INHERITANCE AND CLONING OF CANDIDATE RESISTANCE GENE ANALOGS (RGAs) FOR DOWNY MILDEW IN GRAPEVINE (*Vitis* spp.)

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Crop Production Technology

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การถ่ายทอดลักษณะ และการโคลนกลุ่มของยีนที่มีลำดับเบสคล้ายกับยีนต้านทานโรค (resistance gene analogs; RGAs) ราน้ำค้างในองุ่น (*Vitis* spp.)

นางสาวศิระประภา มหานิล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

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ศิระประภา มหานิล : การถ่ายทอดลักษณะ และการโคลนกลุ่มของยีนที่มีลำดับเบสคล้ายกับยีนต้านทานโรค (resistance gene analogs; RGAs) ราน้ำค้างในองุ่น (*Vitis* spp.) (INHERITANCE AND CLONING OF CANDIDATE RESISTANCE GENE ANALOGs (RGAs) FOR DOWNY MILDEW IN GRAPEVINE (*Vitis* spp.)) อาจารย์ที่ปรึกษา : ศาสตราจารย์ ดร. ไพศาล เหล่าสุวรรณ, 246 หน้า

โรคราน้ำค้างที่เกิดจากเชื้อ *Plasmopara viticola* เป็นโรคที่มีความสำคัญขององุ่น ทั่วโลก รวมถึงประเทศไทยด้วย วัตถุประสงค์สำหรับการศึกษานี้เพื่อ 1) โคลน และจัดกลุ่มของกลุ่มยีนที่มีลำดับเบสคล้ายกับยีนต้านทานโรค (resistance gene analogs; RGAs) จากองุ่นจีโนไทป์ที่มีความต้านทานต่อโรคราน้ำค้างสูง ได้แก่ *Vitis cinerea* B9, *V. rupestris* B38 และจีโนไทป์ที่มีความต้านทานต่อโรคราน้ำค้างในระดับปานกลาง คือ *V. hybrid* 'Horizon' 2) เพื่อทดสอบเครื่องหมายโมเลกุลที่พัฒนามาจาก RGAs ซึ่งมีความเป็นไปได้ที่จะวางอยู่ใกล้ชิดกับอัลลีลที่ทำหน้าที่ในการต้านทานโรคในองุ่น 3) เพื่อทดสอบสมรรถนะการรวมตัวของยีนที่ควบคุมการต้านทานต่อโรคราน้ำค้าง และพัฒนาเชื้อพันธุกรรมที่มีความต้านทานต่อโรคราน้ำค้างในองุ่นรับประทานผลสด การศึกษาครั้งนี้ได้แบ่งการทดลองออกเป็น 2 ส่วน ได้แก่ การปรับปรุงพันธุ์โดยใช้เทคนิคทางชีววิทยาโมเลกุล (molecular breeding) และการปรับปรุงพันธุ์โดยวิธีทั่วไป (conventional breeding) การโคลน RGAs โดยใช้ไพรเมอร์ P-loop/GLPLAL-1 จากองุ่นทั้ง 3 จีโนไทป์ในการศึกษาส่วนแรกได้ RGAs จำนวน 19 โคลน และโคลนเหล่านี้มีลำดับนิวคลีโอไทด์ที่คล้ายคลึงกับ RGAs ขององุ่นจีโนไทป์อื่น และ/หรือในพืชอื่น ในการจำแนกชนิดของ RGAs ที่โคลนได้พบว่า สามารถแบ่งออกเป็น 2 กลุ่ม ตามลำดับกรดอะมิโนที่ N-terminus ซึ่งเป็นลักษณะที่พบโดยทั่วไปในพืชใบเลี้ยงคู่ ได้แก่ *Drosophila Toll* and mammalian *Interleukin-1 receptors* (TIR)-nucleotide binding site (NBS)-leucine-rich repeat (LRR) และ non-TIR-NBS-LRR สำหรับการพัฒนาเครื่องหมายโมเลกุลชนิด simple sequence tag site (STS) สามารถพัฒนาได้ทั้งหมด 17 เครื่องหมายโมเลกุลจาก RGAs จำนวน 19 โคลน ได้มีการทดสอบการกระจายตัวของเครื่องหมายโมเลกุลจำนวน 17 เครื่องหมายนี้ ร่วมกับเครื่องหมายโมเลกุลที่ได้มีการพัฒนามาจาก RGAs ขององุ่นจีโนไทป์อื่นก่อนหน้านี้ ได้แก่ STS จำนวน 6 เครื่องหมายโมเลกุล และ cleaved amplified polymorphic sequence (CAPS) จำนวน 3 เครื่องหมายโมเลกุล ซึ่งเครื่องหมายโมเลกุลเหล่านี้อยู่ในระหว่างการทดสอบหาความเป็นไปได้ที่จะนำมาใช้ร่วมกับการปรับปรุงพันธุ์ให้ต้านทานต่อโรคราน้ำค้างในองุ่น การทดลองส่วนที่ 2 ได้มีการผสมองุ่นจำนวน 9 คู่ผสมในปี พ.ศ. 2547 โดยผสมระหว่างสายพันธุ์พ่อ 3 จีโนไทป์ได้แก่ NY 88.0517.01, NY 65.0550.04 และ NY 65.0551.05 และ สายพันธุ์แม่ที่เป็น *V. vinifera* L. 3 พันธุ์ได้แก่ Black Queen, Carolina Black Rose และ Italia การประเมินโรคในสภาพใบตัด (detached leaf assay) ของลูกผสมจำนวนทั้งหมด 83 ต้น พบว่าลูกผสมเหล่านี้มีศักยภาพในการเป็นแหล่งพันธุกรรมที่มีความต้านทานต่อโรคราน้ำค้าง โดยลูกผสมจำนวน 25.3

เปอร์เซ็นต์ มีความต้านทานต่อโรคราน้ำค้างในระดับสูง และสูงมาก ทำการทดสอบสมรรถนะการรวมตัวทั่วไป (general combining ability; gca) และสมรรถนะการรวมตัวจำเพาะ (specific combining ability; sca) โดยใช้แผนการผสมพันธุ์แบบ North Carolina mating design II (NC II) ผลการวิเคราะห์แสดงให้เห็นว่าการตอบสนองส่วนใหญ่ต่อการเข้าทำลายของโรคราน้ำค้างในประชากรกลุ่มนี้เป็นการแสดงออกของยีนในแบบบวก (additive gene action) เช่นเดียวกับมีค่าประมาณอัตราพันธุกรรม (heritability) เท่ากับ 60.91 เปอร์เซ็นต์ ชี้ให้เห็นว่ายีนที่ควบคุมการต้านทานโรคราน้ำค้างในองุ่นมีการแสดงออกเป็นแบบบวกมากกว่าการแสดงออกของยีนในแบบที่ไม่ใช่แบบบวก (non-additive gene action) คู่ผสม Carolina Black Rose x NY 65.0550.04 ให้ลูกผสมที่มีความต้านทานต่อโรคราน้ำค้างสูงถึง 75.0 เปอร์เซ็นต์ และให้ค่าสมรรถนะการรวมตัวจำเพาะที่แตกต่างอย่างมีนัยสำคัญยิ่งทางสถิติ (-1.09; $P < 0.01$) จัดว่าเป็นคู่ผสมที่มีความเหมาะสมที่สุดสำหรับใช้ในโปรแกรมการปรับปรุงพันธุ์องุ่นให้ต้านทานต่อโรคราน้ำค้างต่อไป

สาขาวิชาเทคโนโลยีการผลิตพืช
ปีการศึกษา 2550

ลายมือชื่อนักศึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

SIRAPRAPA MAHANIL : INHERITANCE AND CLONING OF CANDIDATE RESISTANCE GENE ANALOGs (RGAs) FOR DOWNY MILDEW IN GRAPEVINE (*Vitis* spp.) THESIS ADVISOR : PROF. PAISAN LAOSUWAN, Ph.D. 246 PP.

NBS-LRR/DISEASE RESISTANCE/MARKER-ASSISTED SELECTION/GCA/SCA/ NC II

Downy mildew caused by *Plasmopara viticola* has been recognized as one of the major grape diseases worldwide including Thailand. The objectives of this study were to 1) clone and classify resistance gene analogs (RGAs) from two genotypes highly resistant to downy mildew, *Vitis cinerea* B9 and *V. rupestris* B38, and one moderately resistant genotype, *V. hybrid* 'Horizon' 2) identify candidate markers from RGAs that possibly link to resistance allele(s) in grape and 3) evaluate the inheritance of the disease and develop new table grape germplasm with resistance to downy mildew. This study was divided into two parts according to molecular and conventional breeding. The first part suggested that 19 RGA clones from P-loop/GLPLAL-1 primer pairs of three grape genotypes were similar to nucleotide sequences of RGAs from other grape genotypes and/or other plants. These RGA clones were divided into two groups based on amino acid sequences at N-terminus including, *Drosophila Toll* and mammalian *Interleukin-1 receptors* (TIR)-nucleotide binding site (NBS)-leucine-rich repeat (LRR) and non-TIR-NBS-LRR, as usually found in dicot plants. Seventeen out of 19 RGA clones were developed into simple sequence tag site (STS) markers. Those markers and 6 STS and 3 cleaved amplified polymorphic sequence (CAPS) markers previously developed from other grape species were used for segregation analysis. These molecular markers are

currently being investigated for their potential use in molecular breeding for downy mildew resistance. Nine hybridization crosses were made in year 2004 for the second part. Three resistant genotypes, NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05, were used as male parents while three cultivars of *V. vinifera* L., Black Queen, Carolina Black Rose and Italia, were used as female parents. Eighty-three seedlings were obtained as potentially downy mildew resistance germplasm. The detached leaf assay indicated that 25.3% of the seedlings were highly resistant and resistant to downy mildew. The general combining ability (gca) and specific combining ability (sca) analysis were analyzed by North Carolina mating design II (NC II). The results suggested that additive gene action was primarily responsible for downy mildew resistance in this population. Similarly, the estimated heritability of downy mildew resistance was 60.91%, indicating that the additive gene action was prevalent over the non-additive gene action for downy mildew resistance character. The cross 'Carolina Black Rose x NY 65.0550.04', giving 75.0% resistant seedlings and highly significant sca effects (-1.09; $P < 0.01$), is strongly recommended for downy mildew resistance grape breeding programs in the future.

School of Crop Production Technology

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Student's Signature__________

Advisor's Signature__________

Co-advisor's Signature__________

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Siraprapa Mahanil

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CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Grapes (*Vitis spp*.) are one of the most widely grown fruit crops in the world with significant plantings in Europe, North and South America, South Africa and Australia. This crop has a wide adaptability and can be grown under temperate, sub-tropical and tropical climatic conditions. Grapes are used in the production of wine, brandy, champagne, or non-fermented drinks and are eaten fresh or dried as raisins. Grape seeds contain 6–20% oil, used for edible purposes, soap, anthocyanin pigments, ethanol production and as a linseed substitute. The leaves are edible in some cultures. In 2005, world fresh table grape production was 12.6 million tons, and the major producers were China, Turkey, Italy, Chile, United States, South Africa and Spain (Foreign Agriculture Service, 2005). According to Organization International of Vine and Wine (O.I.V.), world total wine production in 2004 was about 72.47 million of hl (Foreign Agriculture Service, 2005).

At present, grapes are widely grown in Thailand because of their high prices as compared with other crops. Moreover, the tropical conditions offer the advantage of shorter generation time. In these conditions, farmers can harvest twice or three times per year, as compared to temperate climates that allow only one harvest. In 2007, Thailand cultivated approximately 2,329.44 ha and produced 34,626.89 tons of grape per year with the average yield of 16.47 tons/ha (Department of Agricultural

Extension, 2007). Major cultivated area is the central plain including Samut Sakhon (791.68 ha), Ratchaburi (485.60 ha), Saraburi (156.64 ha), Nakhon Pathom (78.40 ha) and Kanchanaburi (26.72 ha). However, the grape industry has now expanded to the northeastern e.g. Nakhon Ratchasima (672.96 ha; Department of Agricultural Extension, 2007). Approximately 87.40% (or 2,377.50 ha) of grape production in Thailand is table grapes such as White Malaga, Cardinal, Kyoho, Beauty Seedless, Early Muscat and Carolina Black Rose. While, the remaining production (12.60% or 340.40 ha) is for wine making. Wine grape varieties in Thailand include Shiraz, Tempranillo and Chenin Blanc (Nilnond, 2001).

Even though grapes are grown widely and expanding rapidly to every region of Thailand, we still need to import fresh grapes, wine and raisins for consumption. Thailand imported fresh grape approximately 8,993 tons or 605.81 million baht, 12,067 tons or 720.27 million baht, 11,468 tons or 709.32 million baht and 13,098 tons or 769.28 million baht from 2003 to 2006, respectively (Office of Agriculture and Economics, 2006). This may due to lower efficiency of grape cultivation in Thailand compared with major grape growing areas of the world. The major cultivation limitations are unsuitable soil, diseases and insect pests, in particular the disease resistant varieties are lacking.

The major diseases in most viticultural countries are downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), anthracnose (*Elsinoe ampelina*), Botrytis rot (*Botrytis cinerea*), black rot (*Guignardia bidwelli*) and crown gall (*Agrobacterium vitis*; Carisse et al., 2006). The important diseases of table grapes in northern Australian vineyard are downy mildew, powdery mildew, Botrytis rot and anthracnose (Oag, 2001). While, Botrytis rot, downy mildew, powdery mildew,

anthracnose, black rot and Pierce's disease (*Xylella fastidiosa*) are the serious diseases in grape cultivation areas in the United States (Texas Winegrape Network, 2004). Similarly, Nilnond (2001) reported that the most important diseases in Thailand included downy mildew, anthracnose, dead arm (*Melanconium fuligeneum*) and Botrytis rot.

