International Journal of Plant Breeding ©2007 Global Science Books

Grape and Grapevine Rootstock Breeding Program in Korea

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ABSTRACT

Several Korean wild *Vitis* species have been collected mainly from mountain areas all over the country, maintained in the vineyard, and used for improving grape cultivars at National Horticultural Research Institute (NHRI), Rural Development Administration (RDA). During the early 1960's, NHRI had developed and released eight table grape cultivars, namely: 'Cheongsoo', 'Hongdan', 'Hongisul', 'Tamnara', 'Jinok', 'Heukgoosul', 'Heukboseok', and 'Suok'. An additional 4 grape cultivars, namely: 'Jarang', 'Sujeong', 'Heukjinjoo', and 'Topas' were developed by the Provincial Grape Experimental Stations and the Gangwon National University. Evaluation and screening procedures for major disease resistance had also been developed for grape seedlings and germplasms. The genetic analysis of seedlessness in berries and application of molecular biology techniques for disease resistance screening provided a viable tool in ensuring a highly efficient grape breeding program. The demand for grafted scions on resistant rootstocks increased with the growing popularity of growing grapes under vinyl houses as well as minimizing vine damage from crown gall and phylloxera infection. Yet, there had been no serious breeding program for grapevine rootstocks being implemented. It was only until the late 1990s that interests and efforts were undertaken by NHRI to develop rootstocks from the native wild grape germplasm suitable to Korean growing conditions.

Keywords: biotechnology, disease resistance, genetics, grape breeding, grape rootstock, wild grape germplasms

CONTENTS

INTRODUCTION

The grape industry in Korea shows a positive trend in terms of increased area and production volume. These increasing trends can be attributed to the ease of its cultivation, higher market price compared to other fruit crops and increasing consumption, it being considered as a healthy food.

Unlike the major producing countries, Korean grapes are produced mainly for table use and only a small portion is used for processing though it had been grown for more than 1,500 years in the country. Different grape cultivars are grown nationwide depending on the prevailing climate. The European cultivars can grow well in the northern areas under over-wintering system like 'hilling' under the ground. However, most American cultivars and their hybrids with European cultivars are more adapted to Korean climatic conditions and can grow without treatment for over-wintering. For instance, the cold-hard cultivar, 'Campbell Early' occupies 74.3% of the total production area while 'Kyoho' occupies 13.1%, and 'Muscat Bailey A', 5.9% or an aggregate 93.3% of the total area cultivated for grape production (MAF 2003).

With the growing demand for the fruit, availability of good quality varieties is necessary to compete with the imported grapes. The cultivation of tetraploid grape cultivars in vinyl greenhouses such as 'Pione', 'Fujiminori', and 'Honey Black' which are noted for their large-sized berries and excellent taste becomes popular as well as the European grapes, namely 'Rizamat', 'Italia', and 'Rosario Bianco' for their high fruit quality. Despite these developments, it was deemed important to further develop new grape cultivars that are well adapted to the Korean climate and growing conditions with emphasis on consumers' quality preferences.

This study deals on developing new grape cultivars with resistance to major diseases as well as the updates on the germplasm collection of wild grapevines, and rootstock breeding program in Korea.

WILD GRAPE GERMPLASM CONSERVATION AND UTILIZATION

Among the 30 Asiatic grape species in eastern Asia, the *Vitis amurensis* Rupr., *V. flexuosa* Thumb, *V. coignetia* Pulliat, and *V. thunbergii* are known to be native to Korea. Although commonly known *V. amurensis* Rupr. has not been cultivated, the edible fruits from this species are collected and used as fresh fruit, juice, and wine in mountain areas. Korean wild grapes were collected mainly from mountain areas all over the country and maintained as grapevine germplasm for breeding particularly those possessing unique charac-

Received: 28 February, 2007. Accepted: 13 April, 2007.

Invited Mini-Review

teristics for wine or juice processing at the National Horticultural Research Institute (NHRI), Rural Development Administration (RDA). This species has been of interest to many consumers as a well-being product because of its physiologically active compounds good for human health. To date, forty six types of wild grapes were evaluated using morphological characteristics of the vines and fruits. The random amplified polymorphic DNA (RAPD) analyses were also employed to determine the relationships among wild grapes cultivars. The RAPD data were obtained using primers URP02, URP07, URP08, and URP12 for cluster analysis using NTSYS. A dendrogram of 46 wild grape samples showed a clear separation from the cultivated grapes 'Campbell Early' and 'Kyoho' (Yun *et al*. 2006b). Park *et al*. (2005b) also evaluated wild grapes collected from Gangwon Province region by RAPD, and selected 'Cheongsan' variety (2005c) to be good for wine making in the Gangwon Provincial Agricultural Technology Institute, Korea.

