博士論文要約（Summary）

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タイトル	Mechanism of how plants sense cold in natural environment s

Plants enhance their freezing tolerance by exposure to low-temperature. This phenomenon is known as cold acclimation (CA). Transient concentration change in cytosolic Ca^{2+} (i.e., Ca^{2+} signal) is widely accepted as a second messenger in CA process, especially in the response to the cooling. Several reports have shown the characteristics of the Ca^{2+} signal, and signal transduction. On the other hand, these studies were based on the artificial cooling and CA treatment, which hardly happens in the field. Therefore, it was difficult to conclude that Ca^{2+} signals could be used in response to small and slow temperature changes in the field.

To observe the Ca^{2+} signal which may be induced in the field condition, I developed the experimental system of cryomicroscope with Yellow Cameleon 3.60 expressing Arabidopsis. The cryomicroscope consists of laser scanning confocal microscope and cryostage / cryochamber. Since the fluorescent intensities of fluoresce protein are affected by the low-temperature, the correction formula was also established to compare the peak value of Ca^{2+} signal in different temperature. Using this system, I investigated the Ca^{2+} signal under several temperature conditions by combining the cooling rate, the start temperature and the cooling time duration. Although the clear Ca^{2+} signal has not been observed by the cooling with 3° C min^{-1} of cooling rate in root in the previous study, I succeeded in observing the Ca^{2+} signal with about 0.5° C min^{-1} of cooling rate in leaf. Since leaf is less responsive to cooling than root, the result updated our knowledge, and strongly supports the hypothesis that the Ca^{2+} signal is used as a messenger to relay the information of cooling from the cold receptor to transcriptional factors. Subsequently, in both root and leaf cells, Ca^{2+} signal rapidly disappear after stopping of the cooling and thereafter under a constant low-temperature; and no Ca^{2+} signal was observed after that. This result suggests that if the

temperature is stable and constant, no Ca^{2+} signal is induced even during 2° C constant CA treatment except the initial temperature change from room temperature to the acclimation temperature. Thus, CA in the field, rich in temperature fluctuation, was performed in Morioka during the winter season to investigate the effect of the temperature changes during the CA process. In CA under field conditions, the expression patterns of *CBF/DREB1s* was distinctly different from in artificial CA. Pharmacological studies with Ca^{2+} channel blockers, $LaCl_3$ and ruthenium red, showed that the Ca^{2+}-induced expression of *CBF/DREB1s* was closely correlated with the amplitude of temperature fluctuation in minutes, suggesting that Ca^{2+} signals regulate *CBF/DREB1s* expression during CA under natural conditions.

To see the effect of the temperature fluctuation in minutes and the temperature cycle of day and night in the controlled environment, the special temperature settings of CA were established. I used the conventional CA at constant 2° C, 10° C-day and 2° C-night of temperature cyclic CA (C- CA), and the 1° C range of temperature fluctuating cyclic CA (FC- CA). The Ca^{2+} channel blockers were used to examine the role of Ca^{2+} signal in each acclimation treatment. Freezing tolerance of the plants after C- CA and CA was almost same, and FC- CA resulted weakest freezing tolerance. On the other hand, the expression level of *CBF/DREB1s* was tended to be higher in order of CA, FC- CA and C- CA plants. In addition, the contribution of Ca^{2+} signal was higher in FC- CA and C- CA for freezing tolerance and FC- CA for gene expression. Furthermore, the Ca^{2+} signal enhanced *CBF1/DREB1B*, *CBF2/DREB1C* and *CBF3/DREB1A* in FC- CA, but suppressed the expression of *CBF2/DREB1C* and *CBF3/DREB1A* in C- CA. These results consistent with the results of experiments of the field CA described above. It had not been reported that Ca^{2+} signaling inhibited the gene expression level of *CBF/DREB1s* so far. Therefore, the experiments of FC- CA and C- CA suggested the existence of a novel Ca^{2+}-*CBF/DREB1s* pathway.

Surprisingly, Surprisingly, despite using Arabidopsis plants grown in a growth chamber, we observed a clear seasonal change in cold-induced Ca^{2+} signals only in roots. Ca^{2+} signals were captured using Arabidopsis expressing Yellow Cameleon 3.60. In winter, two Ca^{2+} signal peaks were observed during a cooling treatment from 20° C to 0° C, but in summer only one small peak was observed under the same cooling condition. In the spring

and autumn seasons, an intermediate type of Ca^{2+} signal, which had a delayed first peak and smaller second peaks compared with the those of winter type, was observed. Volatile chemicals and/or particulates in the air from the outside may affect plants in the growth chamber; this idea is supported by the fact that incubation of plants with activated carbon changed the intermediate-type Ca^{2+} signal to the summer-type. There was also a weak correlation between the seasonal characteristics of the Ca^{2+} signal and the solar radiation intensity. It has been reported that the ethylene concentration in the atmosphere seasonally changes depending on the solar radiation intensity. Ethylene gas and 1-aminocyclopropane-1-carboxylic acid treatment affected the Ca^{2+} signals, the shape of which became a shape close to, but not the same as, the winter type from the intermediate type or the summer type, indicating that ethylene in the atmosphere may be one of several factors influencing the cold-induced Ca^{2+} signal.

Additionally, to study the change of the accumulation level of Ca^{2+} in the Ca^{2+} pools before and after the CA treatments, the soft X-ray spectromicroscopy beamline at the Canadian Light Source is equipped with a state-of-the-art scanning transmission X-ray microscope was used. CA treatment induced the Ca^{2+} accumulation in the extracellular space and vacuole, where is the main Ca^{2+} pools. It is considered that the Ca^{2+} influx through Ca^{2+} channels may be enhanced by increasing the osmolarity difference between cytosol and Ca^{2+} pools. In addition, when Ca^{2+} channel blockers, $LaCl_3$ and ruthenium red, were sprayed to investigate the role of Ca^{2+} signals for the Ca^{2+} accumulation, the blocker spray tended to decrease the Ca^{2+} accumulation after CA treatment, but increased after FC- CA. These results suggest that the cooling without the Ca^{2+} signals may induce the Ca^{2+} accumulation in the extracellular space and the vacuole to fix the Ca^{2+} signaling. Taken together, plants might adapt Ca^{2+} signaling to the ambient temperature by regulating the Ca^{2+} concentration in the Ca^{2+} pools.

In conclusion, this study revealed that: (1) the Ca^{2+} signal is induced by the field-like cooling; (2) Ca^{2+} signals regulate the gene expression of *CBF/DREB1s* positively and negatively depending on the amplitude of temperature fluctuation; (3) the Ca^{2+} signal is suppressed during the summer and modified in the winter by exposure to volatile chemicals such as ethylene; and (4) there was the feedback between Ca^{2+} accumulation in the pools and

Ca^{2+} signaling. Therefore, the Ca^{2+} signaling deeply contribute the sensing of the cold to regulate the physiological process of the CA, and will become a clue to understand how plant sense cold in natural environment.