of leaves · h^{-1} in the whole experiment. 2mmol/L of SFE would make invertase avtivity increase 81units of enzyme activity within 12h. Transcript inhibitor, AMD, could make invertase activity decreased to 119mg reducing sugar · g^{-1} fresh weight of leaves · h^{-1} which was lower 37 units than the invertase treated with SFE. And translation inhibitor, CHI, could make invertase activity declined to 135 mg reducing sugar · g^{-1} fresh weight of leaves · h^{-1}.

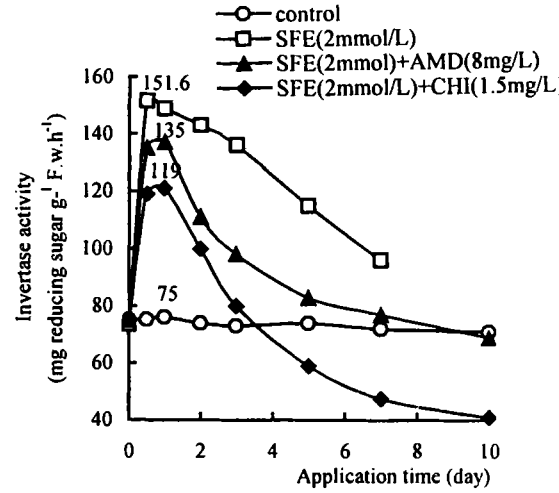

Fig. 7 Inhibition of AMD and CHI to invertase activity

The results during pod-filling, the invertase content promoted by SFE was caused by that SFE could accelerate the process of transcript and translation in the invertase biosynthesis.

3 Discussion

SFE, as an auxiliary of foliar fertilizer, could increase invertase activity obviously. According to the experimental results of permeability of cell membrane, the effective concentration of SFE, AMD and CHI was 2mmol/L, 8mg/L and 1.5mg/L, respectively.

Previous results showed SFE could elevate the activity of invertase in vivo distinctly, which is not caused by the induction of sucrose, but by an increase in invertase content[3].

According to the "central dogma of molecular

biology", protein biosynthesis was the flow of genetic information from DNA through RNA and eventually to protein. Transcript inhibitor (Actinomycin D, AMD) was a peptide antibiotic coming from streptomycin, which could bind tightly to double - helix DNA. Subsequently, DNA could not serve as a template for the synthesis of a complementary strand. AMD could inhibit the transcript process specially, but not inhibit the translation process. CHI was a strong inhibitor to inhibit the translation process specially. Because CHI could bind to 80S ribosome of eukaryote cells so that CHI could inhibit the function of transfer enzymes in the protein synthesis.

Our experiments showed that during both flowering and pod - filling, the increase of invertase content was caused by SFE promoting the de novo synthesis of invertase. SFE could accelerate the flow process of DNA →mRNA. And on the other hand, SFE could accelerate the expression of mRNA→protein. Of cause, the flow of DNA → mRNA could be a more important factor.

The adjustment to enzyme includes that to enzyme activity and to gene level. Adjustment to enzyme activity is realized by changing the structure of enzyme. Previous study showed that the conformation of invertase had not been affected, even at a concentration of 2mmol/L of SFE, according to the result of flurescence measurement[3]. Therefore, the increase of invertase content was not caused by the changes in enzyme conformation.

And adjustment to gene level is realized by changing the velocity of transcription and translation process. According to our experiment, SFE could not change the conformation, but increase the concentration of invertase. Operon theory of Jacob and Monod thought that the structure gene would be closed when aporepressor binds to operator, which would inhibit the transcript of mRNA and corresponding protein would not be expressed. But inducer, such as sucrose, xisted, aporepressor would combine with sucrose. Therefore, operator would bind to structure gene, and the transcript of mRNA would be carried out smoothly. During flowering and pod - filling, SFE would be as an inducer because the structure of SFE contains sucrose. it could combine with aporepressor, which make more structure gene accelerate the transcription of mRNA and the translation of invertase.

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that there is great difference in the four soybean varieties in the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). The controlled plants under low K exhibited an increasing of SOD、POD and CAT activities in different degrees. The activity of SOD appears earlier than that of CAT and POD, but the difference of the activity of CAT and POD is more significant than that of SOD between the effective types. So the difference of potassium nutrient effective types can be expressed by the difference of the protective enzyme systems.

Key words　Low potassium stress; Soybean; Protective enzyme system

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蔗糖脂肪酸酯调节大豆叶片蔗糖酶生物合成机理研究

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摘要　2mmol/L 的 SFE 能显著地提高大豆蔗糖酶的活力。SDS－PAGE 结果表明 SFE 主要通过增加大豆叶片蔗糖酶的生物合成量来大幅度地提高蔗糖酶的活力。用转录抑制剂 AMD(8mg/L)和翻译抑制剂 CHI(1.5mg/L)处理大豆叶片,发现它们都能在较短时间内大幅度地降低蔗糖酶的活性。SFE 对大豆开花期和结荚期的蔗糖酶生物合成量的增加是通过 SFE 促进蔗糖酶的转录水平和翻译水平来实现的,转录起主要作用。

关键词　大豆; 蔗糖酶; 蔗糖脂肪酸酯(SFE); AMD; CHI