Downy mildew is a highly destructive disease of grapes in all grape-growing areas of the world. The causal organism of downy mildew is *P. viticola*. This fungus is an obligate parasite. *P. viticola* becomes active in the spring when oospores germinate to form sporangium. Sporangia are formed in the dark and are disseminated by wind or rain splash. They germinate on host tissues when free moisture is present and then release zoospores. Primary infection occurs when these spores germinate and penetrate the host through stomata. The fungus becomes established as intercellular mycelium and then zoospores released from these structures cause secondary infections, entering the host through stomata on the underside of leaves or lenticels on fruits. As the season progresses, oospores are formed from the mycelium within the host tissues to complete the life cycle. The pathogen attacks all green parts of the grapevine.

First signs of infection on the leaves are angular and light yellow spots. White downy growth composed of fungal threads and spores forms on the underside of the leaves. Shoots, tendrils, or berries may be attacked early in the season when they are tender, causing water-soaked depression and later the appearance of a white moldy growth occurs. The shoots may also be stunted, thickened or turn brown and die. If the berries are attacked, there may be two periods of infection, first infection when the berries are about the size of small peas. Infected berries become soft, shatter easily

and are frequently covered with a white downy growth. Second infection occurs when the nights become cooler. Berries infected at this time become brownish, wither and shatter easily.

This disease causes substantial economic loss such as yield and quality of the fruits. The fungus causes direct yield losses by rotting inflorescences, clusters and shoots. Indirect losses can result from premature defoliation which is a serious problem because it predisposes the vine to the winter. When the weather is favorable and no protection against the disease is provided downy mildew can easily destroy 50 to 75% of a crop in one season (Agrios, 1997). Several applications of fungicides are usually needed to protect grapevine throughout its life cycle. But, chemical control increases the production cost and has negative effects on the environment. Therefore, disease resistance varieties are desirable for all grape producing cultivated areas including Thailand.

The isolation of resistance gene analogs (RGAs) provides the possibility to clone resistance (R) genes from grape. RGAs cloned could also be developed as molecular markers to be used in marker assisted selection (MAS) for resistance to downy mildew or other diseases. Also, grape germplasm for downy mildew resistance which is lacking in Thailand will be useful to grape breeding program for downy mildew resistance in the future. Combined together, these will lead to improved disease resistance levels especially for downy mildew in grape. Consequently, it will reduce the cost for chemical disease control as well as avoid deleterious effects on the environment. This may increase the efficacy of grape cultivation in Thailand.

1.2 The objectives

1. To clone and classify RGAs from two genotypes highly resistant to downy mildew, *V. cinerea* B9 and *V. rupestris* B38, and one moderately resistant genotype, *V. hybrid* 'Horizon'.

2. To identify candidate markers from RGAs that possibly link to resistance allele(s) in grape.

3. To evaluate the inheritance and develop new table grape germplasm with resistance to downy mildew.

1.3 References

Agrios, G.N. (1997). **Plant Pathology.** New York: Academic.

Carisse, O., Bacon, R., Lashier, J. and McFadden-Smith, W. (2006). Identification guide to the major diseases of grape [On-line]. Available: http://www.sci.agr.ca/stjeand/pdf/malad_e.pdf

Department of Agricultural Extension, Ministry of Agriculture and Cooperatives. (2007). Information of Agricultural production [On-line]. Available: http://production.doae.go.th

Foreign Agriculture Service, United States department of Agriculture. (2005). World table grape situation and outlook [On-line]. Available: http://www.fas.usda.gov

Nilnond, S. (2001). Grape production in Thailand. In **Proc. of Grape Production in the Asia-Pacific Region** (pp 70-79). Bangkok, Thailand.

Oag, D.R. (2001). Grape production in Australia. In **Proc. of Grape Production in the Asia-Pacific Region** (pp 70-79). Bangkok, Thailand.

Office of Agricultural Economics, Ministry of Agriculture and Cooperatives. (2006). [On-line]. Available: http://www.oae.go.th/oae

Texas Winegrape Network, Texas Cooperative Extension. (2004). Grape disease management [On-line]. Available: http://www.winegrape.tamu.edu/grow/disease

CHAPTER II

REVIEW OF THE LITERATURE

2.1 Grape (*Vitis* spp.) taxonomy

The genus *Vitis* belongs to the Vitaceae family and is the only economically important genus among 14 genera in this family (Pearson and Goheen, 1988). It is widely distributed in the tropics and subtropics such as eastern Asia, Europe, Middle East and North America.

In general, the family is characterized by climbing habit, terminal buds developing into tendrils and the inflorescence arising opposite a leaf of the node. The flower has five sepals forming the outer part. A petal and a stamen join arising from each of five primordial. The grape flower consists of anther, filament, stigma, style, ovary, ovule and nectary (Figure 1). The cap or calyptra is made up of five petals joined at the top to cover the flower bud. Flower has two ocules and two ovules containing at each ovary. Thus, the developed seeds could be a maximum of four seeds per berry. The types of flower are; 1) hermaphroditic or perfect flower e.g. *V. vinifera* and *V. labrusca*, the stamens of this type are erect and functional pollen is produced in the anthers. The pistil is also functional (Figure 2); 2) pistillate or female flowers whose pistil is well developed and functional but pollen is generally sterile (Figure 2). This type of flower is found on female vines of dioecious species e.g. *V. rotundifolia*, *V. unsoniana* and *V. popenoe* (Kentucky State University, 2005); 3) staminate or male flowers, e.g. those found in some American and Asian species, have erect stamens with functional pollen.

A pistil fails to develop past the stage of immature ovules (Figure 2; Gerrath and Posluszny, 1988; Reisch, 2003).

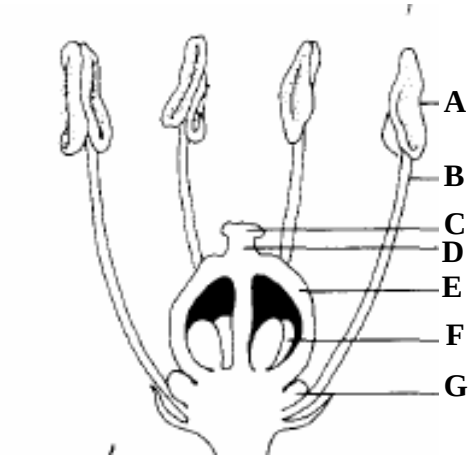


Figure 1. Grape open flower A) anther; B) filament; C) stigma; D) style; E) ovary; F) ovule; G) nectary.

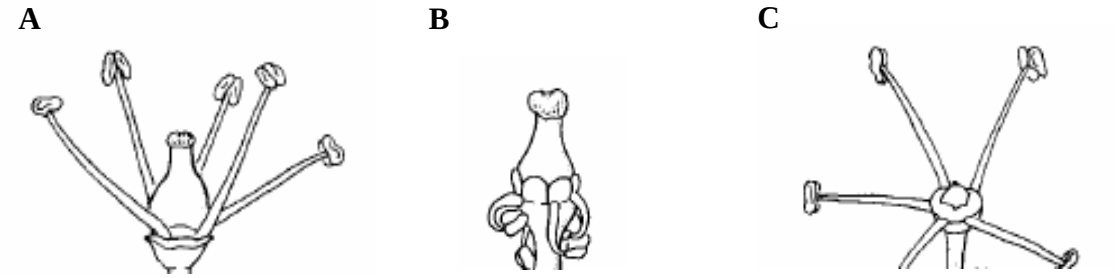


Figure 2. Three types of grape flower A) hermaphroditic or perfect flower; B) pistillate or female flower; C) staminate or male flower.

The cluster consists of a peduncle, pedicels, rachis and berries. The leaf bracts are broad, short scales arising from enlarged base of the petiole. The leaf position is distichous which is disposed in two vertical rows. The characters such as leaf shape, color, surface, contour, margin (dentation) and sinus (space between lobes) could be used to classify many cultivars of grape (Figure 3).

The shoot consists of growing tip, nodes, internodes, buds, tendrils and laterals. Different characters of node, diaphragm or continuous pith, are found in between two subgenera. The subgenus *Euvitis* has wood layer interrupted at each node or diaphragm. While the continuous pith and shoot that has light bark and does not shed are found in subgenus *Muscadinia*.

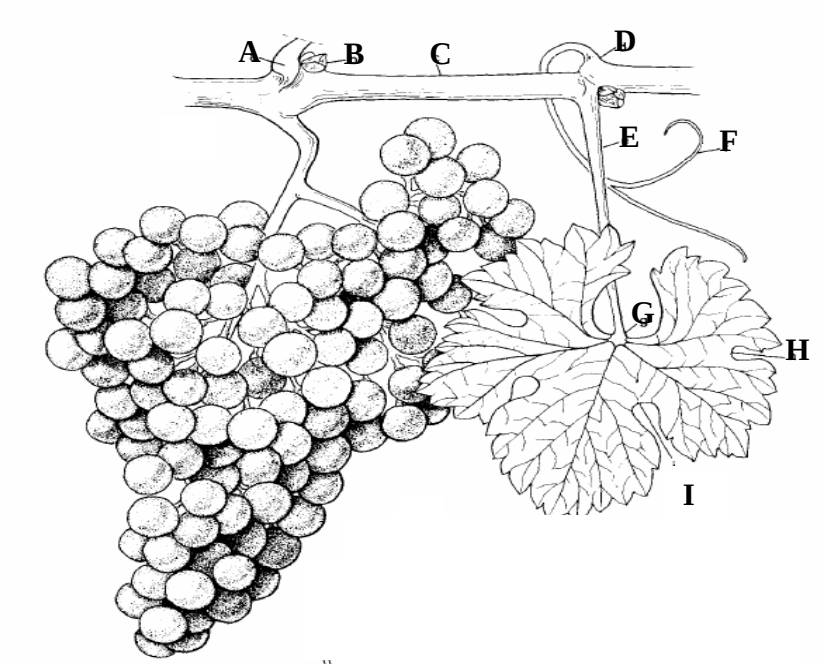


Figure 3. Grape cane, leaf and fruit cluster morphology A) petiole; B) bud; C) internode; D) node; E) petiole; F) tendril; G) petiolar sinus; H) inferior sinus; I) superior sinus.

The genus *Vitis* can be classified into two subclasses include;

I. Subgenus *Euvitis*, 2n = 38

Clusters are elongated; berries adhere to the stem at maturity. Tendrils are forked. The shoots have loose bark in long strips and diaphragms in pith at nodes.

- European wine grape

V. vinifera has an intermittent tendril, thin, smooth shiny leaves with three, five or seven lobes. Berry size varies and shapes are round or oval. Approximately 90% of

world grape production for both wine and table grape is *V. vinifera*. There are at least 5,000 cultivars that are grown worldwide, the major cultivars including Thompson seedless, Flame seedless, Pinot Noir, Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc and Riesling (Reisch and Pratt, 1996). Vinifera grapes require long growing season and high summer temperature to maintain high fruit quality. Ripening season should be free of rainfall and mild winter temperatures (ATTR-National Sustainable Agriculture Information Service, 2007).

- Asian species

The Asiatic group is native from eastern Asia including China, Japan and south into Java. This group includes about 30 species e.g. *V. amurensis* and *V. sylvestris*. *V. amurensis* is the most commonly known species among the group (Alleweldt et al., 1990). This species is cultivated in some areas of northeastern China and Japan. The fruit is collected for use as fresh fruit, juice, wine and jelly (Pieniazek, 1976; Huang, 1980). *V. amurensis* is reported to be excellent source for downy mildew and crown gall resistance in grape breeding program (Szegedi et al., 1984; He and Wang, 1986; Pearson and Goheen, 1988; Eibach et al., 1989; Alleweldt et al., 1990).

- Native American species

They are usually slip skin and inedible. The berries are small and round or nearly round. The Native American species are sources of resistance to diseases and insects that are not found in Vinifera grape. Even though the levels of disease resistance in these wild species are high, their commercial utilization is limited due to their low fruit quality. Wild berries are usually small, have high acidity and undesirable flavors. *V. labrusca* L. or the fox grape is native to the northeast and east of the United States (Figure 4; ATTR-National Sustainable Agriculture Information Service, 2007). This

species is very productive, produces large berried fruit and is cold resistant. The fruit has a strong distinctive flavor (foxy flavor) and slip skin. It is a good source for resistance against downy mildew, anthracnose, powdery mildew and crown gall but not for black rot (Szegedi et al., 1984; He and Wang, 1986; Pearson and Goheen, 1988; Eibach et al., 1989; Alleweldt et al., 1990). Major use of this species is for sweet grape juice and associated product e.g. jelly, jam and some wine (ATTR-National Sustainable Agriculture Information Service, 2007).

V. rupestris Scheele or the sand grape is native from southern Missouri, Illinois, Kentucky and western Tennessee to southwestern Texas, Oklahoma and westward to New Mexico (Figure 4; Reisch and Pratt, 1996; ATTR-National Sustainable Agriculture Information Service, 2007). *V. rupestris* is very resistant to phylloxera, black rot and shows high tolerance but not resistance to nematodes. Due to its resistance to phylloxera, this species is used as a root stock and parent of French-American hybrids. This species is also reported as resistant to downy mildew and botrytis rot (McGrew, 1976; Jabco et al., 1985; He and Wang, 1986; Pearson and Goheen, 1988; Eibach et al., 1989; Alleweldt et al., 1990).

