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An overview of the current research program for haskap (*Lonicera caerulea*), a useful genetic resource in Hokkaido, Japan

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Abstract

There are many useful genetic resources as berry crop candidates in Hokkaido, Japan. Representative plant genetic resources are haskap (*Lonicera caerulea*; Caprifoliaceae), *Rubus idaeus* (Rosaceae), *Actinidia arguta* (Actinidiaceae), and so on. Among them, this manuscript focuses on haskap, showing attractive features for commercial production in the Hokkaido regions. In this manuscript, characterization of the wild genetic resources is described. Both diploid ($2n = 2x = 18$) and tetraploid ($2n = 4x = 36$) were found in Hokkaido. Our study showed that tetraploid haskap populations are widely distributed in Hokkaido, while diploid populations are distributed only in eastern Hokkaido. The factors affecting this distribution pattern are now analyzed. As an attempt to improve haskap accessions, interspecific hybridization has been applied between haskap and Miyama-uguiskagura (*Lonicera gracilipes*), belonging to the same genus as haskap. The interspecific hybrids were fertile and could produce fruits. To characterize the fruit contents of the parental species and interspecific hybrids, liquid chromatography/tandem mass spectrometry (LC/MS) and imaging mass spectrometry were used for detailed analyses.

Key words: haskap, imaging mass spectrometry, interspecific hybridization, LC/MS, *Lonicera caerulea*, *Lonicera gracilipes*

Introduction

Wild genetic resources of haskap (*Lonicera caerulea*) are distributed in northern Eurasia and North America as well as in Japan. Commercial production of haskap fruits has been developed in Hokkaido by using wild haskap accessions. For further utilization and conservation of the wild genetic resources of haskap, further research for genetic diversity is needed. An important characteristic of the wild genetic resources is ploidy level in this plant species. There are diploid ($2n = 2x = 18$) and tetraploid ($2n = 4x = 36$) plants. It is known that these polyploids are distributed in northern Eurasia (Plekhanova et al. 1992). Here, I described the current situation of haskap resources in the Hokkaido region by referring to the study of Miyashita et al. (2011).

Various studies have been conducted to proceed in haskap breeding program and to develop the genetic resources of haskap. Physiological characteristics of seed germination were analyzed in haskap (Phartyal et al. 2009). The seeds of haskap showed morphological dormancy. Endosperm culture was applied to haskap to produce hexaploid plants from the endosperm tissues of tetraploid haskap plants (Miyashita et al. 2009). In this study, an aneuploid plant was produced. This study indicates that the endosperm tissues are useful materials for producing polyploid plants by tissue culture techniques. Various polyploidy haskap plants have been produced by cross-pollination between different polyploid plants (Miyashita and Hoshino 2015). In this study, diploid, triploid, tetraploid, pentaploid, hexaploid and octoploid plants were presented. Interspecific hybrid plants were produced between haskap and the related plants in the same genus (Miyashita and Hoshino 2010). A mechanical harvest machine was examined to harvest haskap fruits efficiently (Fu et al. 2011). Postharvest ripening procedures were tried to develop for transporting immature haskap fruits to distant places (Yamamoto et al. 2014).

Furthermore, we tried to apply interspecific hybridization to improve the traits of haskap. Miyama-uguisukagura (*Lonicera gracilipes*) belongs to the same genus as haskap, and has good-tasting fruit. Therefore, we performed interspecific crosses between haskap and Miyama-uguisukagura. Then, the fruits of interspecific hybrids were analyzed using liquid chromatography/tandem mass spectrometry (LC/MS) and imaging mass spectrometry to evaluate the fruit qualities. In this manuscript, these recent achievements are explained.

Distribution of haskap diploids and tetraploids in Hokkaido

Plekhanova et al. (1992) investigated the ploidy distribution of haskap in northern Eurasia. To clarify the characteristics of the genetic resources of haskap in Hokkaido, we searched for wild haskap to investigate ploidy distribution (Miyashita et al. 2011). Both diploid and tetraploid haskap were found in Hokkaido (Figure 1). The diploid and tetraploid haskap plants were identified with flow cytometry by the measurement of DNA contents. The leaves of the *Capsicum annuum* were used as an internal standard for flow cytometry. The tetraploid plants were widely distributed in Hokkaido. On the other hand, diploid haskap plants were distributed only in eastern Hokkaido. The diploid haskap plants were mainly habited on a wetland in eastern Hokkaido (Figure 2). Currently, only tetraploid haskap plants are used for cultivation. In the future, detailed analyses of the ploidy

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distribution patterns of these genetic resources will be investigated.

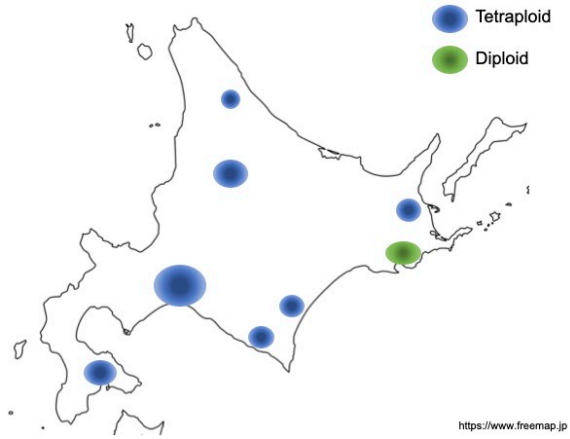

Figure 1 Ploidy distribution pattern of haskap in Hokkaido. Diploid and tetraploid plants are localized.

Figure 2 A typical scene of a wetland in eastern Hokkaido. In this region, the diploid haskap plants are found.

Production of new haskap strains using interspecific hybridization and analysis of the fruit qualities by LC/MS and imaging mass spectrometry

Interspecific hybridization was conducted between haskap and Miyama-uguiskagura to create a variation of haskap fruits. The hybrids could be efficiently produced, when Miyama-uguiskagura and haskap were used as the seed and the pollen parents, respectively. By using the tetraploid Miyama-uguiskagura and the tetraploid haskap for crossing, the interspecific hybrids became amphidiploid. Therefore, the hybrid plants could produce fruits. The fruit morphology showed intermediate traits of the parents (Figure 3). The fruits were analyzed with LC/MS and imaging mass spectrometry to characterize the fruit contents of Miyama-uguisukagura, haskap, and the interspecific hybrids (Fujita et al. 2020a; Fujita et al. 2020b). Miyama-uguisukagura has red and haskap has dark purple fruits, while the interspecific hybrid fruits have intermediate red-purple fruit color. Analysis of anthocyanins revealed that anthocyanins were localized in the skin of the fruits.

Regarding the amount of pigment, the interspecific hybrids tended to be intermediate between the parents.

Figure 3 The fruit of interspecific hybrid between Miyama-uguisukagura and haskap. The fruit shows intermediate characteristics between Miyama-uguisukagura and haskap.

However, quantitative analysis of the various anthocyanins, cyanidin 3,5-diglucoside and peonidin 3,5-diglucoside were higher in concentration than those of the parents. These anthocyanin distribution patterns might involve the fruit morphology. Further research is now in progress to elucidate the changes of anthocyanin accumulation in the hybrid fruits by analyzing biosynthetic pathways of anthocyanins.

Conclusion

This research is needed to conserve the wild haskap genetic resources by evaluating the genetic diversity. For sustainable utilization of haskap genetic resources, “wise use” will be needed based on scientific knowledge.

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