

Identification of Gaeryangmeoru (*Vitis* sp.)'s Origin Using Isozyme Analysis

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Abstract. This study was conducted to infer the origin of Gaeryangmeoru (*Vitis* sp.) which has been known as the progeny obtained by crossing *V. flexuosa* and 'Concord'. As the result of isozyme analysis, the *Gpi-2* genotype of *V. flexuosa* known as the female parent of Gaeryangmeoru, was "gh", and that of 'Concord' known as the male parent of Gaeryangmeoru, was "ij"; however, the *Gpi-2* genotype of Gaeryangmeoru was "bd". The *Pgm-2* genotype of *V. flexuosa* was "cc", and that of 'Concord' was "hh"; however, the genotype of Gaeryangmeoru was "bf". In our study, genotype of Gaeryangmeoru did not include genotypes of *V. flexuosa* and 'Concord' and had the genotypes of European and American Hybrid Group. Therefore, we could infer that the origin of Gaeryangmeoru might be a seedling by a crossing between the species of the European and American Hybrid Group, an imported species bred by a crossing between the European Group and the European and the American Group, or a hybrid between them.

Additional key words: cultivar identification, *Gpi-2*, *Pgm-2*, wild grape

Introduction

Among the Vitaceae plants known, 11 genera and about 700 species, there are four groups of major species were used as economic crops (Westwood, 1978); Europe group (*Vitis vinifera* L.) of Caucasus and the Caspian Sea coasts, North American group (*V. labrusca* L.; *V. champini*; *V. rotundifolia* Michx), European and American Hybrid group (*V. labruscana* B.), and the East Asian group (*V. amurensis* Rupr.; *V. coignetiae* Dulliat; *V. flexuosa* Thunb.; *V. ficifolia* Bunge van. *Sinuate* Hara). In particular, *Vitis amurensis* that belongs to the East Asian group is used as the material for rootstocks for disease resistance and a breeding source of functional grapes, since it has been known to have excellent genetic characteristics regarding freezing and disease resistance, as well as contain highly functional anti-oxidation materials (Brown, 1975; Hwang and Ahn, 1975; Youn et al., 2003).

It has been reported that *V. amurensis*, *V. coignetiae*, *V. flexuosa* and *V. ficifolia* are distributed in Korea (Lee, 1996; Park et al., 2005a). Gaeryangmeoru (*Vitis* sp.) is the most widely cultivated economical crop. Gaeryangmeoru has been cultivated since 1980's, and now is widely used as a material for production of wine and breeding of functional grape species in Korea. It is generally known that Gaeryangmeoru is a species made by the cross between *V. flexuosa* and 'Concord'. However, its origin is uncertain and species name is not yet defined, causing errors such as same names for different species and different names

for the same species (Kim and Kim, 1997; Lee et al., 2002; Lee et al., 2003). For breeding of new grape cultivars using Gaeryangmeoru, its origin needs to be studied and elucidated.

In *Vitis* spp. it has been reported that glucosylphosphate isomerase (GPI) and phosphoglucosylmutase (PGM) are useful sources for different genetic diversity and species using alleles (Gottlieb, 1981; Weeden and Wendel, 1989; Chung and Ko, 1995; Kim and Ko, 1995). Ohmi et al. (1993) reported that GPI and PGM enzymes can be used to differentiate grape species by East Asian group, European group, and North American group depending on the movement between continents. In particular, it has been reported that the polymorphism of *Gpi-2* and *Pgm-2* could be used to confirm the hybrids and polyploids of grapes (Park et al., 2002), and those two isozymes could be used for validation of parents of grape hybrids and the genetic analysis between different species of *Vitis* spp. (Shiraishi et al., 1994).

Therefore, in this study we inferred the origin of Gaeryangmeoru by comparing the band patterns of the isozymes, *Gpi-2* and *Pgm-2*, between *V. flexuosa* known as the female parent and 'Concord' known as the male parent. The results of this study can be useful in grape breeding.

Materials and Methods

Plant material

We used 10 year-old Gaeryangmeoru, *V. flexuosa* Thunb., 'Concord' (*V. labrusca* L.), and *V. amurensis* Rupr. which were planted in a space of 4 x 2 m in an orchard of the

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Gangwondo Agricultural Research and Extension Services for this experiment. In addition, the native wild grapes used in this experiment were the line of native wild grapes classified by the leaf shape among the native wild grapes collected from across the nation since 1999.

Extraction of enzyme

Young leaves of grapevines were gathered from the end of mature shoots of each line in the morning (8 to 9 am). Young leaves (0.2 g) were collected and ground in a mortar with a pestle, in accordance with the enzyme extraction method by Wendel and Parks (1982). The extraction solution and 5% PVP-40 were mixed and samples were finely ground. The samples were centrifuged for 15 minutes at 4°C with 15,000 rpm. Supernatant was taken and used for the experiment.