V. riparia Michx or the riverbank grape is native from Canada to Texas and westward to the Great Salt Lake, Utah (Figure 4; Pierquet and Stushnoff, 1978). It has provided resistance to phylloxera and most fungal diseases e.g. downy mildew, botrytis rot, black rot and powdery mildew (McGrew, 1976; Jabco et al., 1985; He and Wang, 1986; Pearson and Goheen, 1988; Eibach et al., 1989; Alleweldt et al., 1990). The flowers open early and ripenning occurs early producing fruits high in sugar and acid content. Because *V. riparia* can grow rapidly from cutting, it is able to make good stock for grafting (ATTR-National Sustainable Agriculture Information Service, 2007). This

species is extremely winter tolerant and has been used in breeding for winter hardy cultivars such as Marechal Foch and Baco Noir (Reisch and Pratt, 1996).

V. aestivalis Michaux or the summer grape is native from New England to Georgia and westward to Arkansas and Mississippi river (Figure 4; Reisch and Pratt, 1996). *V. aestivalis* does possess resistance to phylloxera and is resistant to powdery mildew (Pearson and Goheen, 1988; Alleweldt et al., 1990; Reisch et al., 1993). The fruit character is desirable. This species is also used as a parent in some French-American hybrids as a source of disease resistance. Norton and Cynthiana are the important cultivars. This grape can produce good quality of wine with the same level of *V. vinifera* (ATTR-National Sustainable Agriculture Information Service, 2007).

V. cinerea Engelm or the graybark grape is native to central and southeastern United States (Figure 4; Reisch and Pratt, 1996). *V. cinerea* var. *helleri* is native from south to central Texas and adjacent Mexico (Spellenberg, 1998). This grape is a source of resistance to *Xiphinema* and phylloxera (Zimmerman and Becker, 1978; Becker, 1988; Becker and Soop, 1990). *V. cinerea* also has a high level of resistance to multiple fungal diseases such as powdery mildew, downy mildew and black rot. However, this species is poor rooting and of low fruit quality (Reisch and Pratt, 1996).

II. Subgenus *Muscadinia*, 2n = 40

The clusters are small with berries that detach one by one as they mature. The tendril is simple (unforked). The shoots have light bark that does not shed, nodes without diaphragm and smooth bark with lenticels. The flower divides this species into two classes; 1) pistillate or female and 2) perfect flowered or hermaphroditic. Their berries are small, loose clusters of 3 – 40 berries and tough skin. The flavor is unique fruity flavor. Fruit colors are bronze, pinkish red, purple and almost black. This species

is a source of resistance for several diseases and insects (Small, 1903; ATTR-National Sustainable Agriculture Information Service, 2007). The popular cultivars are Cowart, Darlene, Hunt, Noble, Jumbo, Carlos, Higgins, Fry, Dixieland, Summit and Scuppernong (California Rare Fruit Growers, 1997).

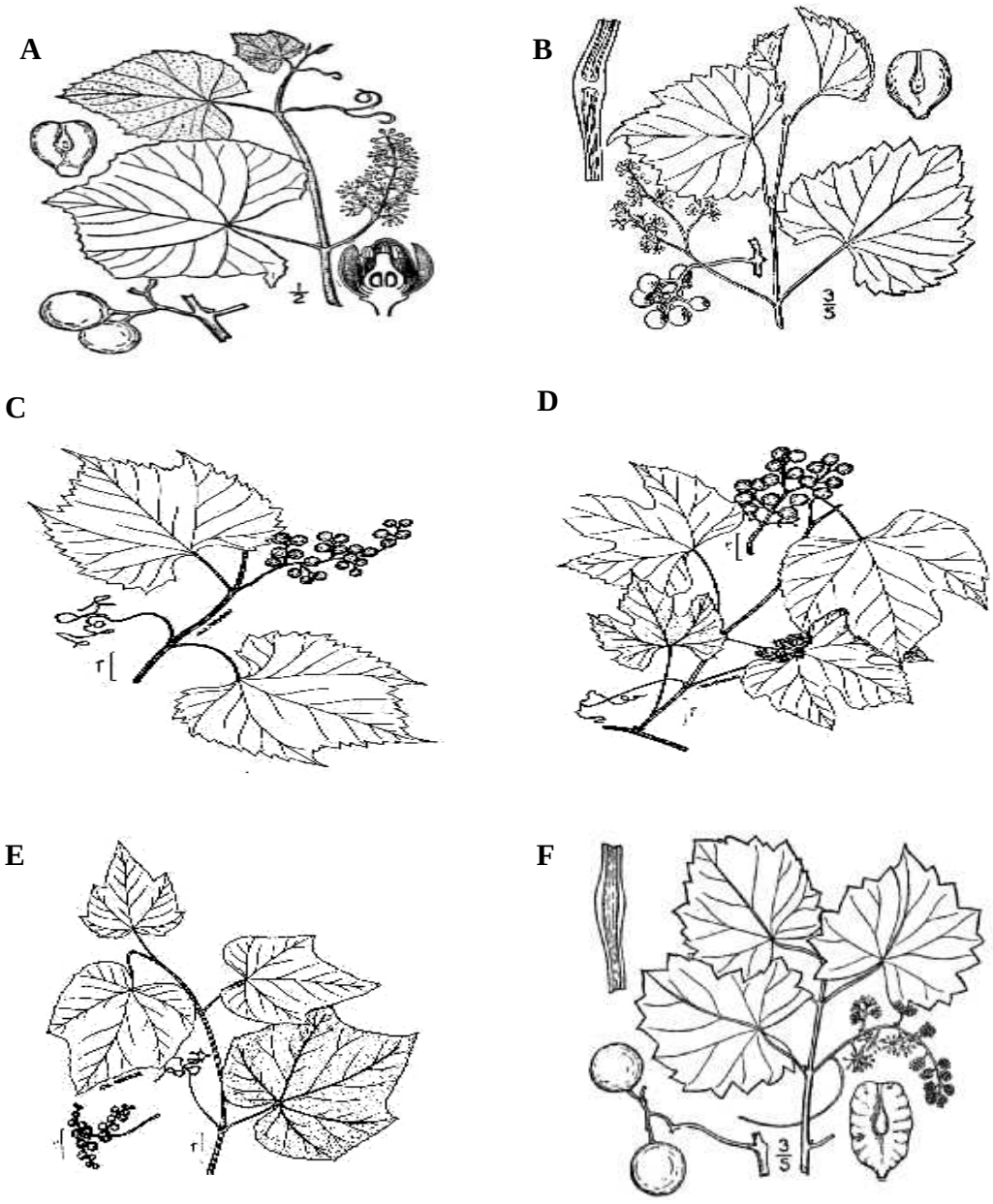


Figure 4. Morphology of American species A) *V. labrusca*; B) *V. rupestris*; C) *V. riparia*; D) *V. aestivalis*; E) *V. cinerea*; F) *V. rotundifolia* (Natural Resources Conservation Service, 2007).

V. rotundifolia Michx, or the Muscadine or the South fox grape is native to the southern Delaware to southern Illinois, south by southwest to northeastern Texas, south to the Gulf and east to the Atlantic (Figure 4; ATTR-National Sustainable Agriculture Information Service, 2007). This species is vigorous, disease tolerant and adapted to the hot and humid summer in southern United States. This species is a source of resistance for several diseases and insects e.g. downy mildew, anthracnose, black rot, rust, powdery mildew, root knot nematode, phylloxera, Pierce's disease and grapevine fanleaf virus (Lider, 1954; Stover, 1960; McGrew, 1976; Olmo, 1976; Mortensen et al., 1977; Bloodworth et al., 1980; Bouquet, 1981; Mortensen, 1981; Jabco et al., 1985; Walker et al., 1985; He and Wang, 1986; Pearson and Goheen, 1988; Eibach et al., 1989; Alleweldt et al., 1990; Walker and Meredith, 1990).

2.2 Plant disease resistance genes

The gene-for-gene interaction describes defense mechanisms used by plants against various pathogens including fungi, viruses, bacteria and nematodes (Keen, 1990; Crute and Pink, 1996; Liu and Ekramoddoullah, 2003). Resistance (R) gene products from plants specifically recognize, either directly or indirectly, avirulence (*Avr*) gene products in a pathogen that leads to defense activation in plants (Flor, 1971). The resistant plant needs three steps to respond to the attack of a pathogen including 1) elicitor of pathogen *Avr* gene must be recognized directly or indirectly by the R gene product; 2) interaction of *Avr* and R gene products activate the defense signal transduction pathway in the plant; 3) activation of the signaling cascade leads to initial defensive response in the plant and then suppression of the pathogen. There are several patterns to suppress the pathogen in plant including programed cell death at the site of pathogen exposure or hypersensitive response (HR), induction of pathogen-related (PR)

gene expression, various physiological events such as calcium ion flux, a burst of reactive oxygen production, cell wall biosynthesis and callose deposition, inhibition of the pathogen's ability to replicate and move systemically and systemic acquired resistance (SAR) against future pathogen attack (Hammond-Kosack and Jones, 1996; Ryals et al., 1996; Dangl and Jones, 2001; Hu et al., 2005).

2.2.1 Two major components of gene-for-gene interaction

The resistant response in plant will occur only if the pathogen possesses an *Avr* gene for which a corresponding R gene exists in the host plant (Staskawicz et al., 1995). Therefore, the loss or alteration to either R gene or *Avr* gene will result in a compatible interaction (susceptibility).

- The avirulence genes

The *Avr* genes are always maintained within a pathogen population despite their obscure function for the pathogen itself (Long and Staskawicz, 1993). Products of *Avr* gene must be recognized by R gene products and lead to activated defense system in plant. Capsid protein (CP), small secreted effector protein and type III secretion (T3SS) are products of *Avr* genes in virus, fungi and bacteria, respectively. For example, Turnip crinkle virus (TCV), causal agent of Turnip crinkle disease, has CP as its elicitor. On the other hand disease resistant genotype, *Arabidopsis* ecotype Di-17, has TCV-interacting protein (TIP) that functions in binding with CP of TCV (Oh et al., 1995). Thus, site-directed mutagenesis within the binding region of TIP caused the loss of ability to bind with CP from TCV. Consequently, the mutant of disease resistant genotype could not induce HR. These data indicated that these two components, TIP (the intracellular receptor) and CP from TCV (elicitor), were essential in resistance response pathway (Gabriel and Rolfe, 1990; Ren et al., 2000).

Knogge (1996) and Grennan (2006) reported that fungi form the haustorium and delivering the elicitors which are small secreted effector proteins e.g. *avr a* and *avr 4* of *Cladosporium fulvum* and *NIP1* of *Rhynchosporium secalis* into the plant apoplast to activate signal transduction pathway. In bacteria, Abramovitch et al. (2006) reported that *Pseudomonas pathovas* form a pilus to deliver the elicitor T3SS protein into plant cells. Moreover, the expression of *hrp*-gfp reporter fusion indicated that *hrp A* and *hrp Y* which are T3SS of *Pseudomonas syringae* and *Ralstonia solonacearum*, respectively have important role in induction of defensive response in plant (Van Gijsegem et al., 1995; Roine et al., 1997; Hueck, 1998; Galan and Collmer, 1999; Aldon et al., 2000).

- The resistance genes

R genes have been studied by either transposon-tagging strategies to destroy biological activity or map-based cloning and transformation to restore the resistance phenotype to verify their roles or function in plant. To date more than 40 R genes have been cloned from several plants such as *Arabidopsis RPS2, RPS5, RPP5* and *RPM1*, flax *L6* and *M*, tobacco *N*, tomato *Cf-9, Cf-2, Cf-4, Cf-5, Pto, Fen, Prf* and *I2*, potato *Gro1, R7* and *Rx*, rice *Xa21* and *Pi9*, wheat *Cre3*, sunflower *PI* and maize *HM* (Johal and Briggs, 1992; Martin et al., 1993; Bent et al., 1994; Jones et al., 1994; Mindrinos et al., 1994; Whitham et al., 1994; Grant et al., 1995; Jones et al., 1995; Lawrence et al., 1995; Song et al., 1995; Dixon et al., 1996; Qu et al., 2006).

R genes have been classified into five classes based on structural characteristics of their protein products (Figure 5; Dangl and Jones, 2001; Di Gaspero and Cipriani, 2003). Five main classes of R genes are 1) extracellular leucine-rich repeat (LRR) with receptor-like kinases (RLKs) and serine-threonine protein kinase domain (e.g. rice *Xa21* and *Arabidopsis FLS2*); 2) LRR anchored to the plasma membrane (e.g. tomato *Cf-9*); 3) proteins containing a nucleotide binding site (NBS) and LRR together with either a

Toll/Interleukin-1 receptor domain (TIR; e.g. tobacco *N* gene) or a coiled-coil structure (CC) or leucine-zipper (LZ; e.g. *Arabidopsis RPS2*); 4) cytoplasmic serine/threonine kinase (e.g. tomato *Pto*); 5) the *HM1* gene encoding a toxin reductase.