GRAPE BREEDING PROGRAMS

Grape breeding was started as early as 1960 by the NHRI, RDA mainly to develop and improve table grape cultivars. In 1990 breeding was done in the Okcheon Grape Experiment Station (OGES), Chungbuk Provincial Agricultural Technology Institute. The Gangwon National University joined the program in 2000.

At the early stage of breeding, foreign cultivars from Japan, Italy, and United States were tested for regional suitability in several provinces of Korea. Conventional breeding through cross pollination was done and starting 1990, new table grape cultivars were developed and released by the NHRI. In 1993, the first Korean-bred cultivar was developed, named 'Cheongsoo', a white, seedless (stenospermocarpic), and an early fruiting table grape which is high yielding with good quality fruits (Lee *et al*. 1994).

Other notable varieties were then developed. 'Hongdan' bred in 1994 and 'Hongisul' in 2000 are red attractive grapes having good fruit quality with high soluble solid, low acidity, attractive skin color, abundant bloom, and no skin cracking (Suh *et al*. 1995; Park *et al*. 2006a).

The NHRI breeding program for disease resistance and cold hardness in 1981 produced the 'Tamnara', hybrid which is a selection in 1998 from the cross 'Campbell Early' × 'Himrod Seedless'. It has a mean TSS of 17.2%, which is 3% higher than the parent 'Campbell Early', and low acidity of 0.39%. This excellent balance between sugar and acidity gives it a good taste (Park *et al*. 2004b).

Another cross from 'Golden Muscat' (2X, *Vitis* sp.) × 'Pione' (4X, *Vitis* sp.) made at NHRI in 1988 produced 'Heukgoosul' (4X) (**Fig. 1**) a selection in 2000 by which has low incidence of berry shattering or good berry set as compared to 'Kyoho'. The mean berry weight is 14.4 g, about 3 g heavier than 'Kyoho', with a mean soluble solid of 18.4°Bx (Park *et al*. 2004a).

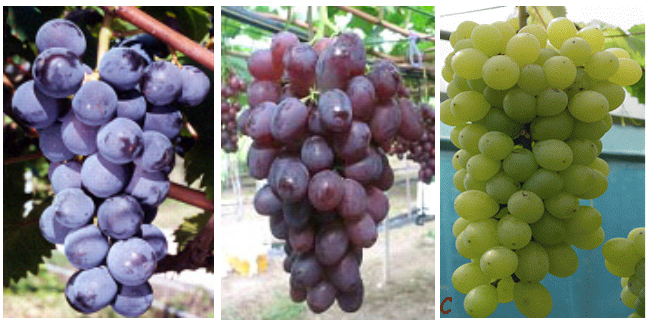

Fig. 1 Fruit appearance of locally-bred grape cultivars. 'Heukgoosul' (left), 'Jarang' (center), and 'Sujeong' (right).

'Heukboseok' (4X), produced from the 1992 NHRI cross 'Beniizu' (4X, *Vitis* sp.) × 'Kyoho' (4X, *Vitis* sp.) and finally selected in 2003 for its large berries with excel-lent fruit skin color and high quality. The mean berry weight is 10.6 g and the mean soluble solidis 18.4°Bx (Park *et al*. 2006b).

The cross 'Kyoho' (4X, *Vitis* sp.) × 'Beniizu' (4X, *Vitis* sp.) started by NHRI in 1992, the 'Suok' (4X) hybrid was finally selected in 2004 for its large berries with excel-lent skin color high quality, and late ripening. It shows a low incidence of berry shattering, resulting in a good berry set compared to 'Kyoho'. The mean berry weight is 10.9 g, and the mean soluble solid is 18.5°Bx (Yun *et al*. 2005).

Selected in 2004, 'Jinok' was produced from the cross 'Delaware' × 'Campbell Early' for disease resistance and cold hardness. The mean berry weight is 6.0 g, 1 g heavier than that of 'Campbell Early'. It has a mean TSS of 15.8%, which is 2% higher than that of 'Campbell Early', and low acidity of 0.49%. The vines are cold tolerant and resistant to downy mildew and anthracnose (Park *et al*. 2006c).

