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SUMMARY OF DOCTORAL THESIS

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Title: Phytochemical Characterization of Major *Allium* Cultivated Vegetable Crops toward Improving Biotic and Abiotic Stress Adaptations

(生物的・非生物的ストレス適応性の改良に向けた主要ネギ属野菜の植物化学的特性評価)

Allium, recognized as the larger genus of monocotyledon plants, has more than 800 species. They are distributed all over the world, from the frozen zone to the dry subtropics. The species' diversity in a certain area has been influenced by local climatic conditions, which has led to natural selection and, also, local consumer preference regarding taste and flavor. Bulb onion (*Allium cepa* L. Common onion group), shallot (*A. cepa* Aggregatum group), Japanese bunching onion (*A. fistulosum* L.), and garlic (*A. sativum* L.) are agricultural commodities for spices and vegetables throughout the world. Both bulb onion and garlic extracts also have high antibiotic activity against some infections in the human body. The nutrients that are beneficial to human health have led to the increase in *Allium* consumption.

The shallot, important *Allium* spice vegetable with high production and demand in Southeast Asia, is interesting and prospective materials for the breeding of bulb onion (*A. cepa* Common onion group). Although of different shapes and sizes, both shallots and bulb onions produce importance phytochemical compounds with high nutraceutical values. Wide-ranging varieties and/or strains of bulb onions and shallots were utilized to understand the variation in chemical compounds responsible for their taste in this study. The bulb samples of 10 F_1 commercial bulb onion varieties (seven short-day and three long-day varieties) from Japan and 12 shallot landraces from abroad (Vietnam: three landraces; Indonesia: nine landraces) were collected as plant materials once a year in 2014 and 2015. The contents of *S*-alk(en)yl-L-cysteine sulfoxides, total flavonoids, and soluble sugars—including fructose, glucose, sucrose, and fructans—were determined for the purposes of finding differences between shallots and bulb onions as well as detecting variations among varieties and/or landraces. While a principal component analysis (PCA) based on results from both 2014 and 2015 could clearly discriminate shallots from bulb onions from a phytochemical perspective, bulb onions mainly had higher monosaccharaides than did shallots. In contrast, shallots produced more polysaccharides than did bulb onions. Flavonoid and PeCSO (*S*-(1-propenyl)-L-cysteine sulfoxide: isoalliin) contents in shallots were higher than those detected in bulb onions, indicating that the stronger pungent, bitter taste could be attributable to excess amounts of these compounds in this tropical plant. In most cases, regression analyses using numerical data of the chemical compounds found in shallots and bulb onions suggested year-to-year correlations between 2014 and 2015.

Highly accurate phytochemical composition analysis might be responsible for positioning *A. cepa* varieties and landraces favorably. For this purpose, the contents of amino acids and several intermediate compounds on the *S*-alk(en)yl-l-cysteine sulfoxides biosynthesis pathway were determined by amino acid analyzer and liquid chromatography tandem-mass spectrometry (LC-MS/MS), respectively, for the bulb samples of shallots and bulb onions. Several important amino acids related to the unique *Allium* taste, such as aspartic acid and glutamic acid, were present at relatively high levels in both bulb onions and shallots. An interesting result found that shallots produced higher contents of amino acids than did bulb onions. Furthermore, the possible *S*-

alk(en)yl-L-cysteine sulfoxides biosynthesis pathway of shallots seemed to be differentiated from the conventional route of bulb onions, since a couple of specific intermediate compounds were detected predominantly in shallots. The direct synthesis of γ-glutamyl-*S*-2-carboxyprpylcysteine from γ-glutamylcysteine seems to take place in shallots, whereas in bulb onions, synthesis occurs in an alternative way via glutathione biosynthesis. In short, the simplified *S*-alk(en)yl-L-cysteine sulfoxides biosynthesis pathway was demonstrated in the comparative study of phytochemical variations between bulb onions and shallots. This mechanism is possibly related to shallots' ability to grow in high temperatures and dry conditions, such as in Indonesia. In that stressful environment, shallots seem to reduce their energy usage by choosing a more efficient biosynthesis pathway. Due to that defense mechanism, shallots could be more resistant to drought and high temperatures.

Japanese bunching onion （*A. fistulosum*) is a widely cultivated species of the genus *Allium* that contains chemical compounds beneficial for human health. During cultivation, unfavorable environmental conditions causing biotic and abiotic stress may affect its productivity and quality. High summer temperatures limit Japanese bunching onion production. Heat stress, followed by biotic stress such as attacks by thrips, can seriously damage Japanese bunching onion production in Japan. A heat-tolerant mechanism could partly be triggered by the increase of some metabolites, such as ascorbic acid and flavonoids, together with the overactivation of antioxidant activity. The high demand for Japanese bunching onions motivates breeders to develop novel heat-tolerant varieties. To evaluate the morphological and phytochemical characteristics of possible Japanese bunching onion heat-tolerant varieties, a two-year experiment was carried out using four varieties in 2013 and nine in 2014. High negative correlations were found between DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and almost all morphological traits, while high positive correlations were discovered between morphological traits and *S*-alk(en)yl-L-cysteine sulfoxides contents. The Japanese bunching onion varieties used in this study could be characterized by the PCA of eight agronomic traits and seven different sets of phytochemical data. 'Kuronegi,' 'Kaminari,' 'NE-15', and 'Fukuichi' were suitable for high-yield production because of their higher total fresh weight during summer cultivation. 'Fuyuhiko' and 'Sanpeinegi' had lower thrips damage as compared with other varieties, showing promising for resistance to the vermin that usually aggravated plants' conditions during the summer.

Allium roylei Stearn (RR, $2n=2x=16$), native to northwest India, is considered the bridging species between *A. cepa* and *A. fistulosum* (FF, $2n=2x=16$). This triangle of species is now providing attractive breeding materials worthy of the investment of considerable time and effort toward cultivar improvement. Interspecific hybridization between *A. fistulosum* and *A. roylei* has successfully produced F_1 hybrids. The chromosome doubling of an F_1 hybrid has produced amphidiploids. After the backcrossing of amphidiploids with *A. fistulosum* twice, the BC_2 generation was obtained with chromosome numbers ($2n$) ranging from 16 to 23. Alien monosomic addition lines (FF+nR, $2n=17$) appeared with the highest frequency. Furthermore, multiple addition lines ($2n=18–23$) were also observed with less frequency. Five alien monosomic addition lines (FF+1R, +3R, +4R, +5R, and +8R) and 10 multiple addition lines were characterized using isozyme and DNA markers. Extrachromosomes from *A. roylei* clearly altered the biochemical characteristics of the multiple addition lines. Variations in *S*-alk(en)yl-L-cysteine sulfoxides, flavonoid, and sugar contents were observed among the multiple addition lines. *Allium fistulosum*−*A. roylei* allotriploids (FFR, $2n=3x=24$) showed significantly higher saponin contents and antifungal activities of saponin extracts against isolates of *Fusarium oxysporum* f. sp. *cepae*, as compared to *A. fistulosum*.

All in all, the results obtained thus far could be exploratory data upon which to build further research to improve the varieties of bulb onions, shallots, and Japanese bunching onions and to enhance their biotic and abiotic stress tolerances and nutraceutical value.