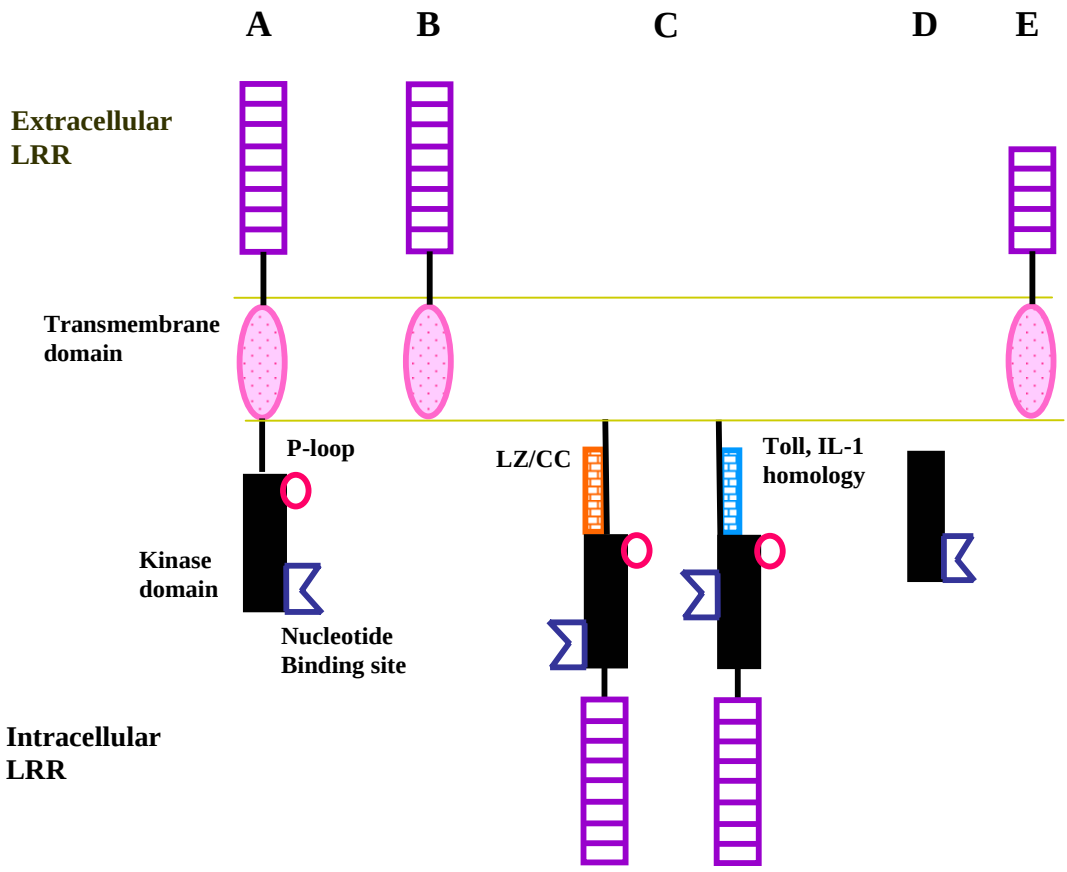


Figure 5. Classes of disease resistance proteins A) extracellular leucine-rich repeat (LRR) with receptor-like kinase (RLK) and serine-threonine protein kinase domain e.g. rice *Xa21* gene; B) LRR anchored to the plasma membrane e.g. tomato *Cf-2* and *Cf-9* genes; C) nucleotide binding site (NBS)-LRR with LZ or CC e.g. *Arabidopsis RPS2* and *RPM1* genes and NBS-LRR with TIR e.g. tobacco *N* gene; D) cytoplasmic serine/threonine kinase e.g. tomato *Pto* and *Fen* genes; E) *HM1* gene encoding a toxin reductase.

- **NBS-LRR type**

The NBS-LRR type is the main class of R genes and makes up approximately 75% of plant R genes. The major domains of this type of R gene are LRR, NBS and TIR or non-TIR domains. These domains are suggested to play important role in defense system of resistant plants. Moffett et al. (2002) demonstrated that the LRR and CC-NBS domains are both required for *Rx* activity which confers resistance against potato virus X (PVX) in the *Solanaceae*. The constructs in which either LRR or CC-NBS had been deleted could not induce HR, while co-expression of both domains resulted in a CP-dependent HR.

- **Function of LRR domains in specific recognition and intracellular signaling**

At C-terminal, LRR domains are thought to have roles in both specific recognition and intracellular signaling (Botella et al., 1998; McDowell et al., 1998; Meyers et al., 1998; Warren et al., 1998; Ellis et al., 1999; Noel et al., 1999; Bittner-Eddy et al., 2000; Banerjee et al., 2001; Dodds et al., 2001). The LRR domain comprises a repeat of approximately 25-38 amino acids of leucine and other hydrophobic residues (Dixon et al., 1998). The repeat unit of leucine causes this segment to fold into β-helix-loops and β-strand-loops. The curved spring tertiary structure of the LRR domain provides a binding surface and has been shown to participate in direct and/or indirect interactions with *Avr* proteins (Jones and Jones, 1997).

The most obvious role of LRR domain in defensive response in plant is specific recognition with *Avr* products. Noutoshi et al. (2005) and Yamasaki et al. (2005) performed functional analysis of LRR domain in *Arabidopsis* by inserting the WRKQQK motif into the LRR domain to generate *slh1* mutant. Alteration in tertiary

structure of LRR domain required for binding with *Avr* gene product was believed to be the effect from this mutation. Therefore, *Arabidopsis* that carried the *slh1* mutation lost the ability to induce an HR in plant. In addition, precise domain swapping in tomato *Cf-9* also indicates that LRR domain has important roles in the interaction of R gene products with products from *Avr* gene (Van der Hoorn et al., 2001; Wulff et al., 2001).

The "Guard hypothesis" is assumed to be the pattern used to recognize *Avr* proteins of LRR domain. A third component is required to mediate resistance via indirect perception of an *Avr* protein (Luderer and Joosten, 2001; Quirino and Bent, 2003). In *Arabidopsis*, *RPP2A* and *RPP2B* genes were needed to cooperate with a third protein to recognize *Peronospara parasitica Avr* protein (ATR; Sinapidou et al., 2004). However, direct interaction between LRR domain and *Avr* protein was also found. The extracytoplasmic LRRs such as pectate lyase, P22 tailspike protein and pertactin were found to bind with *Avr* protein at hydrophobic face (Kobe and Deisenhofer, 1994; Emsley et al., 1996). On the other hand, the extracellular LRR protein was found to bind with *Avr* protein on glycosylation site (Zhang et al., 1995).

As mentioned above, LRR domain is also a candidate motif for participation in signal transduction pathway. Tomato *Cf-4* and *Cf-9* genes have specificity, KKxx motif, resides at LRR domain. The extended loops of this motif have been suggested to play a role in dimerization or interaction with either upstream or downstream signal components (Hammond-Kosack and Jones, 1996; Jones and Jones, 1997). Warren et al. (1998) and Banerjee et al. (2001) also reported the indirect evidence that LRR may contribute to activation of downstream signaling as well as pathogen recognition.

- Role in signal transduction pathway and specific recognition of NBS domain

At the N-terminus, NBS domain is presumed to play a major role in the signal transduction pathway in resistant plant. Highly conserved motifs among family members have been found in NBS domain. The backbone of the NBS domain mostly consists of P-loop/Kinase-2/RNBS-C/RNBS-D/GLPL (Traut, 1994). These conserved motifs are implicated in binding with *Avr* product leading to signaling transduction (Pan et al., 2000). Zhang et al. (2004) reported that P-loop plays a critical role in the NBS domain, due to the lack of HR induction in tobacco with point mutations at the P-loop of *RPS4* gene. The kinase-2 domain is presumed to co-ordinate metal ion (Mg^{2+}) binding that is required for phospho-transfer reaction (Traut, 1994). Moreover, the P-loop and kinase-2 motifs have been well characterized in the NBS domain as ATP- and GTP-binding proteins which allow the NBS membrane fusion protein to disassemble with other proteins e.g. proteins at downstream signaling transduction pathway (Traut, 1994; May et al., 2001).

In addition, NBS domain also plays a role in pathogen recognition. Even though, highly conserved motifs have been found in NBS domain. But, the sequences between conserved motifs are highly diverse among plants. These diverse sequences are presumed to influence elicitor-specific recognition between R gene and *Avr* gene and/or with downstream proteins in various signaling pathways (Aarts et al., 1998; Noir et al., 2001).

- **The TIR and non-TIR at N-terminus**

The NBS-LRR group can be subdivided into two classes according to types of domain at the NBS region 1) the TIR that contains a domain resembling the *Drosophila Toll* and mammalian *Interleukin-1 receptors* and 2) the non-TIR which contains CC or LZ. The non-TIR group e.g. *RPM1* and *RPS2* are found distributed in both monocot and

dicot plants (Aarts et al., 1998; Meyers et al., 1999; Pan et al., 2000). Both CC and LZ motifs were found in non-TIR subclasses. The CC motif is a bundle of two to five helices with two interacting hydrophobic amino acids at the helix-helix interface and functions in downstream signaling (Lupas, 1996; Century et al., 1997; Parker et al., 1997). On the other hand, the LZs found in *RPM1*, *RPS2* and *Prf* exist as monomers in uninfected condition, however they can exist as dimers or multimers from activation of pathogen. These data suggest that LZ motif has the ability to dimerize with themselves through specific interaction with other proteins (Landschulz et al., 1988).

The TIR subclass including such genes as *N*, *L6* and *RPP5* are found only in dicot plants. The mechanism of TIR protein activation of the signaling transduction pathway is still unknown; however, the sequence homology was found between TIR in plants and animal innate immunity and apoptosis proteins. The similarity of the TIR domain in these systems points to similar function in activating a signaling transduction pathway (Hammond-Kosack and Jones, 1997). Moreover, other than the potential of TIR to initiate signal transduction, this domain may also play a role in pathogen recognition. As described in the studies of 13 alleles of flax *L6* and *L7*, pathogen recognition was changed by variation in the TIR domain (Ellis et al., 1999; Falk et al., 1999).

The non-TIR and TIR genes have shown differences in their defense response to pathogens. In *Arabidopsis*, TIR sequences operate through an *eds-1* dependent pathway, while some of non-TIR sequences operate through the *ard-1* pathway (Aarts et al., 1998). These data suggest that TIR or CC motifs play a role in the bifurcation of the signal transduction pathway (Aarts et al., 1998).

- R gene evolution

The defense response in plant is activated by specific interaction between R gene in plant and *Avr* gene of pathogen. The domains that are assumed to play a role in specific recognition with *Avr* gene are LRR and TIR/non-TIR domains. Therefore, increased ability of these domains in recognition of *Avr* gene or product of the pathogen facilitated defensive response to several races of the pathogen and/or different pathogens (Zhou et al., 2004).

The diversifying selection, the unequal-crossing over, transposable elements, interlocus recombination, chromosome breaking and chromosome rearrangement were reported as mechanisms for R gene evolution to generate diversity in LRR and TIR/non-TIR domains (Michelmore and Meyers, 1998; Ellis et al., 2000; Richter and Ronald, 2000; Young, 2000). Moreover, pathogen virulence, pathogen and plant populations also involve in evolution of R genes as well (Zhou et al., 2004). The unequal crossing over was proposed to generate the divergent of *T2* gene in *Arabidopsis* (Vision et al., 2000). While, Ellis et al. (1999) showed that high rates of allelism that have been observed in flax *L* locus resulted from recombination within clusters and chromosome rearrangements. Consequently, ten of eleven identified alleles of flax *L* locus can induce defensive response to different races of flax rust (Ellis et al., 1999).

Indeed, LRR domain is assumed to play important role in cell specificity recognition making its sequence highly divergent (Parniske et al., 1997; Meyers et al., 2003; Zhou et al., 2004). Therefore, evolution of R gene by diversifying selection mainly occurs at LRR domain (Mondragon-Palomino et al., 2002). However, TIR/non-TIR motif that also plays a role in cell recognition has been evolutionary as well. Interestingly, the diversifying selections have been found in non-TIR more often than TIR especially in rice genome (Zhou et al., 2004).

In rice, there was no significant increase in number of similar or almost identical genes within non-TIR-NBS-LRR whereas copies of similar TIR-NBS-LRR genes were duplicated (Zhou et al., 2004). It was found that numbers of non-TIR subclasses were larger than TIR subclasses approximately 10 times (480 non-TIR from total 535 NBS-LRR genes in rice; Bai et al., 2002). This high degree of diversifying found among non-TIR genes in rice could not be explained by single duplication, but was mainly caused by an increase in divergent gene group such as diversifying selection and/or expansion of diversity (Zhou et al., 2004). Moreover, phylogenetic studies of NBS-LRR genes from diverse taxa including *Arabidopsis thaliana*, legume species e.g. *Glycine max* and *Medicago truncatula* and other plant families indicated that the non-TIR subfamily has greater sequence diversity than the TIR subfamily (Cannon et al., 2002).

2.2.2 Resistance gene analogs

Resistance gene analogs (RGAs) are groups of genes whose sequences are partially homologous to resistance genes. They may function in resistance against similar or different pathogens, or other function(s) unrelated to resistance. Thus RGAs have led to discovery of resistance genes against diseases caused by fungi, viruses, bacteria and nematodes (Hammond-Kosack and Jones, 2000; Taler et al., 2004). Therefore, RGAs have been intensely studied and have high potential to improve disease resistance levels in several plants.

Most RGAs have been found in the NBS-LRR group (Table 1). The conserved motifs in the NBS domain (i.e. P-loop and GLPL) have sequence properties that facilitate isolation of RGAs from various plants. RGAs have been successfully cloned by a polymerase chain reaction (PCR) based approach with degenerate oligonucleotide primers designed from those domains.