In 2005, eight new table grape cultivars have been developed and released for grape growers. 'Jarang' (selected in 2005; **Fig. 1**) is one of the chance seedlings kept in the vineyard of Okcheon Grape Experimental Station, Chungbuk Provincial Agricultural Technology Institute. The fruit has a black skin and ripens in late September with a mean weight of 8.6 g, mean TSS of 16.4%, and low acidity (0.50%).

Three new grape varieties, 'Sujeong', 'Heukjinjoo', and 'Topaz', were developed from the cross between tetraploidy and diploidy in Gangwon National University (GNU), Korea (2005). 'Sujeong' (**Fig. 1**)produced from the cross 'Kyoho' × 'Thompson seedless' shows dense fruit setting with moderate but vigorous vines at early development stage. The green-skinned berry weighs 8 g with a mean TSS of 19.6% in the cluster of 500~600 g. Although slight cracking of berries was observed, healthy clusters with large seedless berries could be treated by gibberellic acid (GA_3) once a year.

'Heukjinjoo' resulting from the cross 'Kyoho' x

Table 1 Fruit characteristics of grape cultivars developed in Korea.

Variety	Cluster weight (g)	Berry weight (g)	Berry skin color	Soluble solids (°Bx)	Developed in
Cheongsoo	250	4.2	white	16.0	NHRI
Hongdan	315	5.2	red	17.2	NHRI
Tamnara	323	7.5	black	17.2	NHRI
Hongisul	325	5.9	red	16.3	NHRI
Jinok	320	6.0	black	15.8	NHRI
Jarang	450	8.6	black	16.4	OGES
Sujeong	743	6.3	white	19.6	GNU
Heukjinjoo	446	3.9	black	21.1	GNU
Topas	577	8.4	Red	20.8	GNU
Campbell Early	324	5.5	black	15.0	USA
Heukgoosul	450	14.4	black	18.4	NHRI
Heukboseok	452	10.6	black	18.4	NHRI
Suok	445	10.9	black	18.5	NHRI
Kyoho	450	11.6	black	17.1	Japan

'Thompson seedless' gives high yield with moderately vigorous vines. The clusters weighing more or less than 500 g with about 80 berries are preferred to prevent cracking incidence of berries. The berries have crispy flesh with 19.3% TSS, physiologically active compounds for human health, and long shelf life.

The cross 'Kyoho' × 'Sekirei' produced 'Topaz' which is highly productive and has dense berry setting with low shattering giving an excellent cluster appearance. A cluster of red scarlet berries weighs 500~600g. The berries have crispy flesh with a mean TSS of 21% giving it an excellent taste with low acidity. The thick skins prolong the berries' shelf life long (**Table 1**).

Grapevine cultivars bred by the NHRI, GNU, and OGES are recommended as replacement for 'Campbell Early' and 'Kyoho', (two leading table grapes in Korea). DNA markers and kits for identification of these cultivars have been developed to avoid confusion as well promote the growing of these grape cultivars by the farmers (Lee *et al*. 2006). At present, although the wine industry is not yet well developed as it is carried out by only few growers or cooperatives on small scale, there is a good prospect for the development of grape cultivars suitable for wine making and adaptable to Korean environment.

GRAPE GENETICS AND BIOTECHNOLOGY

Efforts have been done to establish the genetics of grapes and come up with an efficient biotechnology procedure for its breeding program. Seeded grape cultivars 'Campbell Early', 'Golden Muscat', and 'Tano Red' were separately crossed with the stenospermocarpic seedless grape cultivar 'Himrod' and the F_1 progenies gave 3:1, 15:1, and 3:1 ratios of seeded to seedless plants, respectively. Among the cross combinations, the 'Campbell Early' × 'Himrod' produced the highest proportion of seedlessness at 33%. This result suggests that the seedlessness in grapes is controlled by complex genes, although it has been known to be a recessive character governed by dominant genes (Park *et al*. 2003).

