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Enhancing freezing tolerance of *Brassica napus* L. by overexpression of a stearoyl-acyl carrier protein desaturase gene (*SAD*) from *Sapium sebiferum* (L.) Roxb.

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ABSTRACT

Sapium sebiferum (L.) Roxb. is an important woody oil tree and traditional herbal medicine in China. Stearoyl-acyl carrier protein desaturase (SAD) is a dehydrogenase enzyme that plays a key role in the transformation of saturated fatty acids into unsaturated fatty acids in oil; these fatty acids greatly influence the freezing tolerance of plants. However, it remains unclear whether freezing tolerance can be regulated by the expression level of *SsSAD* in *S. sebiferum* L. Our research indicated that *SsSAD* expression in *S. sebiferum* L. increased under freezing stress. To further confirm this result, we constructed a *pEGAD-SsSAD* vector and transformed it into *B. napus* L. *W10* by *Agrobacterium tumefaciens*-mediated transformation. Transgenic plants that overexpressed the *SsSAD* gene exhibited significantly higher linoleic (18:2) and linolenic acid (18:3) content and advanced freezing tolerance. These results suggest that *SsSAD* overexpression in *B. napus* L. can increase the content of polyunsaturated fatty acids (PUFAs) such as linoleic (18:2) and linolenic acid (18:3), which are likely pivotal in improving freezing tolerance in *B. napus* L. plants. Thus, *SsSAD* overexpression could be useful in the production of freeze-tolerant varieties of *B. napus* L.

1. Introduction

Sapium sebiferum (L.) Roxb. is not only one of the most important native woody oil trees but also an important traditional herbal medicine in China, where it has been cultivated for over 14 centuries [1]. Previous studies have suggested that the nut oil concentration in dry *S. sebiferum* L. seeds is as high as 65.76% [2]. The concentrations of oleic acid, linoleic acid, linolenic acid, and other unsaturated fatty acids are surprisingly high (up to 90%) [3]; thus, *S. sebiferum* L. nut oil is considered an ideal material for the production of biodiesel, and Japanese scientists have termed it the “green bomb” [4].

Freezing stress is a major challenge for plants in many zones [5–8]. Especially in temperate regions, freezing temperature is one of the major limitations to plant growth, distribution and crop productivity [9]. Exposure of plants to low non-freezing temperature will increase tolerance to freezing stress both in short term as in annual herbaceous plants and seasonally as in over-wintering herbaceous and woody plants [10]. Despite the fact that *B. napus* L. is an overwintering oil crop, freezing stress can still affect plant development and ultimately lead to a decrease in production [8,11–13].

It is generally known that both unsaturated and saturated fatty acids are present in plants; only unsaturated fatty acids such as hexadecatrienoic (16:3), linoleic acid (C18:2) and linolenic (18:3) acids play a vital role in the freezing tolerance of plants [8,14]. Many studies have shown that an increased content of linoleic acid (C18:2) could promote plant resistance to cold or freezing stress. In alfalfa roots the proportion of linoleic acid (C18:2) increases during low temperature acclimation [15]. Abdallah and Palta reported that the content of C18:2, an important compound for improving plant freezing tolerance, could be enhanced through cold acclimation [16]. In woody tissues such as mulberry bark, plasma membrane phospholipids underwent a dramatic increase in the leve1 of unsaturation during cold acclimation in fall and winter, primarily due to an increase in the proportion of 18:2 [17,18]. An increase in C18:2 and a decrease in C18:3 was detected in the cold acclimating potato species (*Solanum commersonii*), which was associated with increased freezing tolerance during cold acclimation [18].