In grape, RGAs were studied by Di Gaspero and Cipriani (2002, 2003) using two disease resistant species, *V. amurensis* and *V. riparia*. The work focused on two classes of R genes, NBS-LRR and Serine/Threonine kinase (STK) genes, which are two of the known classes of R genes in plants and occur in large multigene families. Four degenerate primer pairs and nine degenerate primer pairs that were developed from four conserved motifs in NBS-LRR genes were used to clone grape RGAs in Di Gaspero and Cipriani (2002) and (2003), respectively. The conserved motifs in NBS-LRRs genes that were used to develop degenerate primers included P-loop, kinase-2, kinase-3 and GLPL. In total, 126 unique sequences of RGAs in NBS-LRR group were derived from two grape species (Di Gasporo and Cipriani, 2002, 2003). Those cloned showed significant similarity to known R genes or RGA-like sequences from other plants (Di Gasporo and Cipriani, 2002, 2003). Three RGA subclasses; non-TIR (CC type), non-TIR (LZ type) and TIR, were found at ratio of 1:1:3, respectively based on phylogenetic tree analysis (Di Gaspero and Cipriani, 2003).

Although, the NBS-LRR is a group of R gene that seems to be exclusively devoted to defense response in plants, the STK group of R genes is also implicated in a wide rage of signaling pathway (Meyers et al., 1999). Therefore, five combinations of primers that were developed from conserved motif of STK-like gene such as *Pto*, *Fen*, *Lrk10*, *Xa21* and *FLS2* were also used to clone RGAs from *V. amurensis* and *V. riparia* (Di Gaspero and Cipriani, 2003). Among the seventy three unique sequences derived from two grape species, 53 sequences matched STK group genes of other plant in the GenBank (Di Gaspero and Cipriani, 2003). These data indicated that those five primer combinations had approximately 73% efficiency in selective amplification of STK-like genes. Fifty three grape STK group RGAs were classified into four subclasses including RLCKs, LRR-RLKs, S-RLKs and lec-RLKs. These four subclasses have been reported

to play a role in signaling pathway and/or defense response in plant (Meyers et al., 1999; Pastuglia et al., 2002)

Table 1. List of RGAs that have been studied in several plants.

Plant	Reference
Potato (*Solanum tuberosum*)	Leister et al. (1996)
Arabidopsis (*Arabidopsis thaliana*)	Parker et al. (1997); Meyers et al. (2002); Sinapidou et al. (2004); Noutoshi et al. (2005) ; Faigon-Soverna et al. (2006)
Tomato (*Lycopersicon esculentum)*	Parniske et al. (1997); Hu et al. (2005)
Wheat (*Triticum aestivum*)	Seah et al. (1998)
Barley (*Hordeum vulgare*)	Yu et al. (2000)
Sunflower (*Helianthus annuus)*	Gedli et al. (2001); Radwan et al. (2005)
Chickpea (*Cicer arietinum*)	Huettel et al. (2002)
Medicago truncatula	Zhu et al. (2002)
Grape (*Vitis* spp.)	Donald et al. (2002)
	Di Gaspero and Cipriani (2002), (2003)
Soybean (*Glycine max)*	Penuela et al. (2002); Gao et al. (2005)
Sugar beet (*Beta vulgaris)*	Hunger et al. (2003)
White pine (*Pinus monticola)*	Liu and Ekramoddoullah (2003)
Cotton (*Gossypium hirsutum)*	He et al. (2004)
Rice (*Oryza sativa*)	Zhou et al. (2004); Qu et al. (2006)
Melon (*Cucumis melo)*	Joobeur et al. (2004)
Lens species (*Lens* spp.)	Yaish et al. (2004)
Apple (*Malus sylvestris*)	Baldi et al. (2004)
Peach (*Prunus davidiana*)	Decroocq et al. (2005); Lalli et al. (2005)
Apricot (*Prunus armeniacu)*	Soriano et al. (2005)
Avena species (*Avena* spp.)	Irigoyen et al. (2006)
Sugar pine (*Pinus lambertiana)*	Jemstad et al. (2006)
Wild rice (*Zizania latifolia*)	Chen et al. (2006)
Poplar (*Populus tremula*)	Zhang et al. (2006)

2.2.3 Resistance gene analog polymorphism

Due to the high probability of finding RGAs clustered in plant genome plus their function in disease resistance, the markers developed from RGA sequences may have high possibility of co-localizing with quantitative trait loci (QTL) for disease resistance in plants (Toojinda et al., 2000; Yan et al., 2003). RGA sequence could be converted into at least three markers including restriction fragment length polymorphism (RFLP), cleaved amplified polymorphic sequences (CAPS) and sequence tagged site (STS). Based on RFLP technique, this type of marker is defined by a specific enzyme-probe combination. The genomic DNA will be digested with series of enzymes and then hybridized with sequence from RGA clone. The polymorphisms occur because the sequence of the probe is homologus to restriction fragments of different sizes (Severson et al., 1993; Toroser et al., 1995). For CAPS marker, a short genomic sequence is amplified using specific primers developed from RGA sequence. Restriction enzyme digestion patterns on short genomic DNA fragment will provide polymorphisms among genotypes. This technique is detected easily by agarose gel electrophoresis (Konieczny and Ausubel, 1993). On the other hand, the STS marker is performed by single strand conformation polymorphism (SSCP) technique. This technique is a PCR approach using primers based on conserved RGAs domains, high resolution polyacrylamide gel electrophoresis and sensitive silver staining (Chen et al., 1998). Those types of markers based on RGA sequences are also called resistance gene analog polymorphism **(**RGAP).

The RGAP technique has allowed identification of markers linked to disease resistance genes in several plants such as grape, barley, rice, sunflower, wheat, common bean and *Avena* species (Chen et al., 1998; Toojinda et al., 2000; Dodds et al., 2001; Gedil et al., 2001; Pflieger et al., 2001; Yan et al., 2003; Irigoyen et al., 2006; Miklas et al., 2006).

In grape, the sequences of representative RGAs cloned from *V. amurensis* and *V. riparia* were converted into RFLP and STS markers (Di Gaspero and Cipriani, 2002, 2003). Several RFLP markers were developed including rgVrip064, rgVamu012 and rgVamu026. The rgVrip064 with a pair of restriction enzymes (*Taq*I and *Dde*I) gave a clear polymorphism between two classes (resistant and susceptible to downy mildew) of *Vitis* accessions (Di Gaspero and Cipriani, 2002). The 650 bp in *Tag*I digested DNA and 2000 bp in *Dde*I digested DNA were present in resistant wild species e.g. *V. amurensis* and *V. cinerea* and tolerant hybrid e.g. *V. hybrid* cv. Regent and *V. hybrid* cv. Bianca. While, those bands were absent in susceptible cultivars of *V. vinifera* (cv. Cabernet Sauvignon, Chardonnay and Merlot; Di Gaspero and Cipriani, 2002). Forty five STS markers were developed from the NBS-LRR and STK-like classes of RGAs. Those molecular markers were used to analyze the variation in the nucleotide sequences of RGA-STS in 22 *Vitis* accessions including disease resistant genotypes e.g. *V. amurensis*, *V. berlandieri*, *V. champinii*, *V. cinerea*, *V. labrusca*, *V. riparia*, *V. rupestris*, *V. shuttleworthii*, *V. yeshanensis* and *V. rotundifolia*, tolerant genotypes e.g. *V. hybrid* cv. 14/1, *V. hybrid* cv. Roesler, *V. hybrid* cv. Regent, *V. hybrid* cv. Seyval and *V. hybrid* cv. Bianca and susceptible genotypes e.g. *V. armata*, *V. girdiana*, *V. vinifera* cv. Sultanina, *V. vinifera* cv. Sauvignon, *V. vinifera* cv. Chardonnay and *V. vinifera* cv. Merlot (Di Gaspero and Cipriani, 2003). The SSCP analysis showed polymorphisms between resistant and susceptible genotypes for both NBS-LRR and STK classes. These molecular markers are now being investigated for potential link or co-segregation with disease resistant alleles such as those of powdery mildew and downy mildew in grape by Di Gaspero and his co-worker (Di Gaspero, personal communication).

RGA sequences have also been isolated from *V. rotundifolia.* The two CAPS markers, GLP1-12 and MHD145, were converted from RGA clone of *V. rotundifolia*.

These markers were observed to co-segregate with a powdery mildew resistance gene, resistance to *Uncinula necator*1 (*Run1*) which is a single dominant gene controlling powdery mildew resistance in *V. rotundifolia* (Donald et al., 2002; Baker et al., 2005).

In apple, RGAs were cloned by degenerate oligonucleotide primers based on conserved motifs including P-loop and GLPL or kinase-2 in the NBS domain. Those RGAs were cloned and converted into CAPS and SSCP markers. Markers were tested for segregation in a cross between two apple cultivars 'Fiesta x Discovery' and were found to be present in 12 out of 17 linkage groups. Some of RGA markers were located proximal to known loci containing genes or QTL for disease resistance. The marker ARGH34 was located on linkage group 1 very close to *Vf* which confers resistance to *Venturia inaequalis*. The marker ARGH20 was mapped to linkage group 11 which is also the location of QTL for fruit scab resistance (Seglias and Gessler, 1997; Liebhard et al., 2003). In addition, linkage group 2 which is believed to carry *Vr2* that confers resistance to apple scab (*V. inaequalis)* also contains two RGA markers, ARGH17 and ARGH3, along with a simple sequence repeat (SSR) marker already determined to co-segregate with *Vr2* gene (Baldi et al., 2004). Moreover, R genes for apple scab have been mapped on linkage group 2 including *Vbj*, *Vh8* and *Vr*/*Vh2* (Gygax et al., 2004; Bus et al., 2004, 2005).

Naik et al. (2006) reported an apple genetic map composed of 46 apple expressed sequence tagged site (E-STS), eight RGAs, 85 SSRs from apple and peach and 88 random amplified polymorphic DNA (RAPD) in an 'Antonovka debnicka (Q12-4) x Summerred' population. Interestingly, one and two RGAs have been found located on linkage groups 2 and 11, respectively. The presence of different RGAs in the same location (linkage groups 2 and 11) in different mapping population ('Fiesta x Discovery' and 'Antonovka debnicka (Q12-4) x Summerred') suggests that RGAs have high

potential to be linked or closely associated with disease resistance in apple (Baldi et al., 2004; Naik, 2006).

In wheat, the 16 RGA markers were used to determine co-segregation with *Yr5* and *Yr9*. These R genes confer resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat. The *Yr5* gene has been located on chromosome 2B while *Yr9* is located on chromosome 1B. The 16 RGA markers have been mapped onto the same populations which are BC_7:F_2 and BC_7:F_3 progeny between resistant *Triticum spelta* albuin with recurrent susceptible parent, Avocet. Two RGA markers include Xwgp-17 and Xwgp-18 co-segregate completely with *Yr5* on chromosome 2B (Yan et al., 2003). Xwgp-8 was mapped to chromosome 1B on which *Yr9* is also located, in addition 12 RGA markers were found closely related to *Yr9* gene (Shi et al., 2001). Interestingly, some of RGA markers have similar DNA sequences to other R genes in related plant species. For example, RGA marker, Xwap11, which is located on chromosome 1B about 18.6 cM away from *Yr9*, has similar sequence to acetyl-coenzyme A carboxylase gene in *Triticum aestivum*. Also, the rice gene for resistance to bacterial blight caused by *Xanthomonas campestris* pv. oryzae, *Xa1* and *Xa21*, have homologous sequences with Xwgp-8 (Shi et al., 2001).

Similarly, 44 RGA clones were derived from sugarcane and developed into 31 RGA markers. The 31 and 17 RGA markers were mapped in sugarcane and sorghum, respectively. Three RGA markers from sugarcane including RGA-Q8, RGA-Q18 and RGA-Q50 were shown to co-segregate with brown rust resistance located on linkage group G. Those three RGA markers also mapped to the same linkage group that had been previously reported as having a QTL for disease resistance in sorghum (Mclntyre et al., 2005). These data support the capacity of RGA markers to be linked with disease resistance and the advantage of applying RGA markers across related plants species.

2.3 *Vitis* map

V. vinifera is by far the most widely grown grape in the world but it is highly susceptible to several diseases. Therefore, resistant varieties have been developed from grape breeding programs by combining the desirable resistance genes from American and Asian species with superior fruit quality from *V. vinifera*. However, long generation times, plant size, inbreeding depression and clone heterozygosity are limitations of grape breeding. Therefore, creating grape linkage map could provide a valuable tool for breeding program employing marker assisted selection (MAS). There are several types of molecular markers that were used to create grape linkage map, particularly RAPD, SSR and amplification fragment length polymorphism (AFLP; Millam and Spoor et al., 1999; Dalbo et al., 2000; Doligez et al. 2002; Subudhi and Nguyen, 2004).

RAPD is a type of molecular marker that is based on the differential PCR amplification of DNA sample using short oligonucleotide primers (Williams et al., 1990). This technique uses a single oligonucleotide (approximately 10 nucleotide long) to amplify DNA of random location in the plant genome. The number of amplified products is directly related to the number and orientation of sequences that are complementary to the primer in the genome (Thormann et al., 1994). RAPD was mainly used in first grape linkage map created by Dalbo et al. (2000). This molecular marker has great advantages in terms of cost and time for development (Williams et al., 1990).