Stenospermocarpic seedless grape cultivars have been used for introducing seedlessness in the breeding programs. Low proportion and high variation of seedless genotypes in the progenies require the development of new techniques to breed seedless cultivars using stenospermocarpic grapes. Park *et al*. (2003) tried to develop novel ovule culture techniques using stenospermocarpic seedless cultivars to improve the breeding efficiency of seedless grape cultivars. Park *et al*. (2005) reported the optimal harvesting and excising time of ovules from berries, and germination media composition under the light to simulate 16-h photoperiod at 24 ± 1°C for *in vitro cultures* of stenospermocarpic grape cultivars 'Cheongsoo', 'Himrod', and 'Benizawa' to obtain whole plantlets. Among them, 'Himrod' was the most responsive to ovule culture. In GNU, Park *et al*. (2001) reported an efficient method for the endosperm and embryo rescue of aneuploid seeds obtained from 3X × 2X and 3X × 4X crosses in grapes. They reported further that the development of seed embryo and endosperm from 3X × 2X and 3X × 4X crosses was inferior than the seeds from 2X × 2X and 4X × 4X crosses. Embryos were excised aseptically and cultured onto germination protocol consisting of MS medium and 1 M benzyl adenine.

Physical emasculation of grapes has been considered to be very difficult requiring time and hard labor. Yun *et al*. (2006c) tested several chemical treatments that can be used for an efficient emasculation of stamens the leading grape cultivar, 'Campbell Early'. Among the seven chemicals tested, the most promising is the benzoic acid (500 ppm) or gibberrelic acid (100 ppm) since when sprayed at full bloom the vine produced 100% impotent pollen, prevented ovule development and seed germination after hand pollination. Male and female sterility in diploid plants was examined by Park *et al*. (2001). Self-pollinated berries from two diploid plants, BRi9211 and BRi9214, obtained from the cross 'Muscat Bailey A' × 'Rizamat 4X', were reported to contain no seeds but GA_3 treatment promoted the growth of berries by the increased cluster weight and berry set.

The cloning and identification of genes for disease resistance provide insights into disease resistance mechanisms in plants and also serve as novel sources for developing disease resistant varieties. However, current methods employed to identify genes that are expressed during plant disease resistance responses are labor intensive and encounter high incidence of false positives. To avoid these problems, the project employed a new and accurate method of reverse transcription-polymerase chain reaction (RT-PCR). This method involves annealing of control primers (ACPs) to identify the genes expressed specifically in grapevine leaves infected with *Agrobacterium vitis*. Twenty ACPs were used to identify and sequence 12 differentially expressed genes (DEGs). Basic Local Alignment Search Tool (BLAST) searches identified 14 genes. Of the known genes, six were selected and further characterized using RT-PCR analysis to assess their specific expression in the *A. vitis*-infected plant. Results of the analysis suggest that the ACP system is a very useful tool for identifying specific disease-resistance genes in grapes. Further analysis of the cloned genes will provide insights into the molecular basis of disease resistance expression (Yun *et al*. 2006a).

EVALUATING RESISTANCE AGAINST MAJOR DISEASES

Progress had been attained in developing a system for screening disease resistance against downy mildew, anthracnose, crown gall, gray mold, and ripe rot of grape cultivars and seedling lines.

In screening resistance to downy mildew (*Plasmopara viticola*), the plants inoculated with 5×10^4 spores/ml were incubated in a moist chamber for 48 to 72 h at 24-28°C and observed for symptom development. These plants were then evaluated under greenhouse condition resembling those of field conditions (Yun *et al*. 2001). Of the tested grape cultivars, *Vitis vinifera* was reported to be more susceptible to downy mildew than *V. vinifera-labrusca* and *V. vinifera-labrusca-aestivalis* hybrids. In *V. vinifera-labrusca* hybrids, tetraploid cultivars were more susceptible than diploid culivars.

Anthracnose (*Elsinoe ampelina*) incidence in grapevines is severe during warm and humid conditions. Yun *et al*. (2003a) reported a rapid anthracnose screening system for resistant cultivars and progenies even at early plant development stage. Spray inoculation by 10^5 spores/ml suspension then incubation in a moisture chamber for 48 to 72 h were optimum for symptom development. Scoring for lesion on the third or fourth leaf was efficient enough to distinguish the anthracnose-resistant cultivars from susceptible ones. The results of bioassay using culture filtrates of the pathogen, *E. ampelina*, were consistent with that of pathogen inoculation and screening in the vineyard (**Fig. 2**) suggesting that the culture filtrates of the pathogen could be used as an alternative to pathogen inoculation or to field tests in evaluating grapes' resistance to anthracnose (Yun *et al*. 2007). The patterns of salicylic acid and resveratrol content in the leaves after pathogen inoculation or culture filtrate treatment of *E. ampelina*. were evaluated using high

Fig. 1 Necrosis symptoms (left) and tumor formation (right) developed after inoculation of *E. ampelina* culture filtrates and *A. vitis* 10^9 cfu/ml, respectively. In this order, from left to right: resistant, moderately resistant, and sensitive.

performance liquid chromatography. Salicylic acid and resveratrol content of resistant cultivar 'Campbell Early' increased rapidly one hour after inoculation but no change was noted in downy mildew and anthracnose-susceptible cultivars, 'Rizamat' and 'Kyoho'. Similar pattern of salicylic acid and resveratrol contents was observed in cell free culture filtrate treated with anthracnose pathogen (Rho *et al*. 2005).

