(Format No. 3)

SUMMARY OF DOCTORAL THESIS

Name: Vu Quynh Hoa

Title: Exploitation of Indigenous *Allium* Germplasm in Southeast Asia and Its Application to Practical Cytogenetic Studies
（東南アジアにおける在来ネギ属遺伝資源の探索とその実用細胞遺伝学研究への応用）

Allium cepa is one of the most economically important species in the section *Cepa* of the genus *Allium*. It contains two main groups: the common onion group (onion) and the Aggregatum group (shallot). Onion is the most important *Allium* vegetable crop, with large numbers of cultivars developed and grown in many parts of the world. Meanwhile, shallot is an economically important spice crop cultivated mainly in low-latitude areas such as south-east Asia. Onion breeding has been based on F_1 hybrid seed production since the discovery of cytoplasmic male sterility (CMS) in onion with S cytoplasm and two homologous recessive genes, namely CMS-S. The wide use of the F_1 hybrid has resulted in a narrow genetic base in onion. Crossbreeding of shallot has been poorly examined because the main propagation system is based on division. However, the breeding of shallot would be also very promising because shallot can set seeds and possesses a close genetic relationship with onion. The current breeding goal of *A. cepa* has been to develop new varieties with some favorable characteristics, such as male sterility, health-enhancing qualities, and disease resistance. This study was focus on a shallot germplasm collection derived from Vietnam and various countries and a threatened wild species, *Allium roylei*, to exploit novel breeding materials for *A. cepa*. The objectives of the present study were 1) to characterize intraspecific variation within a shallot germplasm collection based on chemical components and their relationships with antioxidant activities in shallot, 2) to evaluate antifungal effects of several chemical compounds in shallot and to assign the chromosomal location of genes in shallot related to Fusarium wilt (FW) resistance using a complete set of *A. fistulosum* - shallot monosomic addition lines (MALs), and 3) to develop alloplasmic and alien monosomic addition lines (AMALs) in *A. cepa* using *A. roylei* as a donor species.

Wide variation was observed in the quantitative analyses of the chemical contents in shallot germplasm. Shallots with high contents of polyphenols, saponins, and quercetins were found in southern Vietnam and other low-latitude countries. Meanwhile, those possessing fairly high *S*-alk(en)yl-L-cystein sulfoxide and sugar contents were observed in northen Vietnam. Qualitative analysis of saponins via thin layer chromatography (TLC) did not show clear variation among shallot strains, but polymorphism was observed between shallot and other *Allium* species such as *A. roylei*. The principal component analysis could clearly discriminate shallot strains by their geographical origins. All shallot strains showed potent antioxidant activities in the DPPH assay. The highest antioxidant capacity was found in the strains possessing relatively high polyphenol, quercetin, and saponin contents. Significant correlations were found between antioxidant capacity (IC_{50}^{-1}) and four groups of chemical compounds (polyphenols, quercetins, saponins, and ACSOs) (r = 0.41 - 0.59). A strong correlation was found between IC_{50}^{-1} and quercetin contents (r = 0.59, $p < 0.01$). The six *Fusarium*-inoculated shallot strains seemed to be adequately resistant against the disease and the levels of resistance may be related to the saponin content in the bulb tissues.

The antifungal effects of hexane, butanol, and water extraction fractions from bulbs of shallot on 35 isolates of *F. oxysporum* were examined using the disc-diffusion method. Only the

hexane and butanol fractions showed high antifungal activity, while no fungal inhibition was observed in the water fraction. Inoculation tests with *F. oxysporum* f. sp. *cepae* isolated from *A. fistulosum* were carried out in shallot and the eight *A. fistulosum* - shallot MALs (FF+nA, n=1~8). Shallot showed no symptom of disease after inoculation. Moreover, the phenolic content of the roots and the saponin content of root exudates released into a hydroponic medium of inoculated shallot increased to much higher levels than those of the control at three days after inoculation and tended to reach normal contents at 28 days after inoculation. Application of freeze-dried shallot root exudates to seeds of *A. fistulosum* soaked in a spore suspension of *F. oxysporum* resulted in protection of seedlings against infection. Among eight MALs and *A. fistulosum*, FF+2A showed the highest resistance to FW. This MAL also showed a specific saponin band derived from shallot on the TLC profile of saponins in the eight MALs. The chromosome 2A of shallot might possess the genes related to FW resistance.

A. roylei was employed as the donor species for producing two different plant materials in order to explore its new possibilities for the following breeding purposes: 1) the development of cytoplasmic substitution lines and 2) the production of AMALs in *A. cepa*. The chromosomes of a single F_1 plant between *A. roylei* ('97175', female) and shallot ('86208') were doubled, and then backcrossing with shallot ('Chiangmai') or bulb onion ('Banchusei') was performed to produce BC_1 as allotriploids. One allotriploid plant was used for backcrossing with bulb onion ('Sapporo-Ki') as the seed parent (BC_2: BR line) and selfing (BC_1F_2: SR line). Another plant was used for backcrossings with bulb onions ('Kitami Kohai 39 go' and 'Sapporo-Ki') as pollen parents (BC_2: RB and SY lines). BC_2 showed $2n$=16, 17, 18, 19, 22, 23, and 24. Diploid plants were recorded with the highest frequency (110 plants), followed by the monosomic plants (48 plants), in a total of 169 BC_2 and BC_1F_2 plants. For the development of cytoplasmic substitution lines, BC_2 plants possessing 16 chromosomes in the RB line (68 plants) were checked for pollen fertility in Yamaguchi, Japan, and then backcrossed with bulb onion again to evaluate their seed-setting characteristics. The pollen fertility of diploid BC_2 ($2n$=16) ranged from 0% to more than 10%. A large number of plants showed no pollen fertility (0%). In Kagawa and Hokkaido, most of the plants were completely pollen-sterile, while the percentages of seed sets in BC_2 were high enough to reproduce BC_3 seeds. The results revealed that the substitution of the *A. roylei* cytoplasm would be useful for the development of a novel CMS line in *Allium*.

For the production of AMALs, 48 BC_2 and BC_1F_2 plants with $2n$=17 were used for the characterization of alien chromosome from *A. roylei* via chromosome-specific markers (three isozymes and ten EST markers). A complete set of eight AMALs (CC+nR, n=1~8) with different frequency was obtained: CC+1R (one plant), CC+2R (one), CC+3R (two), CC+4R (one), CC+5R (two), CC+6R (one), CC+7R (two), and CC+8R (14). All the plants could form dormant bulbs in different sizes and colors. In genomic *in situ* hybridization analysis, CC+1R, +3R, +4R, +5R, +7R, and +8R showed an entire extrachromosome from *A. roylei* in the integral diploid background of *A. cepa*. A single recombination between *A. cepa* and *A. roylei* was observed on the extrachromosome in CC+6R. All alloplasmic AMALs showed high or complete pollen sterility. Only the autoplasmic CC+4R possessed relatively high pollen fertility. Downy mildew screening in the field showed higher resistance in *A. roylei*, a hypo-allotriploid (CCR-nR, $2n$ = 23), and an allotriploid (CCR, $2n$ = 24). Meanwhile, no complete resistance was found in some AMALs examined. The novel complete set of AMALs would be potential material not only for the breeding of *A. cepa* but also for genetic studies of *A. roylei*.

All the results obtained open new prospects to conduct further biochemical and genetic studies in *A. cepa* gene pool including wild species and develop novel varieties in *A. cepa* .