The SSR technique amplifies 1-6 bp unit of repeat polymorphisms or microsatellites in the plant genome. SSRs are abundant in plant genome. Therefore, the SSR markers are useful in description of variation between populations and individuals (Millam and Spoor, 1999). These markers exhibit co-dominant inheritance and are multi-allelic, both features making them suitable for use in heterozygous species

(Millam and Spoor, 1999). SSR has been very useful in the amplification of the microsatellites in the *Vitis* genome using primers complementary with the highly conserved sequences at flanking regions (Sefc et al., 1999; Di Gaspero et al., 2000; Rossetto et al., 2002). The identification of grape varieties based on SSR polymorphisms is useful for management of grape collections and control of the trade of plant materials (Grando and Frisinghelli, 1998; Lamboy and Alpha, 1998; Franks et al., 2002). Today, grape linkage maps from several labs containing high number of SSR markers facilitate the integration of these maps to generate a common reference map for grapevine (Grando and Frisinghelli, 1998; Di Gaspero et al., 2000).

AFLP uses PCR combined with RFLP. This method operates by digesting the various genomic DNA with restriction enzymes. Then two short adapter sequences are ligated to the ends of digested fragments. The primers for PCR are obtained from the sequences of adapters. Thus the amplified fragments are usually from throughout the plant genome (Zabeau and Vos, 1993). This marker is a dominant marker which prevents the accurate detection of heterozygotes (Westman and Kresovich, 1997; Subudhi and Nguyen, 2004). However, the AFLP method gives the high level of polymorphisms and is consistent in different genetic background of parents and population. Grando et al. (2003) reported that average distance among AFLPs in linkage map is 10 cM. This indicated the potential of AFLP markers to be distributed throughout the grape genome and their usefulness in detecting QTL.

The first grape linkage map was constructed using 227 RAPD, 25 SSR, 12 AFLP and 4 CAPS markers on 'Horizon x Illinois 547-1 (Ill. 547-1)' population (Dalbo et al., 2000). The second grape linkage map was created by Doligez et al. (2002) with an objective to detect QTL for seedlessness by using mapping population from a cross between two partially seedless genotypes, MTP2223-27 (Dattier de Beyrouth x 75

Piroang) and MTP2121-30 (Alphonse Lavallee x Sultanine). The molecular markers presented on the consensus map included 250 AFLP, 44 SSR, three isozymes, two RAPD, one sequence-characterized amplified region (SCAR) and one phenotypic marker (seedlessness). A third grape linkage map using a cross between *V. vinifera* cv. Moscato Bianca and *V. riparia* accession Wr 63 utilized 21 SSR and 19 AFLP (Grando et al. 2003). Adam-Blondon et al. (2004) reported the fourth grape linkage map constructed from a total of 340 SSR markers which were successfully amplified in a full sib population from a cross between *V. vinifera* cv. Syrah and *V. vinifera* cv. Grenache. The fifth grape linkage map was created by using the progeny of cross between *V. vinifera* cv. Riesling and *V. vinifera* cv. Cabernet Sauvignon. This consensus map contained 152 SSRs and one Expressed Sequence Tag (EST) marker (Riaz et al., 2004).

Recently, only few molecular markers linking to QTL for powdery mildew resistance in grape were published (Dalbo et al., 2000; Dalbo et al., 2001). One RAPD marker, CS25b (LOD score = 6.56, R^2 = 0.41), and one AFLP marker, AfAA6 (LOD score = 6.53, R^2 = 0.38), showed the highest association with powdery mildew resistance in 'Horizon x Ill. 547-1' population (Dalbo et al., 2000). Moreover, CAPS markers, $CCS25_{997}$/*Rsa*I and STS-AA61/*Hae* III, were developed from those markers and tested for segregation in four populations including 'Horizon x Ill. 547-1', NY 88.0517.03 (Horizon x Ill. 547-1) x Traminette (Joannes-Seyve 23-416 x Gewurztraminer), NY 88.0517.03 x NY 73.0136.17 (NY33277 x Chancellor) and J.S. 23-416 x Ill. 547-1 were found closely associated with resistance in all crosses. Therefore, MAS using these markers was found to be highly efficient especially in crosses with high segregation distortion ratios (Dalbo et al., 2001). Pauquet et al. (2001) also reported other AFLP markers that were found to co-segregate with powdery mildew resistance. Specifically, three markers, EMhb1, EMbd4 and EMfd3, were believed to associate with *Run1* gene.

The presence of these markers in the resistant genotypes and absence in susceptible genotypes of the BC_4 and BC_5 populations between *V. vinifera* and *V. rotundifolia* indicated that these markers are good candidates for use in MAS.

However, the QTL for other major diseases in grape such as downy mildew have not been published yet. Therefore, Doligez et al. (2006) created the integrated genetic map by using five previously published populations to place as many transferable markers as possible on one single map. The results from this integrated map will increase potential for the development of molecular markers that co-segregate with disease resistance alleles in grape. The five populations included 96 full-sib progeny from two reciprocal crosses between *V. vinifera* cv. Syrah and Grenache from INRA Montpellier, France (Adam-Blondon et al., 2004), 114 self-pollinated progeny of *V. vinifera* cv. Riesling from INRA Colmar, France (Adam-Blondon et al., 2004), 46 full-sib progeny from a cross between *V. vinifera* cv. Chardonnay and Bianca from University of Udine, Italy (Di Gaspero et al., 2005), 139 full-sib progeny from a cross between two table grape genotypes, MTP2223-27 (Dattier de Beyrouth x 75 Pirovano) and MTP2121-30 (Alphonse Lavallee x Sultanine) from INRA Montpellier, France (Bouquet and Danglot, 1996), and 153 full-sib progeny of a cross between *V. vinifera* cv. Riesling and Cabernet Sauvignon from University of California, Davis, CA, USA (Riazal., 2004). A total of 502 SSR and 13 PCR-based markers were mapped onto 19 linkage groups (2n = 38) as shown in Figure 6. The total length of the integrated map was 1,647 cM with an average of 3.3 cM distance between neighboring loci. The consensus map contained approximately 50.1% of SSR markers which shared similarity in at least two populations. The similar SSR positions in different maps will provide information on stability of QTL in different genetic backgrounds and environments.

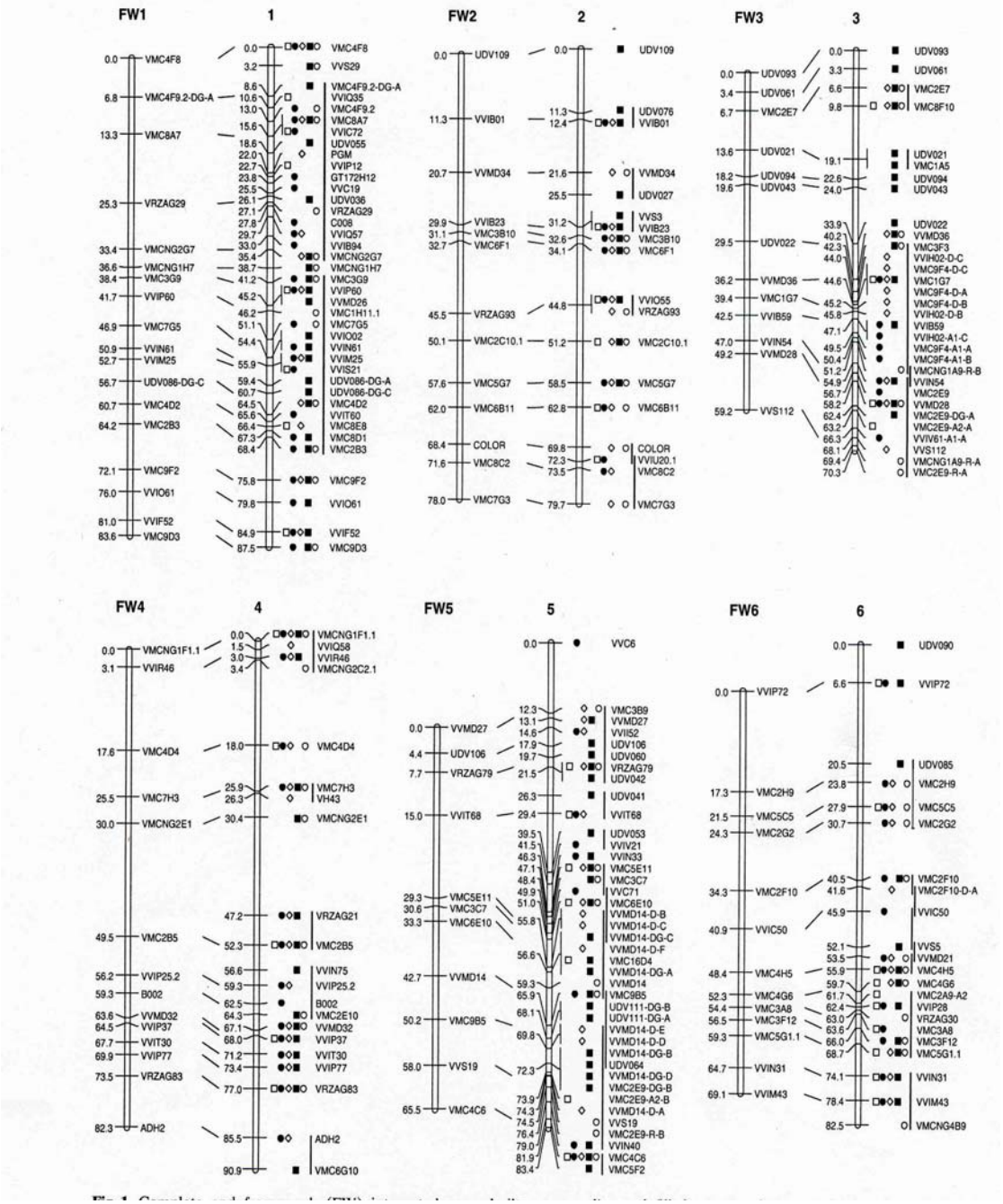


Figure 6. Complete and framework integrated maps built from five different grapevine populations (Doligez et al., 2006).

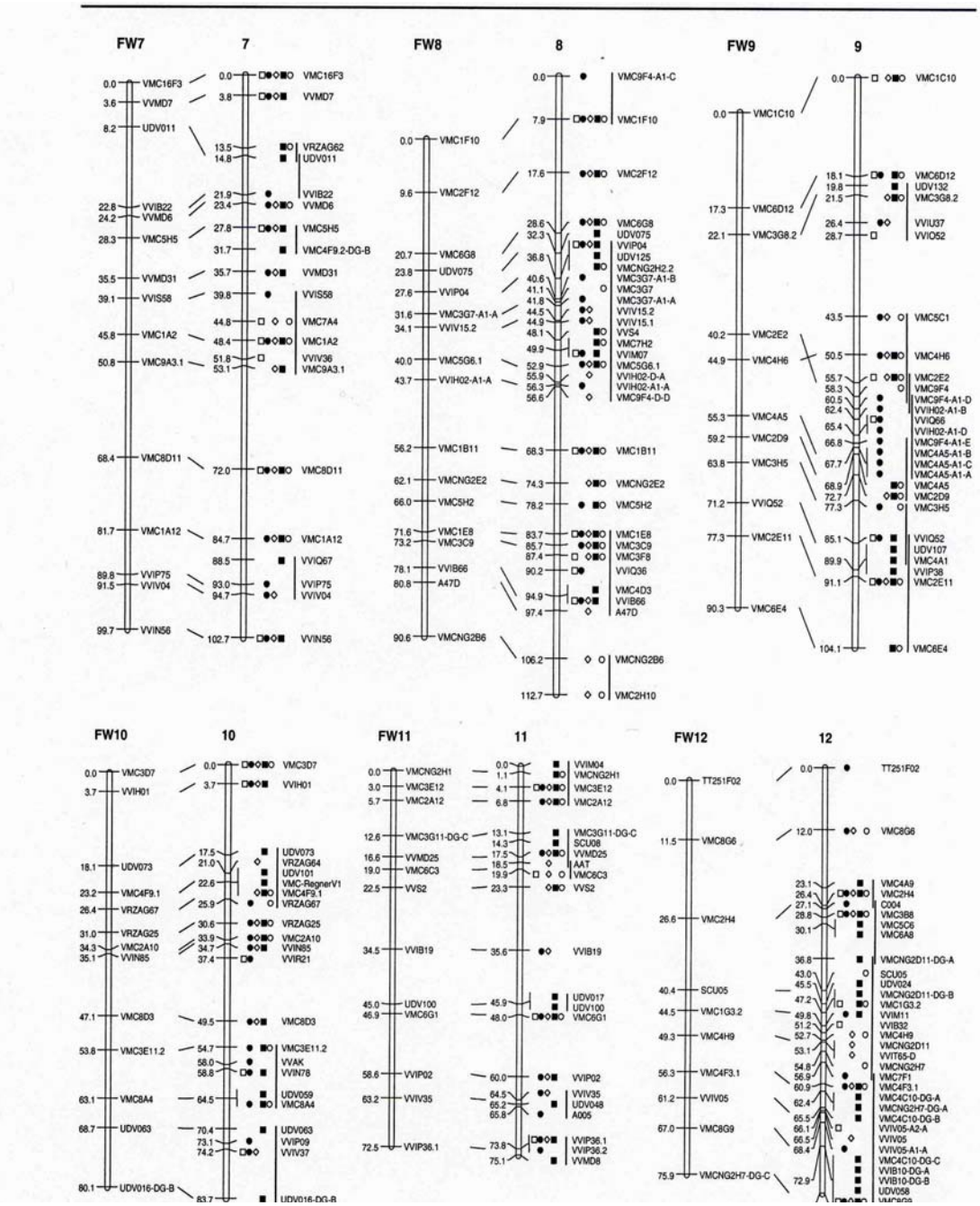


Figure 6. Complete and framework integrated maps built from five different grapevine populations (continued; Doligez et al., 2006).