In screening resistance to crown gall, the biggest tumors were found in plants inoculated by *Agrobacterium vitis*, the pathogenic bacteria adjusted to 10^9 cfu/ml at 26°C with 3.1 mm^3-sized holes (Yun *et al*. 2003b). Among the 27 grape rootstocks inoculated with *A*. *vitis* strain, the 'Gloire', '140R', '101-14M', '3309C', and '333EM' were observed to be resistant (Rho *et al*. 2003). Rho *et al*. (2005) reported that resistance reaction against crown gall was closely related to the development of secondary phloem in grapevine stems leading to tumor formation and can be seen in a confocal laser scanning microscope (CLSM) 2 months after greenhouse inoculation with *A*. *vitis*. Crown gall resistance screening was done for 49 *Vitis* genotypes consisting of 29 European, 6 American, and 14 *Vitis* hybrid cultivars. While most European cultivars were found susceptible, many American cultivars such as 'Campbell Early', 'Concord', 'Concord Seedless', 'Delaware', and 'Sheridan' were resistant (Rho *et al*. 2006).

Severe incidence of ripe rot and gray mold also occurred in the grapevines in warm and humid climate regions like Korea. An attempt to develop an efficient screening system for resistance against ripe rot and gray mold was done by pathogen inoculation of grapevines. Lesion development in grapevines was significantly affected by temperature and pathogen inoculation method of. Lesion scoring was effective on the detached 5~7 leaves inoculated with mycelial agar blocks of *Colletotrichum gloeosporioides* and *C*. *acutatum* at 30°C for ripe rot resistance screening. On the other hand, inoculation of the detached 5~7 leaves with spore suspension of *Botrytis cinerea* at 24°C was effective in screening resistance against gray mold under laboratory condition (Yun *et al*. 2006d).

GRAPEVINE ROOTSTOCKS IN KOREA

The *V*. *vinifera* is the most popular grape species being widely planted particularly on a commercial scale plantings all over the country. Although its vine can be easily rooted for planting, grafting is opted because the vines are very susceptible to phylloxera, a soil-borne insect. In Korea, phylloxera occurs in the vineyard once in 20-30 years, and induces damages to the grapevines. Despite the moderate resistance of the leading cultivars, 'Campbell Early' to insects, grafting on phylloxera-resistant rootstock is still employed in grape production.

Damages of vines from crown gall infection also prompt the use of grafted scions on resistant rootstocks. To date, grape cultivation in vinyl houses increases so that vigorous grapevines are needed for continuous production of high quality fruits every year. The '5BB' and 'SO4' varieties were considered good sources of rootstocks because of its ability for deep soil penetration, a positive hydrotropism, and therefore ensure an efficient uptake of water and nutrients from the soil. Until 2000, there had been no breeding program for grapevine rootstocks, but it was only recently that NHRI showed interests and efforts to breed for suitable rootstocks using the native wild grape germplasms.

OTHER ADVANCES AND DEVELOPMENTS IN BIOTECHNOLOGY

Because it is very easy to propagate vines by cutting, a few results from *in vitro* propagation system studies have been reported in production of grapevine nursery stocks. Kim and Paek (1981) reported that varietal behaviors among 8 major grapevine cultivars in callus formation were very different, ranging from 46.6% in 'Delaware' to only 3.2% in 'Early Niabell', and that as maximum callus formation occurred in cultures grown on Murashige and Skoog's (MS) medium with 2 mg/l kinetin + 0.5 mg/l IAA + 0.5 mg/l NAA, NAA seems to be more effective than IAA or IBA when the concentration of kinetin is fixed. Hwang and Kim (1990) investigated the effects of plant growth regulators on *in vitro* growth of differentially chilled grape shoots in 'Muscat Bailey A' (MBA) and 'Riesling' grapes, and reported that bud break of both cultivars increased with the additional chilling, showing the peak percent bud break with 10 weeks' chilling at 4°C, but percentage of buds grown over 5 cm was highest with 8 weeks' chilling, and that increase in shoot growth of 'Riesling' by prolonged chilling was recognized only when explants were grown on basal and 1 M BA-supplemented MS medium. Shoot tips derived from the cuttings of 'Campbell Early' grapes were cultured on MS media and their *in vitro* growth and nutrient uptake were investigated for selecting optimal medium composition (Cha *et al*. 1991).

