

学位論文全文に代わる要約 Extended Summary in Lieu of Dissertation

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学位論文題目 :

Title of Dissertation

Chloroplast DNA structure, diversity and genetic variation of hosta, and analysis of their essential macro-micro mineral content

(ギボウシ属植物の葉緑体DNA構造, 多様性と遺伝的変異及び主要な多量・微量ミネラルの分析)

学位論文要約 :

Dissertation Summary

Hosta, a member of Asparagaceae family, is a shade-tolerant perennial garden plant. It is also recognized as plantain lilies, and giboshi in Japan. It is preferable to the gardeners for its striking foliage. It is mostly used as plant for landscape and sometimes as vegetables. It has many varieties, cultivars, and hybrids and these have dissimilar leaves. A number of hostas are also grown in many parts of Japan and Shikoku area as well. Some of hosta communities appear as stable conditions while others have rigorous fluctuations. Molecular techniques widely used for genetic structure, diversity, evolutionary status and genetic variation among taxa using dissimilar genes and DNA-based markers. Despite great phenotypic diversity of hostas, it has little information regarding genetic organization. Now a day, people consume a range of foods for taste and health benefits. Agriculturists have already added several plants from non-cultivated resources in the vegetable lists and these are asparagus, globe artichoke, Jerusalem artichoke, rhubarb, bunching onion, Egyptian onion etc. A percentage of world populations face malnutrition that can be resolved by adding more plants from non-cultivated resources in human diet. Hostas have the potentiality to be nutritious vegetable like asparagus. Concerning the facts for the hostas, the objectives of this study was to analysis of the genetic structure, diversity, variation and their leaf mineral content of a number of hosta taxa.

We collected leaves of hosta plant from different locations around Shikoku Island for the study and recorded the GPS data for each sample collection sites. We used newly emerged leaves for cpDNA extraction and mineral content analysis. We amplified cpDNA intergenic regions with a pair of primers *trnS* (GCU) and *trnG* (UCC). Amplified PCR products were sequenced (399 in total) using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and sequence data were deposited in DNA Data Bank of Japan (DDBJ) under the accession number LC152207 to LC152287. We used RAPD 10mer Kit A (OPA-01 to OPA-20) for amplification as well. In this case, we used 1000 kb DNA ladder for gel electrophoresis and obtained binary data for the presence (1) or absence (0) of band from gel image analysis. The leaf samples were dried and powdered and then acid digestion was done. We used a Phoenix™ CEM microwave muffle furnace (Tokyo, Japan) at 580°C for 6 hrs for ashing. We added approximately 5 ml of HCl : H₂O₂ (1:1) solution to the ash and heated at 80°C for the approx. 1-2 ml volume. The solutions were filtered and prepared 100 ml solution. We took 1000 µl of filtered solution and diluted for a final volume of 50 ml, which were used for the inorganic analysis. We determined mineral content of the samples using Induced Coupled Plasma Spectroscopy (Japan) fitted with an automatic sampler (Japan) for macro minerals (K, P, Ca and Mg) and micro minerals (Fe, Zn, Mn and Cu). The absorption values were calculated, and converted into ppm and percentages as well. We used a number of statistical softwares for the dissimilar data analysis.

We found the negative values for Tajima's D (-2.263), Fu and Li's D (-5.181) and Fu and Li's F (-4.810) test thus indicated the increase of the population size. *H. sieboldiana* and *H. kiyosumiensis* populations showed

the maximum haplotype diversity (1.0), and *H. alismifolia* showed maximum nucleotide diversity (π : 0.012). Tajima's D, Fu and Li's D and Fu and Li's test were not significant at $p > 0.10$ level of significance. The different numbers of polymorphic sites were found for each taxon. The maximum F_{ST} divergence (52.7%) was between *H. tardiva* and *H. kikutii* var. *caput-avis* populations, and *H. sieboldiana* and *H. tardiva* populations (52.2%) as well, which means these pairs were greatly differentiated from each other. The divergences of population of each taxon were shown in NJ phylogenetic tree. The numbers of haplotypes were different from taxon to taxon. From the analysis of RAPD markers, we found maximum number of bands for *H. longissima* (40), and the second highest was for *H. sieboldiana* and *H. sieboldii* (35) while minimum for *H. nakaiana* (14). Maximum genetic distance (G_D : 0.655) and minimum genetic identity (G_I : 0.520) were for *H. sieboldiana* - *H. longissima*, and between *H. sieboldii* - *H. longissima* as well. Conversely, minimum and maximum G_D and G_I values were found for *H. kikutii* var. *polyneuron* and *H. longipes* var. *caduca* (0.071 and 0.931, G_D and G_I respectively). A dendrogram comprising twelve hosta taxa and UPGMA cluster showed average-linkage relationships among them. From the macro and micro minerals content analysis, we found that the maximum leaf K was in *H. alismifolia* (4.05%) and *H. montana* (3.87%), and P was in *H. nakaiana* (0.34%) and *H. tardiva* (0.29%). Higher Ca, Mg, Mn, Zn, Cu, and Fe content was in *H. sieboldii* (1.15%), *H. nakaiana* (794.12 ppm), *H. longissima* (133.77 ppm), *H. nakaiana* (334.52 ppm), *H. longissima* (5.95 ppm) [statistically alike with *H. nakaiana* (5.62 ppm) and *H. montana* (5.52 ppm)], and *H. sieboldii* (251.95 ppm), respectively. *H. montana* and *H. sieboldii* showed 0.23% and 0.21% of P, while 606.68 ppm and 603.95 ppm Mg, respectively but *H. montana* contained very low Ca (0.17%). *H. alismifolia* had second highest Mg and Fe content and *H. longissima* showed second highest leaf Zn content.

The hosta population is increasing irrespective to their species or cultivars in Shikoku areas. RAPD provided genetic variations of the entire genome for these hostas. Phenotypically dissimilar hostas can vary in a few loci, whereas phenotypically alike genotypes can differ completely. Results of the micro-macro minerals analysis of the current study, *H. alismifolia*, *H. sieboldii*, *H. nakaiana*, *H. longissima* and *H. montana* can be considered as a regular dietary source of minerals more specifically for their K, Ca, Fe, P, Mg, Zn content. Lastly, we found frequent inter-taxon genetic variation in the hosta population around the Shikoku area, their genetic variability and diversity, and amount of the leaf macro- and micro-mineral content.