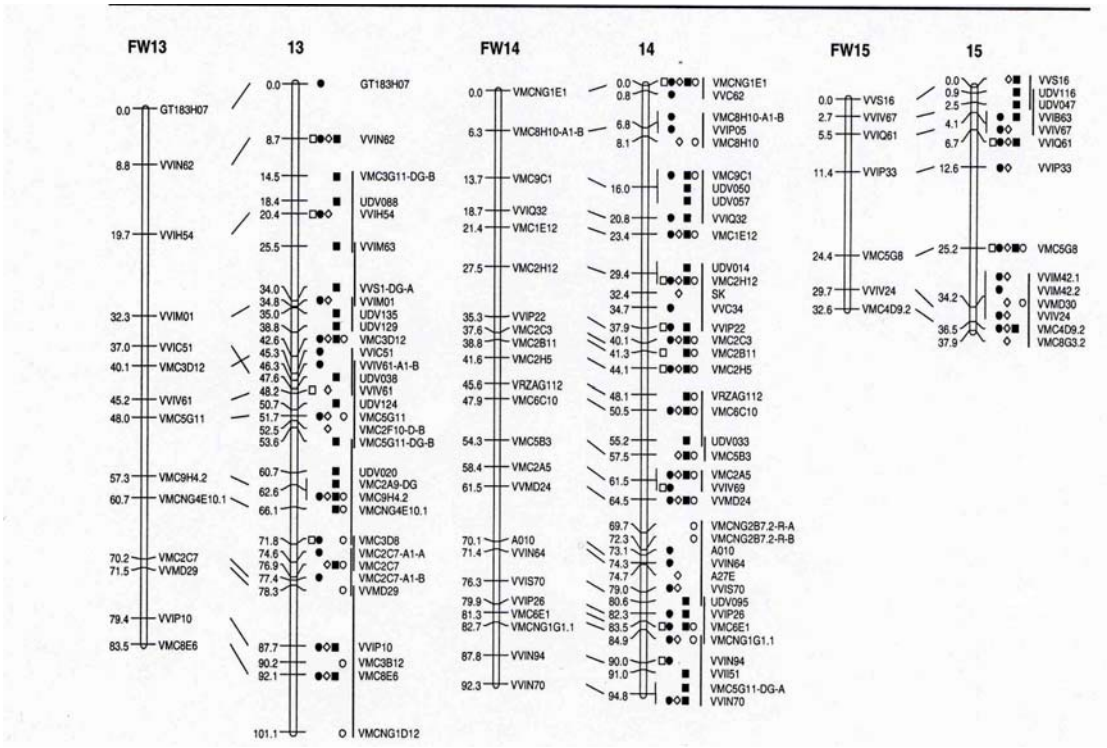


Figure 6. Complete and framework integrated maps built from five different grapevine populations (continued; Doligez et al., 2006).

2.4 Grape disease resistance and general and specific combining ability

2.4.1 Grape disease resistance

Presently, the *Vitis* species with the most significant production in the world is *V. vinifera* due to its high quality and ability to adjust to a wide range of climate conditions. However, *V. vinifera* is highly susceptible to several diseases including downy mildew. Differences in resistance levels are found among varieties and possibly even among clones (Föex, 1981; Reynier, 1989).

Many American and Asian *Vitis* species were reported as resistance to important grape diseases such as downy mildew, powdery mildew and anthracnose (Table 2; Boubals, 1959; Langcake and Lovell, 1980; He and Wang, 1986; Eibach et al., 1989; Alleweldt et al., 1990).

Table 2. Sources of resistance or tolerance to important grape diseases (modified from Reisch and Pratt, 1996).

Disease	Source of resistance or tolerance
Downy mildew (*P. viticola*)	*V. riparia, V. rupestris, V. lincecumii, V. labrusca, V. amurensis, V. rotundifolia, V. yenshanensis, V. pseudoreticulata, V. piasezkii, V. romanetii, V. flexuosa, V. bryoniifolia*
Powdery mildew (*Oidium necator*)	*V. aestivalis, V. cinerea, V. riparia, V. berlandieri, V. labrusca, V. rotundifolia*
Anthracnose (*Elsinoe ampelina*)	*V. simpsoni, V. smalliana, V. shuttleworthii, V. labrusca, V. rotundifolia, V. munsoniana*

Most grape breeding programs frequently aim at developing new disease resistant varieties by combining the desirable resistant characteristic from American species with the high fruit quality of *V. vinifera*. The "French-American hybrids" were first introduced in 1860 (Reisch and Pratt, 1996). *V. labrusca* and other species native to the host range of phylloxera were hybridized with Vinifera grape to produce resistant rootstocks to phylloxera. Apparently, some of the hybrids had both phylloxera resistance and good quality attributes and could be used for commercial production. Later, Boubals (1998) obtained hybrid table grapes of *V. aestivalis* or *V. rupestris* and *V. vinifera* with variable resistance levels to downy mildew. However, the candidate hybrids which could be grown without application of fungicides still needed fruit quality improvement. Complex hybrids between European and American species were also effective in wine grape breeding programs. The cultivars of "French-American hybrids" that were used for commercial wine production include Marechal Foch, Vidal Blanc,

Chambourcin and Seyval (Barrett, 1956). The hybrids from second backcrosses of *V. vinifera* x *V. rotundifolia* had tolerance to some important diseases and insect pests of *V. vinifera* and provided high fruit quality. Thus, the quality of wine from this program was acceptable to several standards of wine grapes (Olmo, 1971). *V. riparia* has also proven itself to be a good source of gene(s) for disease resistance. Combination of *V. riparia* with *V. vinifera* resulted in vines that were resistant and also capable of making quality wines (Hemstad and Luby, 1998). Other American species such as *V. rupestris*, *V. cinerea* and *V. rubra* have been incorporated in both table and wine grape breeding programs to improve downy mildew resistance.

The grape breeding programs at New York State Agricultural Experiment Station (NYSAES), Cornell University have been making annual crosses of American species with European grape (Reisch, 2003; personal communication). Three cross categories of these breeding programs are 1) highly disease resistant (e.g. Ill. 547-1, *V. labrusca*, *V. rupestris*, *V. cinerea* and *V. aestivalis*) x *V. vinifera*; 2) highly disease resistant x high quality interspecific hybrids (e.g. NY 65.0550.04, NY 88.0517.01 and NY 88.0517.03); 3) highly disease resistant x highly disease resistant (different sources). The hybrids were planted in no-spray vineyard. Disease evaluation and other characteristics such as trunk injury, vine vigor and crop load were observed yearly (Reisch, 2003). Similarly, Peterlunger et al. (2003) made crosses between European grape and American or Asian species with the aim to introgress disease resistance gene(s) into European grape. About 28 crosses were made resulting in a few to 136 progenies per cross. These hybrid progenies are being investigated for potential to develop new resistant hybrid.

2.4.2 General and specific combining ability

The mating designs are classified into diallel cross, North Carolina (NC) designs I, II and III, line x tester and partial diallel (Comstock and Robinson, 1952; Griffing,

1956). The mating design is a system to produce large number of progenies that allow estimation on mean performance (Falconer and Mackay, 1960). The values being expressed as deviation from the overall means performance of crosses are described by general combining ability (gca) effects. While the expected values of any two lines in combination are described by specific combining ability (sca) effects (Falconer and Mackay, 1960).

The variance of gca ($\sigma^2_{GCA}(M) = ½ FV_A + ¼ F^2V_{AA} + \ldots$; $\sigma^2_{GCA}(F) = ½ FV_A + ¼ F^2V_{AA} + \ldots$) provides diagnosis of a predominant role for additive or non-allelic interaction effects of genes. The additive gene action is a form of an allelic interaction in which dominance is absent, resulting in a heterozygote that is intermediate in phenotypes between homozygotes for the alternative alleles. Differences in gca are based on additive variance and A x A interactions in population. On the other hand, differences of sca variance ($\sigma^2_{SCA} = ½ F^2V_{AA} + F^2V_D + F^3V_{AD} + F^4V_{DD} \ldots$.) is attributable to the non-additive genetic variance (Griffing, 1956; Falconer and Mackay, 1960). Non-additive gene action occurs when the heterozygous genotype is not intermediate in phenotypic values to the homozygous genotypes. The additive or non-additive gene action will help locate the parents and crosses that are responsible in bringing about a particular type of gene action and the best strategy for a plant breeding program (Dabholkar, 1992).

In grape, the diallele analysis was used to evaluate gca and sca effects of downy mildew resistance gene(s) from 25 cross combinations among resistant and susceptible selections from germplasm of different genetic compositions of *V. labrusca* (6% to 88%), *V. vinifera* (12% to 88%) and *V. rupestris* or *V. riparia* (0% to 42%; Brown et al., 1999). The gca estimates as well as sca estimate were highly significant. However, gca estimates were approximately 7.7 – 17.5 times higher than sca estimate. This indicated

that additive gene action was considered to be the most important genetic influence on resistance to downy mildew (Brown et al., 1999). Moreover, seedlings derived from crosses between two susceptible parents (e.g. A-1046 x A-1702) showed higher rates of downy mildew when compared with seedlings from resistant x resistant and resistant x susceptible crosses (Brown et al., 1999). The data suggested that downy mildew resistance gene(s) could be introgressed from resistant genotypes into European grape to enhance downy mildew resistance.

Wu and Metheson (2004) performed a series of 20 sets of 6 x 6 half diallel mating experiments to estimate tree diameter of radiata pine (*P. radiata*). The four combinations which had significant gca effect showed 52.9% additional deployment gain. In addition, ten combinations that had significant gca as well as sca effects had an additional deployment gain of 46.0%.

In elite maize (*Z. mays*) the R genes controlling resistance against *Phaeosphaeria maydis* and southern corn rust (*Puccina polysora*) were studied. Complete diallel crosses of 10 elite maize lines identified three lines with the greatest potential for hybrid synthesis based on higher gca for yield and moderate resistance to *P. maydis* (Paterniani et al., 2000). Navabi et al. (2003) applied the one-way diallel cross for studies of leaf rust (*P. triticina*) resistance in wheat (*T. aestivum*). The studies showed that additive gene action was a major component of variation due to the significance of gca. The estimate of narrow-sense heritability of adult-plant resistance to leaf rust ranged from 0.67 to 0.97. Consequently, the resistance gene(s) from resistant genotypes could be easily transferred into their progenies. Moreover, it is highly likely that the breeding program would be successful as indicated by high percentage of heritability (67 - 97%). Owolade (2006) performed complete diallel mating among nine resistant cassava (*Manihot esculenta*) and nine susceptible genotypes. The results suggested that additive

and non-additive gene effects were responsible for cassava anthracnose (*Collectotrichum gloeosporiodes*) resistance. Similarly, Owolade et al. (2006) reported that additive, non-additive and possibly epistatic gene actions were important for gene(s) controlling cassava anthracnose resistance. The line x tester analysis of 13 cassava genotypes suggested that the most resistant variety, I63397, had the largest significant negative gca effects for resistance among the lines. The cross of 'I63397 x TME-8' which had significantly high negative sca effects was recommended for developing a resistant hybrid.

2.5 References

Aarts, M.G.M., te Lintel Hekkert, B., Holub, E.B., Beynon, J.L., Stiekema, W.J. and Pereira, A. (1998). Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. **Mol. Plant Microbe. Interact.** 11:251-258.

Abramovitch, R.B., Anderson, J.C. and Martin, G.B. (2006). Bacterial elicitation and evasion of plant innate immunity. **Nat. Rev. Mol. Cell. Biol.** 7:601-611.

Adam-Blondon, A.F., Roux, C., Claux, D., Butterlin, G., Merdinoglu, D. and This, P. (2004). Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. **Theor. Appl. Genet.** 109:1017-1027.

Aldon, D., Brito, B., Boucher, C. and Genin, S. (2000). A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. **EMBO. J.** 19:2304-2314.

Alleweldt, G., Spiegel-Roy, P. and Reisch, B. (1990). Grape (*Vitis*). Quoted in J.N. Moore and J.R. Ballington. (1996). Genetic resources of temperate fruit and nut crops. **Acta. Hort.** 290:291-337.

ATTR-National Sustainable Agriculture Information Service. (2007). Grape: organic production [On-line]. Available http://www.attra.ncat.org

Bai, J., Pennill, L. A., Ning, J., Lee, S.W., Rama lingam, J., Webb, C.R., Zhuo, B., Sun, Q., Nelson, J.C., Leach, J.E. and Hulbert, S. H. (2002). Diversity in nucleotide binding site-leucine rich repeat genes in cereals. **Genome Res.** 12:1871-1874.

Baker, C.L., Donald, T.M., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., Thomas, M.R. and Dry, I.B. (2005). Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. **Theor. Appl. Genet.** 111:370-377.

Baldi, P., Patocchi, A., Zini, E., Toller, C., Velasco, R. and Komjanc, M. (2004). Cloning and linkage mapping of resistance gene homologues in apple. **Theor. Appl. Genet.** 109:231-239.