Lee *et al*. (2000) collected 84 superior spontaneous mutants in the major grape cultivars growing in Korea and identified their mutations in ripening time and fruit size. However, in Korean grape breeding programs, there has been no report regarding the chemical or physical treatment to create mutants for developing new varieties.

Yu and Ko (1972) reported that gibberellin (1 ppm) among tested growth regulators were more effective than kinetin, thiourea, and IAA on rest breaking of grape buds of 'Campbell Early'. Lee (1983) suggested that growth regulators with other chemicals played in induction of parthenocarpy, fruit set, ripening and coloration of grape fruis. He reported that GA_3, streptomycin or 4-CPA induced formation of seedless berries in 'Delaware', 'Muscat Bailey A' and 'Kyoho' grape cultivars, that parthenocarpy by GA treatment was strikingly stabilized by combined application with BA, atlox BI or 4-CPA, and that daminozide and ethephon effectively increased berry-set in 'Kyoho', 'Pione' and 'Concord' grape cultivars. GA treatment, thinning and trimming of cluster, and pinching of shoots are applied to attain large and regular sized berry and cluster with excellent skin color in Korean vineyards. Grape growers can develop seedless berries from seeded cultivars by dipping clusters in GA solutions, because of consumer's preference for seedless grapes. GA treatment can also improve berry setting of tetraploid cultivars such as 'Kyoho' and 'Pione'.

Korean grape growers apply fertilizer separately. Basal fertilizers are applied in dormant season, from November to March. Fertilizers at this time are slowly released manures supplemented with nitrogen, phosphorous, potassium, and lime for the high soil pH. Other fertilizers applied, are nitrogen and potassium during the growing season, May or June for berry enlargement. After harvest, grape growers supply a small amount of fertilizers for recovery of vine vigor. The amount of fertilizer depends on the soil type, the age of vine, and cultivars. Tetraploid and *vinifera* cultivars require less nitrogen to suppress excess of growth than *labrusca* or *labruscana* because they grow very vigorously even in a soil with less nitrogen.

Directed genetic modification of existing grape cultivars by the introduction of single genes would result in improved genotypes. Genetic transformation system offers significant opportunities for improvement of grape while allowing the continued use of major growing cultivars of economic significance in Korea. However, from several years ago, lots of efforts to develop a transformation system of grapevines has concentrated on major cultivars, 'Campbell Early' and 'Kyoho' in Korea University, NHRI, and Gyeongpook National University. Significant progress has not been made in developing the necessary molecular techniques such as direct regeneration system by *Agrobacterium* infection onto leaf or petiole tissue of grapevines with green florescent protein (GFP) gene as a reporter gene, and further research is still required.

CONCLUSION

Within the span of 46 years of grape breeding in NHRI, GNU, and OGES, more than ten varieties have been developed. Among the significant achievements is the attainment of the most important goal in grape breeding, that is the development of novel seedless grape and large-sized berry cultivars which are cold tolerant and resistant to major diseases such as downy mildew, anthracnose, crown gall, and ripe rot. Although high quality wine making is difficult due to the weather conditions of the country, grape cultivar for wine making is also envisioned to provide solution to over supply of grape berries. The level of knowledge and skills in grape processing may still be limited, but continuous training of specialists in other countries having such advanced skills is being planned. Financial support is also needed for research and development projects dealing on high quality grape production and processing. Grape production is generally considered to be very difficult because it requires special vineyard production practices and takes a long time for the vine to adapt to the prevailing climatic conditions in the growing area it is still deemed necessary to develop different varieties to ensure high profitability from grape production. Therefore, it is envisioned that the grape breeding program can provide the growers with noble and locally-bred varieties rather than only a few.

ACKNOWLEDGEMENTS

We thank the following for research support: Rural Development Administration, Agricultural R&D Promotion Center, and National Horticultural Research Institute, and the following colleagues for the study: Lee DK, Lee JS, Suh HS, Rho JH, Jeong SB (NHRI), Park SM (GNU), Park YS (GATI), and Kim YH (OGES).

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