(Format No. 3)

SUMMARY OF DOCTORAL THESIS

Name: PRASAJITH KAPILA DISSANAYAKE

Title: Use of *Allium* alien monosomic addition lines to investigate chlorophyll degradation mechanism in stored Japanese bunching onion
(ネギ属異種染色体添加系統を用いた葉ネギの貯蔵中におけるクロロフィル分解機構の解明)

.....

SUMMARY

Japanese bunching onion (*Allium fistulosum* L.; JBO genome FF), or welsh onion, is an important green vegetable in East Asia and to a certain extent in the USA. The shelf life of JBO leaves diminished with loss of greenness or yellowing of leaves as a consequence of chlorophyll (Chl) degradation, which seems to be the main factor in yellowing of stored JBO leaves.

Green leaves of JBO, stored at 25°C, turned yellow within 3 days, whereas no changes occurred at 4°C. Pheophytin (Phy) *a*, chlorophyllide (Chlide) *a*, pheophorbide (Pheide) *a* and $C13^2$-hydroxychlorophyll (OHChl) *a* were the main derivatives of Chl *a* and diminished at 25°C concomitant with leaf yellowing, whereas no significant reductions were observed at 4°C. The activities of Chl-degrading peroxidase and Mg-dechelation increased significantly at 25°C but not at 4°C. Chl degradation was accelerated and Chl derivative levels (mainly Phy *a*, Chlide *a*, Pheide *a* and OHChl *a*) were diminished in JBO leaves stored at 25°C, suggesting that the derivatives were rapidly converted into their following forms.

A series of alien monosomic addition lines (AMALs; FF+1A to FF+8A) of JBO, with extra chromosomes from shallot (*A. cepa* L., Aggregatum group; genome AA) provide a genetic resource for the improvement of JBO, based on their physiological and morphological characteristics, and difference in appearance in relation to the extra chromosomes. Chl degradation may also be affected by these additional chromosomes. Therefore, these AMALs could be used to elucidate the mechanism of Chl degradation in JBO, based on differences in Chl degradation among the AMALs. Year-round changes in Chl content in JBO and in the AMALs were also detected. The Chl content in FF was significantly influenced by the alien chromosomes from shallot. In particular, the Chl contents of FF+4A or FF+5A, were higher than in FF that chromosomes 4A and 5A from shallot had a larger influence on the formation of Chl than the other chromosomes. Chl contents in FF+3A and FF+5A decreased greatly in the leaves of AMALs and JBO during storage at 25° C, whereas in FF+4A reduction of Chl content was lowest compared to the control, FF. Chlide *a*, OHChl *a*, Pheide *a*, and Phy *a*, as derivatives of Chl *a*, were present mainly during storage of JBO and the AMALs. Phy *a* levels increased with Chl degradation, especially in

FF+3A (rapid Chl-degrading line), while OHChl *a* levels in FF and FF+3A showed a decline during storage. In addition, Chlide *a* levels in all lines diminished during storage. Moreover, the activities of Chl-degrading enzymes, especially Chl-degrading peroxidase and Mg-dechelation, also progressively increased during storage at 25℃.

More interestingly, in JBO leaves, during Chl degradation, presence of Phy *a* was prominent, which was not recorded in other horticultural crops. The formation of Phy *a* in stored JBO leaves were further investigated. By incubating the reaction mixture of crude enzyme extract and Chl *a*, Phy *a* was directly formed by Mg-dechelating action. Especially in FF+3A, the formation of Phy *a* was high than FF and FF+4A. Electron microscopic observation of plant cells elucidated the formation of plastoglobuli in chloroplast and its movement from chloroplast to vacuole in JBO. These observations indicated that Phy *a* could be, in part, formed non-enzymatically by the acidic condition in vacuole. Especially, a large number of plastoglobuli in vacuole of FF+3A might relate to the high Phy *a* formation with Chl degradation.

Thus, all these findings suggest that Chl *a* could be degraded, in part, through Phy *a*, as well as Chlide *a* and OHChl *a*, and the vacuole along with the chloroplast might be an important site for Chl degradation in JBO during storage.