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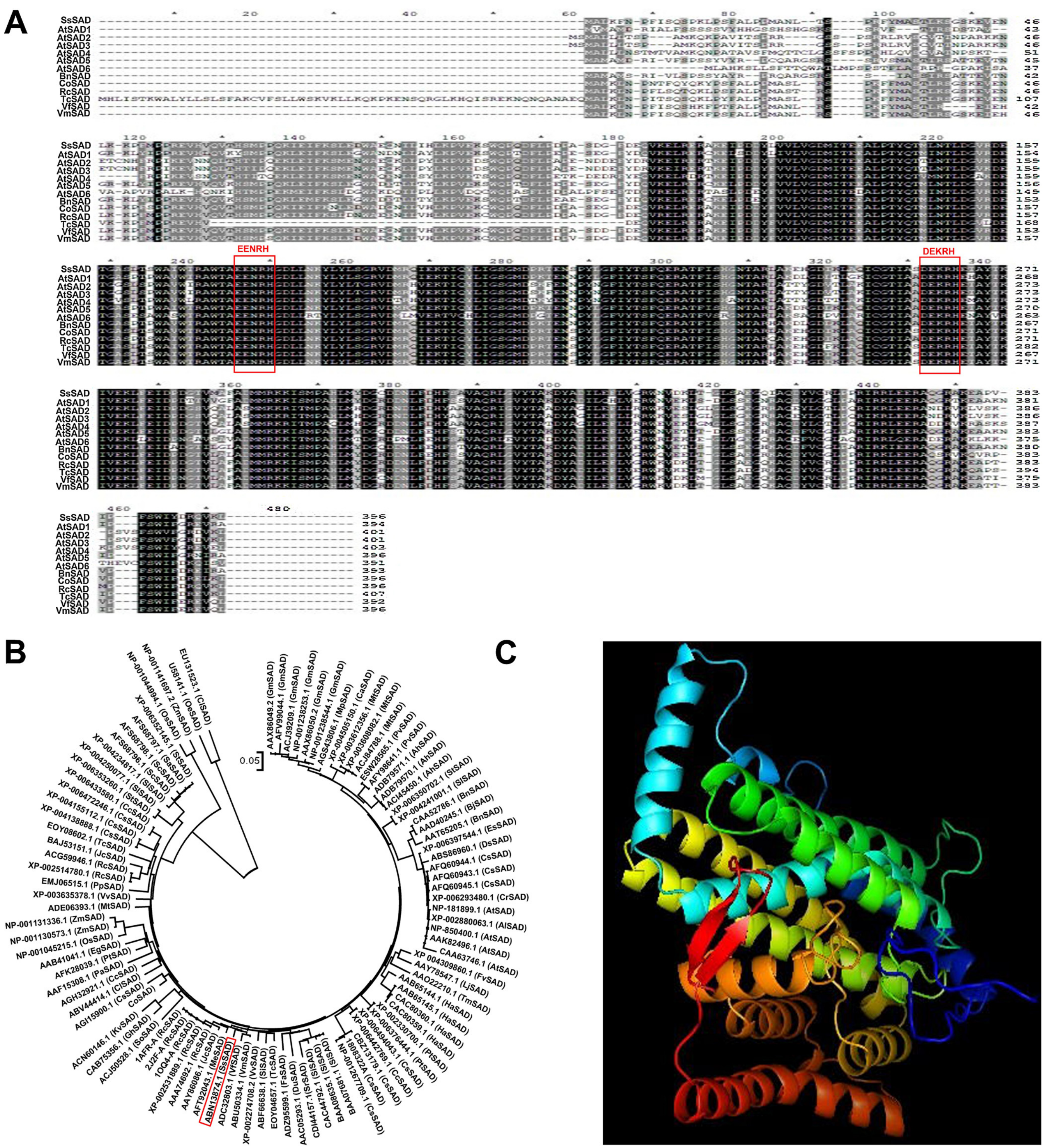

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C18:2 content exhibits seasonal variation in Scots pine (*Pinus sylvestris*) [19], cranberry (*Oxycoccos*) [16], and rice (*Oryza sativa*) [20], suggesting that it is strongly correlated with freezing tolerance. Plant freezing tolerance largely depends on the homeostasis between unsaturated and saturated fatty acids [21–23]. Unsaturated fatty acids are synthesized from saturated fatty acids by fatty acid desaturases, which convert single bonds to double bonds [24,25] and the extent of desaturation of individual fatty acids is usually regulated genetically and environmentally [25–27]. Stearoyl-acyl carrier protein desaturase (SAD) catalyzes the dehydrogenation of stearoyl-ACP (18:0-ACP) to generate oleoyl-ACP (18:1-ACP) by adding a double bond between carbon atoms 9 and 10 into the fatty acid chain, and this product, 18:1-ACP, is typically a substrate of polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2) and linolenic acid (C18:3) [8,27,28]. It follows that SAD is a key rate-limiting enzyme in the unsaturated fatty acid biosynthetic pathway and is crucial in the determination of the lipid transition from saturated to unsaturated fatty acids. The introduction of the first double bond into fatty acids in the cell membrane is necessary for the change from a gelatinous to a crystalline state [29]. SAD plays an important role in deciding the proportion of saturated to