# In Vitro Evaluation of Korean Wild Grapes for Disease Resistance

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## Abstract

*We tested disease resistance in the following Korean wild grapes as anthracnose, brown leaf spot, gray mold, and ripe rot. Wild grapes (Vitis flexuosa, V. amurensis, V. coignetiae) showed a lower infection rate than conventional cultivars ('Campbell Early' and 'Kyoho') in the leaf disease tests (anthracnose, brown leaf spot). In the gray mold test, wild grapes showed lower infection rate than conventional cultivars, but in the ripe rot test, V.coignetiae showed a high infection rate. Our results showed that the wild Korean grapes are more resistant to general disease in Korea, which is useful for finding a parent for disease resistance breeding.*

**Keywords**: Disease resistance, in-vitro test, wild grape

## *1. Introduction*

Korean wild grapes are grown in most of the mountain regions and show a broad range of disease resistance. Wild grape bunches have a high acidity, poor fruit set, small berry size and different ripening in the same bunch at harvest. Wild grapes are not used like table grapes but are just used as medicine or for making traditional wines. Korean wild grapes are reported to have five different kinds of species: 'Gaemeoru' (*Ampelopsis brevipedunculata*), 'Wangmeoru' (*V. amurensis* Rupr.), 'Meoru' (*V. coignetiae* Pulliant.), 'Saemeoru' (*V. flexuosa* Thunb.), and 'Gamagwemeoru' (*V. ficifolia*) (Lee 1980). *V. amurensis* was found in northeastern China and Korea but not found in Japan. *V. coignetiae* was found in Korea and Japan but not found in China (Wan et al. 2008; Nakagawa et al. 1991; Park et al. 2005). *V. flexuosa* are widely distributed species in East Asia. However, some of the classification studies reported that *V. coignetiae* is similar to *V. amurensis* in their morphology, RAPD band pattern (Goto-Yamamoto et al. 1998), and SSR marker (Hur et al. 2012). Now we can classify not only the morphology but also the genomic sequences of these species. Recently, several studies have reported that Korean wild grapes have many antioxidative, nitrate scavenging, and antibacterial activities (Lee et al. 2010; Choi et al. 2006; Won and Kim 2012). For this reason, we cross commercial cultivars and the Korean wild grape species to breed more valuable and adaptable cultivars. Some severe diseases occur in Korean vineyards annually, such as downy mildew (*Plasmopara viticola*), anthracnose (*Elsinoe ampalata*), brown leaf spot (*Pseudocercospora vitis*), ripe rot (*Colletotrichum acutatum*), and gray mold (*Botrytis cinerea*).

The Korean wild grapes have a nonhost resistance to general disease in Korea because they have adapted their traits to the regional climate for a long time. In this experiment, we tested several disease resistances of the Korean wild grape species to compare with other common grape cultivars for disease resistance grape breeding.

## *2. Materials and Methods*

### 2.1 Korean Wild Grapes

We used three different species of Korean wild grapes: 'Saemeoru' (*V.flexuosa*), 'Wangmeoru' (*V.amurensis*), and 'Meoru' (*V.coignetiae*) to compare with the disease infection rate, of the conventional grape cultivars 'Campbell Early' (*V.labrusca* hybrid) and 'Kyoho'(*V.vinifera* hybrid). Leaf and bunches were randomly collected in a germplasm repository (Gangwon-do Agricultural Research and Extension Service; Chuncheon in Korea) at harvest.

### 2.2 Leaf Test

We modified the leaf inoculation method from Hong et al. (2008), and Yun et al. (2001). Fresh leaves from Korean wild grape and cultivar leaves were washed with 70% ethanol for 30 seconds, 0.4% NaOCl for 1 minute, and washed twice with distilled water (D.W.). Sterilized leaves were cut into five to seven small pieces with scissors and then placed on a water soaked paper towel in a clear plastic box. An anthracnose (*Elsinoe ampelina*) spore suspension was prepared from the fresh mass of spores grown on a mycelial mat on potato dextrose agar media for one month. Fresh spores were transferred to 5 ml D.W. in a conical tube and then adjusted to concentration (1 x $10^6$/cfu) with D.W. The brown spot disease (*Pseudocercospora vitis*) spore was grown in the same way as the anthracnose spores for one month. Then, it was transferred to a conical tube and adjusted to concentration (1 x $10^6$/cfu) with D.W. For leaf inoculation, we made three to five wounds on the adaxial side of the leaf with a stubby pencil, and each leaf was inoculated with a pipette of 10 ul of each spore suspension. Total inoculation sites numbered 24 each species. After inoculation, we covered the plastic boxes and sealed them with plastic wrap and placed them in the incubator at 25℃ for one week. Infection rate was evaluated by browning color with spores at the inoculation sites. Inoculation was replicated twice.

### 2.3 Berry Test

Surface sterilization for each Korean wild grape and cultivar berry used the same method as the leaf tissue preparation. A total of 25 sterilized berries from each species with pedicles were placed on a water-soaked paper towel in a plastic box. Gray mold (*Botrytis cinerea* ) and ripe rot (*Colletotricum acutatum* ) disease were grown separate mycelial mat on the potato dextrose agar media for one week. Spores were scraped with 10 ml of D.W. They were transferred to a conical tube and adjusted to concentration (1 x $10^6$/cfu) with D.W. 10 ul of each spore suspension was used to inoculate each berry with a pipette on the sterilized berry surface. After inoculation, we covered the plastic boxes and sealed them with plastic wrap and placed them in the incubator at 25℃ for one week and The infection rate was evaluated by berry skin color turning brown with spores or rot in the inoculation site. Inoculation rate data was analyzed for the significance of differences by using Tukey's HSD method with the 'R' program (ver 3.2.0; The R Foundation for Statistical Computing). The experiment replicated twice.

