Accumulation of polymethoxyflavones in plants of the subfamily Aurantioideae including wild citrus

○Fatema Tuz Zohra1) • M. Yamaguchi2) • A. Fujisawa2) • Y. Itami2) • A. Noutomi3) • N. Kotoda1) 2)
(1) The United Graduate School of Agricultural Sciences, Kagoshima Univ. 2) The Graduate School of Agriculture, Saga Univ. 3) Saga Prefectural Fruit Tree Experimental Research Station

【Objective】
The genus Citrus belongs to the subfamily Aurantioideae of Rutaceae family which included orange, lemon, mandarin, grapefruit and lime, and has high nutritional value. Polymethoxyflavones (PMFs), a class of flavonoids, occur exclusively in citrus. The most common PMFs, nobiletin, tangeretin, sinensetin and heptamethoxyflavone, are derived from citrus (Fig. 1). Among the PMFs, Nobiletin attracts the greater attention for its potential role of health benefited functions. Our current research was carried out with a view to study the distribution and analyze the mode of segregation of PMFs in leaves of Hassaku × Ponkan and different PMFs abundant cultivar of citrus.

【Materials and Methods】
Leaves were collected from the plants preserved in the germplasm collection at Saga University and quickly frozen to liquid nitrogen. To analyze the accumulation and distribution of PMFs, freeze dried 50 mg of leaves powder were extracted with 500 μl of 80% MeOH solvent at thrice and finally adjust the extracted sample to 2.5ml. Using a syringe (TERUMO, Terumo syringe 10 ml, SS-10 LZP) and a 0.45 um filter (RephiQuik Syringe Filter, Rephile Bioscience), the extract was filtered through a vial for HPLC and measurement was performed by HPLC (JASCO) with a GL Science Inertsil ODS-3 (4.6 mm × 33 mm, 3 μm), detected UV wavelength was 254 nm.

【Results and Discussion】
Among the 10 citrus cultivars, Mukakukishu, Benibae, Yoshida Ponkan and Ogimi Kugani accumulated higher amount of Nobiletin. Moreover, Binkitsu, Shiranui and Seinan no hikari were contained medium amount of Nobiletin. Though, Nobiletin was contained at low level, in the case of Kiyomi, Aoshima and Amanatsu cultivar (Fig. 2). The content of nobiletin per 1 g dry weight of Hassaku and Yoshida Ponkan, which are parents of the seedling analyzed in this study, was 0.029 mg/gDW and 3.02 mg/gDW, respectively (Fig. 3). Furthermore, wild plants closely related to the genus Citrus were also analyzed in this study to compare with those in the genus Citrus.

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Fig. 1 Polymethoxyflavones (PMFs) are found in citrus spp.

Fig. 2 Accumulation of Nobiletin in leaves of different citrus cultivar

Fig. 3 Histogram of Nobiletin of Hassaku × Yoshida Ponkan