Electrophoresis and enzyme analysis

Electrophoresis for enzyme analysis was followed according to a method described by Wendel and Parks (1982), with slight modification. For culture medium, electro starch of the same volume was used instead of hydrolyzed starch (Nacalai Tesque, Inc. Kyoto, Japan). For electrophoresis, horizontal starch gel and a tris/citrate buffer were used. The extracted sample was absorbed into a filter paper cut in the size of 0.3 cm × 0.5 cm, which was inserted into a horizontal gel prepared in advance. Then it was electrophoresed at 4°C and 10 W for about 8 hours. After electrophoresis, the gel was cut in 1 mm thickness at the anode, and dyed using the two isozymes GPI and PGM in accordance with the method of Wendel and Parks (1982).

Allozyme naming rule

The genetic analysis lines that move to the anode were

marked by 1, 2, 3, 4, and so on in accordance with the International Society of Biochemistry (Webb, 1984), and the anode marked at each position or fast-moving allozymes were marked as alphabets a, b, and so on.

Results and Discussion

An analysis of the isozyme *Gpi-2* of *V. flexuosa*, *V. amurensis*, and Gaeryangmeoru indicates that the parts where isozyme reaction occurred ranged from 20 to 50 mm of moving distance. Seven genotypes, including b, d, f, g, h, i, and j, were presented. The genotype of *V. flexuosa* known as the female parent of Gaeryangmeoru was found to be “gh” and that of “Concord” known as the male parent was found to be “ij”. The genotype of Gaeryangmeoru known to be bred by them as its parents was “bd”, whereas the genotypes of *V. amurensis*, GW-45 and GW-202, were “fg” and “gg” respectively (Fig. 1). Shiraishi et al. (1994) reported that the genotypes of the East Asian Group were c, d, f, and g. The genotype of ‘Concord’ in the North American group was “ij”, and the genotypes of the European and American Hybrid Group (*V. labruscana* B.) were a, b, c, d, e, i, j, k, and m. Therefore, results of isozyme analysis of the East Asian Group and the North American

Table 1. Grape cultivar and wild grapes used in this study.

Plant name	Plant group
GW-48 GW-178	Asia species (<i>Vitis flexuosa</i> Thunb.)
GW-45 GW-202	Asia species (<i>Vitis amurensis</i> Rupr.)
Concord	American species (<i>Vitis labrusca</i> L.)
Gaeryangmeoru	Interspecific hybrid (<i>Vitis</i> sp.)

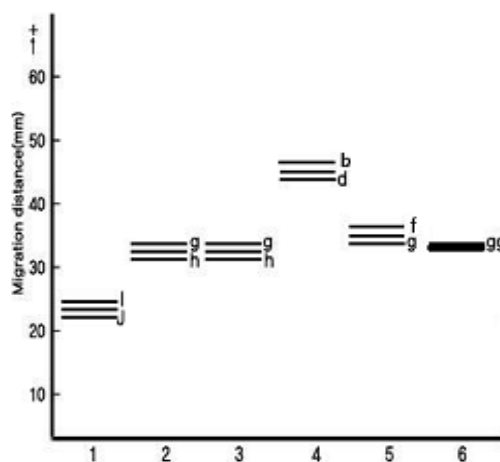
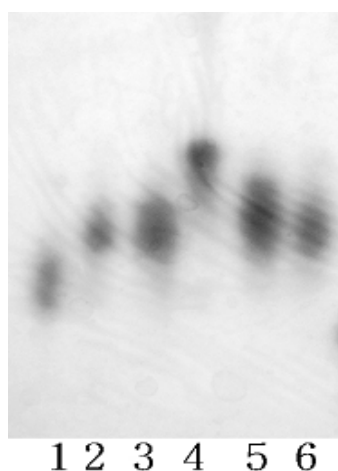


Fig. 1. *Gpi-2* zymograms of lines used in this study (left) and position of homodimeric allozymes of *Gpi-2* (right). Anode is toward the top of the figure. 1, ‘Concord’; 2, GW-48; 3, GW-178; 4, Gaeryangmeoru; 5, GW-45; 6, GW-202

Group were identical, when our results were compared with the report by Shiraishi et al. (1994). Furthermore, in the basis of results described by Park et al. (2002), we can infer that the genotype of the seedling bred through the crossing between “gh”, the genotype of *V. flexuosa* estimated to be the female parent of Gaeryangmeoru, and “ij”, the genotype of ‘Concord’, will be “gi”, “gj”, “hi”, and “hj” (Loukas et al., 1983; Weeden et al., 1988; Messina et al., 1991; Ohmi et al 1990; Ohmi et al., 1993; Park et al., 2002). However, the genotype type of Gaeryangmeoru was “bd” which was not found in East Asian species. Therefore, it is suggested that “bd” of the genotype of Gaeryangmeoru originated from the European species and the North American Hybrid Group, on the basis of a report described by Shiraishi et al. (1994).