### *3. Results and Discussion*

In vitro test results showed the infection rate of grape anthracnose as follows: 'Saemeoru' (*V.flexuosa*) 0%, 'Wangmeoru' (*V. amurensis*) 0%, 'Meoru' (*V. coignetiae*) 8.3%, 'Campbell Early' 0%, and 'Kyoho' 4.2%. (Table 1). Previous results have shown that European grapes (*V. vinifera*) are more susceptible to anthracnose than *V.labrusca* grapes (Yun et al. 2001), and *V.amurensis* and *V. flexousa* have been shown to be disease resistant species in the Chinese wild grapes (Wang et al. 1998). Our results are similar to several previous anthracnose tests, meaning that the Korean wild grape has a resistance to anthracnose disease on leaves. In vitro test results, grape showed infection rate of brown spot disease the species as follows: 'Saemeoru' 0%, 'Wangmeoru' 0%, 'Meoru' 8.3%, 'Gamaguimeoru' 0%, 'Campbell Early' 20.8%, and 'Kyoho' 0%. (Table 1). Generally, brown leaf spot disease severely infected the 'Campbell Early' cultivar (Jung et al. 2009), but other European grapes (*V. vinifear*) were rarely infected (Peason and Goheen. 1988). The Korean wild grapes had a resistance to brown leaf spot disease in the test. The Korean wild grapes showed that their resistance was not specific to each disease. In this result, 'Meoru' (*V. coignetiae*) had a tolerance to anthracnose and brown leaf spot disease. However, those leaf disease were not detected in infected lesions of 'Saemeoru' (*V.flexuosa*) and 'Wangmeoru' (*V. amurensis*), but these results need to be compared with field evaluation data.

In the berry results, we did not find significant differences in gray mold infection rate in the wild species (Table 2). However, ripe rot on 'Meoru' (*V. coignetiae*) showed a higher infection rate (33.3%) than other wild grape species. But 'Saemeoru' (4.2%) and 'Wangmeoru' (0%) showed a lower infection rate than conventional cultivars 'Campbell Early' (25.0%) and 'Kyoho' (20.8%). *B. cinerea* (gray mold), is a strong pathogen in the various plants, but the Korean wild grape tolerates. The ripe rot is more widely spread to other grapevines because they make a mass of conidiospores on the acervulus in the lesion and spread quickly with rain drops (Agrios 2005). We only used the pathogen *C. acutatum* in the ripe rot experiment, but former research reported that there are two different pathogens (*C. acutatum* and *C. gloeosporioides*) which cause ripe rot disease in Korea (Hong et al. 2008). The classification of two different pathogens between *C. acutatum* and *C. gloeosporioides*, is not clear from morphological traits. For this reason, many isolates of *C. acutatum* have been wrongly identified as *C. gloeosporioides*. As mentioned, Korean wild grapes have a general disease resistance, but 'Meoru' (*V.coignetiae*) species needed additional tests for disease resistance to *C. gloeosporioides*.

In the genetic analysis results by RAPD and SSR marker, 'Saemeoru' (*V. flexuosa*) is classified with *V.riparia*, which has a broad range of disease resistance and tolerance of environmental stress (Hur et al. 2012). Our results showed that the wild Korean grapes are more resistant to general disease in Korea except 'Meoru' (*V.coignetiae*) in the ripe rot test results. Wild species have a high resistance to pest, and many environmental stresses. However, in some cases, wild species have a tolerance that is different from resistance. Generally, wild species have a high level of non-specific disease resistance but are not immune. Our experiment create extreme conditions to test resistance to these disease. In our results, the Korean wild grape species showed a different disease infection rate against the various pathogens. Even though our experiment was done in vitro; it is useful for choosing a parent for disease resistance breeding. Moreover, it will be possible to compare our data with field survey data to find disease development factors related to climate.

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**Table 1: Leaf Disease Resistance of the Korean wild Grapes and Cultivars in Vitro Evaluation**

| Name | Species | Origin in Korea | In vitro test result (Infection rate, %) | |
|---|---|---|---|---|
| | | | Anthracnose (*Elsinoe ampelina*) | Brown Leaf Spot (*Pseudocecospora vitis*) |
| Saemeoru | *V. flexuosa* | Hongcheon | - | - |
| Wangmeoru | *V. amurensis* | Taebak | - | - |
| Meoru | *V. coignetiae* | Chungnam | 8.3 a$^{y}$ | 8.3 b |
| Kyoho | *V. vinifera hybrid* | - | 4.2 a | - |
| Campbell Early | *V. labrusca hybrid* | - | - | 20.8 a |

$^{y}$. Means with the same letter are not significantly different at the 5% by Tukey's HSD.

**Table 2: Berry Disease Resistance of the Korean wild Grapes and Cultivars in Vitro Evaluation**

| Name | Species | Origin in Korea | In vitro test result (Infection rate, %) | |
|---|---|---|---|---|
| | | | Gray Mold (*Botrytis cinerea*) | Ripe Rot (*Colletotrichum acutatum*) |
| Saemeoru | *V. flexuosa* | Hongcheon | 8.3 a$^{y}$ | 4.2 b |
| Wangmeoru | *V. amurensis* | Taebak | 8.3 a | - |
| Meoru | *V. coignetiae* | Chungnam | 20.8 a | 33.3 a |
| Kyoho | *V. vinifera hybrid* | - | 16.7 a | 20.8 ab |
| Campbell Early | *V. labrusca hybrid* | - | 16.7 a | 25.0 ab |

$^{y}$. Means with the same letter are not significantly different at the 5% by Tukey's HSD.