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# Functional Characterization of *CsRCI2D/G* under Temperature Stress in *Camelina sativa* L.

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**[Introduction]**

*Camelina sativa* L. is a potential oilseed crop and belongs to Brassicaceae. Seed oil of camelina contains high amount of unsaturated fatty acid which has a suitable bioenergy crop. In addition, it has short period from seedling to harvest (about 90 days) and requires relatively low agricultural inputs. Rare Cold Inducible 2 (RCI2) proteins are known to be induced by abiotic stresses and localized at plasma membrane in model plant. RCI2 proteins have two hydrophobic transmembrane domains and can be classified into two groups by presence or absence of C-terminal tail. Our previous study reported that *RCI2* genes were induced by cold stress. However, functional study of *RCI2s* was not remained clear under high temperature stress. In this study, we investigated characteristics of *CsRCI2D* and *CsRCI2G* under high and low temperature stress.

**[Materials and Methods]**

To analyze the localization of *CsRCI2D, CsRCI2G* and *CsRCI2D* no-tail, 35S:CsRCI2s-YFP fusion protein, the recombinent vector CsRCI2s:YFP was infiltrated in 3 weeks old tobacco leaves. After 36 hour, fluorescence was observed using confocal scanning microscope (Leica, Jena, Germany). To analyze transcriptional level of *CsRCI2D* and *CsRCI2G*, one week old camelina seedlings that are grown on hydroponics were treated at each 2°C and 37°C for 24 hours. To understand tolerance of transgenic camelina, electrolyte leakage was measured under freezing stress and chlorophyll contents in four weeks old camelina leaves treated with heat stress was used.

**[Results and Discussions]**

YFP fluorescence signal in tobacco leaves was observed in plasma membrane. Expression level of *CsRCI2G* was significantly increased under cold stress, while mainly *CsRCI2D* was not. In contrast, expression level of *CsRCI2D* was increased under heat stress but not in *CsRCI2G*. In order to test the temperature tolerance of *CsRCI2G* overexpressed plants, we investigated electrolyte leakage. The result showed that when freezing stress was treated at −5°C to −7°C, electrolyte leakage of WT was higher than transgenic plants. This suggests that *CsRCI2G* can reduce damage of membrane under freezing stress. In addition, to analyze the tolerance of *CsRCI2D* overexpressed plants under heat stress, we investigated chlorophyll contents of WT and transgenic camelina after high temperature treatment. Under normal condition, chlorophyll contents were higher in *CsRCI2D* overexpressed plants but were similar in WT and *CsRCI2D* no-tail. However, after high temperature treatment, the results showed no differences between WT and transgenic plants. These results suggest that overexpression of *CsRCI2* genes could improve the tolerance under temperature stress.

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