The results of *Pgm-2* analysis exhibited that the isozyme reaction was presented from 20 to 50 mm on a gel, with the five genotypes (b, c, f, h, and g). The genotype of *V. flexuosa* was characterized as “cc” and that of “Concord” was characterized as “hh”. The genotype of Gaeryangmeoru was “bf”, whereas the genotype of *V. amurensis*, GW-45 and GW-202, was “cc” (Fig. 2). Shiraishi et al. (1994) reported that the genotypes of the East Asian Group were characterized as a, c, e, and h; the North American Group as g, h, k, and j; and the European and American Hybrid Group as b, e, f, g, h, i, and j. Therefore, when the results of this experiment were compared with the report by Shiraishi et al. (1994), the results are similar for the East Asian Group and the North American Group. Furthermore, in the basis of the experimental results by Park et al. (2002), we can infer that the genotype of the seedling bred through

the crossing between “cc”, the genotype of *V. flexuosa* estimated to be the female parent of Gaeryangmeoru, and “hh”, the genotype of ‘Concord’, will be “ch” (Loukas et al., 1983; Weeden et al., 1988; Messina et al., 1991; Ohmi et al 1990; Ohmi et al., 1993; Park et al., 2002). However, the genotype of Gaeryangmeoru was “bf” which was not found in the East Asian Group and the North America Group like *Gpi-2*. According to the report by Shiraishi et al. (1994), the genotype of Gaeryangmeoru belongs to the European Group and the European and American Hybrid Group, and the genotype “bf” originates from the European and American Hybrid Group. In summary of the experimental results for *Gpi-2* and *Pgm-2*, the genotype of the Gaeryangmeoru did not include the genotype of the *V. flexuosa* nor the genotype of ‘Concord.’ This result is identical to the report that the Gaeryangmeoru had no phylogenetic relationships with *V. amurensis*, *V. flexuosa*, *V. coignetiae* and *V. ficifolia* which are the wild grapes of the East Asian Group, but had phylogenetic relationships with the European species of grapes, in the basis of the classification of phylogenetic relationships among East Asian Group, European and American species, European Group, and the European and American Hybrid Group of Gaeryangmeoru using RAPD analysis (Kim et al., 2006; Park et al., 2005b).

Therefore, if we infer the origin of Gaeryangmeoru using the isozyme analysis with the results of this experiment, it is thought to be a seedling by crossing between the species of the European and American Hybrid Group, or an imported species bred from a crossing between the European Group and the European and the American Group, or a hybrid between them.

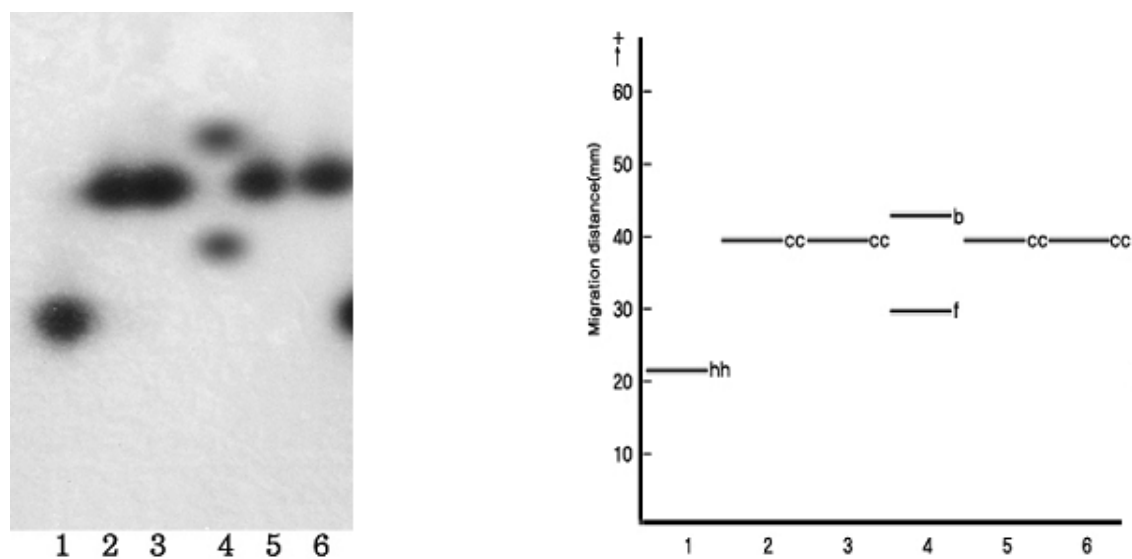


Fig. 2. *Pgm-2* zymograms of lines used in this study (left) and position of homodimeric allozymes of *Pgm-2* (right). Anode is toward the top of the figure. 1, ‘Concord’; 2, GW-48; 3, GW-178; 4, Gaeryangmeoru; 5, GW-45; 6, GW-202

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