Banerjee, D., Zhang, X.C. and Bent, A.F. (2001). The leucine-rich repeat domain can determine effective interaction between *RPS2* and other host factors in *Arabidopsis RPS2*-mediated disease resistance. **Genetics** 158:439-450.

Barrett, H.C. (1956). The French hybrid grapes. **Natl. Hort. Mag.** 35:132-144.

Becker, H. (1988). Boerner: the first rootstock immune to all phylloxera biotypes. In **Proc. 2nd Int. Cool Climate Viticulture and Oenology Symposium** (pp 51-52). Auckland, New Zealand.

Becker, H. and Soop, E. (1990). Rootstocks with immunity to phylloxera and nematode resistance. In **Proc. 5th Int. Sym. on Grape Breeding** (pp 294). Pfalz, Germany.

Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J. and Staskawicz, B.J. (1994). *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. **Science** 265:1856-1860.

Bittner-Eddy, P.D., Crute, I.R., Holub, E.B. and Beynon, J.L. (2000). *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. **Plant J.** 21:177-188.

Bloodworth, P.J., Nesbitt, W.B. and Barker, K.R. (1980). Resistance to root knot nematodes in *Euvitis* x *Muscadinia* hybrids. In **Proc. 3rd Int. Sym. Grape Breeding** (pp 275-292). University of Califonia, USA.

Botella, M. A., Parker, J.E., Frost, L.N., Bittner-Eddy, P.D., Beynon, J.L., Daniels, M.J., Holub, E.B. and Jones, J.D.G. (1998). Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. **Plant Cell** 10:1847-1860.

Boubals, D. (1959). Contribution a l'etude des causes de la resistance des Vitacees au Mildiou de la Vigne et de leur mode de transmission rereditaire. **Ann. Amelior. Plantes** 9:5-233. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Boubals, D. (1998). Grapevine genetics and breeding facing the challenges of the 3rd millennium. In **Proc. of the 7th Int. Sym. on Grapevine Genetic and Breedin** (pp 25-27). Montpellier, France.

Bouquet, A. (1981). Resistance to grape fanleaf virus in *Muscadinia* grape inoculated with *Xiphinema index*. **Plant Dis.** 65:791-793.

Bouquet, A. and Danglot, Y. (1996). Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). **Vitis** 35:17-25.

Brown, M.V., Moore, J.N., McNew, R.W. and Fenn, P. (1999). Inheritance of downy mildew resistance in table grapes. **J. Amer. Soc. Hort. Sci.** 124:262-267.

Bus, V., Van de Weg, W.E., Durel, C.E., Gessler, C., Calenge, F. and Parisi, L. (2004). Delineation of scab resistance gene cluster on linkage group 2 of apple. **Acta. Hort.** 663:57-62.

Bus, V., Rikkerink, E.H.A., Van de Weg, W.E., Rusholme, R.L. and Gardiner, S.E. (2005). The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. **Mol. Breed.** 15:103-116.

California Rare Fruit Growers. (1997). Muscadine grape [On-line]. Available http:// www.crfg.org/pubs/ff/muscadinegrape.html

Cannon, S.B., Zhu, H., Baumgarten, A.M., Spangler, R., May, G., Cook, D.R. and Young, N.D. (2002). Diversity, distribution, and ancient taxonomic relationships within the TIR and non-TIR NBS-LRR resistance gene subfamilies. **Jour. Molec. Evol.** 54:548-562.

Century, K.S., Shapiro, A.D., Repetti, P.P., Dahlbeck, D., Holub, E. and Staskawicz, B.J. (1997). NDR1, a pathogen-induced component required for *Arabidopsis* disease resistance. **Science** 278:1963-1965.

Chen, M., SanMiguel, P. and Bennetzen, J.L. (1998). Sequence organization and conservation in *sh2/a1*-homologous regions of sorghum and rice. **Genetics** 148:435- 443.

Chen, Y., Long, L., Lin, X., Guo, W. and Liu, B. (2006). Isolation and characterization of a set of disease resistance gene analogs (RGAs) from wild rice, *Zizania latifolia* Griseb. I. Introgression, copy number lability, sequence change, and DNA methylation alteration in several rice-Zizania introgression lines. **Genome** 49:150-158.

Comstock, R.E. and Robinson, H.F. (1952). Estimation of average dominance of genes. In G.W. Gowen (ed.). **Heterosis**. Iowa: Iowa State College.

Crute, I.R. and Pink, D.A.C. (1996). The genetics and utilization of pathogen resistance in plants. **Plant Cell** 8:1747-1755.

Dabholkar, A.R. (1992). **Elements of Biometrical Genetics.** New Delhi: Ashok Kumar Mittal Concept.

Dalbo, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M. and Reisch, B.I. (2000). A gene controlling sex in grapevines placed on a molecular marker-based genetic map. **Genome** 43:333-340.

Dalbo, M.A., Ye, G.N., Weeden, N.F., Wilcox, W.F. and Reisch, B.I. (2001). Marker-assisted selection for powdery mildew resistance in grapes. **J. Amer. Soc. Hort. Sci.** 126:83-89.

Dangl, J.L. and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. **Nature** 411:826-833.

Decroocq, V., Foulongne, M., Lambert, P., Le Gall, O., Mantin, C., Pascal, T., Schurdi-Levraud, V. and Kervella, J. (2005). Analogues of virus resistance genes map to QTLs for resistance to sharka disease in *Prunus davidiana*. **Mol. Gen. Genomics** 272:680-689.

Di Gaspero, G., Peterlunger, E., Testolin, R., Edwards, K.J. and Cipriani, G. (2000). Conservation of microsatellite loci within the genus *Vitis*. **Theor. Appl. Genet.** 101:301-308.

Di Gaspero, G. and Cipriani, G. (2002). Resistance gene analogs are candidate markers for disease-resistance genes in grape (*Vitis* spp.). **Theor. Appl. Genet.** 106:163-172.

Di Gaspero, G. and Cipriani, G. (2003). Nucleotide binding site/leucine-rich repeats, *Pto*-like and receptor-like kinases related to disease resistance in grapevine. **Mol. Genet. Genomics** 269:612-623.

Di Gaspero, G., Cipriani, G., Marrazzo, M.T., Andreetta, D., Prado Castro, M.J., Peterlunger, E. and Testolin, R. (2005). Isolation of (AC)*n* microsatellites in *Vitis vinifera* L. and analysis of genetic background in grapevines under marker assisted selection. **Mol. Breed.** 15:11-20.

Dixon, M.S., Jones, D.A., Keddie, J.S., Thomas, C.M., Harrison, K. and Jones, J.D.G. (1996). The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. **Cell** 84:451-459.

Dixon, M.S., Hatzixanthis, K., Jones, D.A., Harrison, K. and Jones, J.D.G. (1998). The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. **Plant Cell** 10:1915-1925.

Dodds, P.N., Lawrence, G.J. and Ellis, J.G. (2001). Six amino acid changes confined to the LRR beta-strand/beta-turn motif determine the difference between the *P* and *Ps* rust resistance specificities in flax. **Plant Cell** 13:163-178.

Doligez, A., Bouquet, A., Danglot, Y., Lahogue, F., Riaz, S., Meredith, C.P., Edwards, K.J. and This, P. (2002). Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. **Theor. Appl. Genet.** 105:780-795.

Doligez, A., Adam-Blondon, A.F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglud, D., Meridith, C.P., Riaz, S., Roux, C. and This, P. (2006). An integrated SSR map of grapevine based on five mapping populations. **Theor. Appl. Genet.** 113:369-382.

Donald, T.M., Pellerone, F., Adam-Blondon, A.F., Bouquet, A., Thomas, M.R. and Dry, I.B. (2002). Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. **Theor. Appl. Genet.** 104:610-618.

Eibach, R., Diehl, H. and Alleweldt, G. (1989). Untersuchungen zur vererbung von resistenzeigenschaften bei reben gegen *Oidium tuckeri*, *Plasmopara viticola* und *Botrytis cinerea*. **Vitis** 28:209-228. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Ellis, J.G., Lawrence, G.J., Luck, J.E. and Dodds, P.N. (1999). Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. **Plant Cell** 11:495-506.

Ellis, J., Dodds, P. and Pryor, T. (2000). Structure, function and evolution of plant disease resistance genes. **Curr. Opin. Plant Biol.** 3:278-284.

Emsley, P., Charles, I.G., Fairweather, N.F. and Isaacs, N.W. (1996). Structure of *Bordetella pertussis* virulence factor P.69 pertactin. **Nature** 381:90-92.

Faigon-Soverna, A., Harmon, F.G., Storani, L., Karayekov, E., Staneloni, R.J., Gassmann, W., Mas, P., Casal, J.J., Kay, S.A. and Yanovsky, M.J. (2006). A constitutive shade-avoidance mutant implicates TIR-NBS-LRR proteins in *Arabidopsis* photomorphogenic development. **Plant Cell** 18:2919-2928.

Falconer, D.S. and Mackay, T.F.C. (1960). **Introduction to Quantitative Genetics.** Harlow: Longman.

Falk, A., Feys, B.J., Frost, L.N., Jones, J.D.G., Daniels, M.J. and Parker, J.E. (1999). *EDS1*, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. **Proc. Natl. Acad. Sci. USA** 96:3292-3297.

Flor, H.H. (1971). Current status of the gene-for-gene concept. **Annu. Rev. Phytopathol.** 9:275-296.

Föex, G. (1981). **Cours Complet de Viticulture**. Paris. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Franks, T., Botta, R. and Thomas, M.R. (2002). Chimerism in grapevines: implication for cultivar identity, ancestry and genetic improvement. **Theor. Appl. Genet.** 104:192-199.

Gabriel, D.W. and Rolfe, B.G. (1990). Working models of specific recognition in plant-microbe interactions. **Annu. Rev. Phytopathol.** 28:365-391.

Galan, J.E. and Collmer, A. (1999). Type III secretion machines: bacterial devices for protein delivery into host cells. **Science** 284:1322-1328.

Gao, H., Narayanan, N.N., Ellison, L. and Bhattacharyya, M.K. (2005). Two classes of highly similar coiled coli-nucleotide binding-leucine rich repeat genes isolated from the *Rps1-k* locus encode *phytopthora* resistance in soybean. **Mol. Plant-Microbe Interact.** 18:1035-1045.

Gedil, M.A., Slabaugh, M.B., Berry, S., Johnson, R., Michelmore, R., Miller, J., Gulya, T. and Knapp, S.J. (2001). Candidate disease resistance genes in sunflower cloned using conserved nucleotide-binding site motifs: genetic mapping and linkage to the downy mildew resistance gene *PI*1. **Genome** 44:205-212.

Gerrath, J.M. and Posluszny, U. (1988). Morphological and anatomical development in the Vitaceae, II: floral development in *Vitis riparia*. **Can. J. Bot.** 66:1334-1351.

Grando, M.S. and Frisinghelli, C. (1998). Grape microsattelite markers: sizing of DNA alleles and genotype analysis of some grapevine cultivars. **Vitis** 37:79-82.

Grando, M.S., Bellin, D., Edwards, K.J., Pozzi, C., Stefanini, M. and Velasco, R. (2003). Molecular linkage map of *Vitis vinifera* L. and *Vitis riparia* Mchx. **Theor. Appl. Genet.** 106:1213-1224.

Grant, M.R., Godiard, L., Straube, E., Ashfield, T. and Lewald, J. (1995). Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. **Science** 269:843-846.

Grennan, A.K. (2006). Plant response to bacterial pathogens overlap between innate and gene-for-gene defense response. **Plant Physiol.** 142:809-811.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. **Austral. J. Biol. Sci.** 9:463-493.

Gygax, M., Gianfranceschi, L., Liehard, R., Kellerhals, M., Gessler, C. and Patocchi, A. (2004). Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata jackii*. **Theor. Appl. Genet.** 109:1702-1709.

Hammond-Kosack, K.E. and Jones, J.D. (1996). Disease resistance gene-dependent inducible plant defense mechanisms. **Plant Cell** 8:1773-1791.

Hammond-Kosack, K.E. and Jones, J.D. (1997). Plant disease resistance genes. **Annu. Rev. Plant Physiol. Mol. Biol.** 48:575-607.

Hammond-Kosack, K.E. and Jones, J.D. (2000). Response to plant pathogens. In B.B. Buchanan, W. Gruissem, R.L. Jones (eds.) **Biochemistry and Molecular Biology of Plants**. Rockville: American Society of Plant Physiologists.

He, P. and Wang, G. (1986). Studies on the resistance of wild *Vitis* species native to china to downy mildew, *Plasmopara viticola* (Berk. et Curtis) Berl. et de Toni. **Acta. Hort. Sinica** 13:17-24.

He, L., Du, C., Covaleda, L., Xu, Z., Robinson, A.F., Yu, J.Z., Kohel, R.J. and Zhang, H.(2004). Cloning, characterization, and evolution of the NBS-LRR-encoding resistance gene analogue family in polyploidy cotton (*Gossypium hirsutum* L.). **Mol. Plant-Microbe. Interact.** 11:1234-1241.

Hemstad, P.R. and Luby, J.J. (1998). Utilization of *Vitis riparia* for the development of new wine varieties with resistance to disease and extream cold. In **Proc. Of the 7th Int. Sym. on Grapevine Genetic and Breeding** (pp 487-490). Montpellier, France.

Hu, G., deHart, A.K.A., Li, Y., Ustach, C., Handley, V., Navarre, R., Hwang, C., Aegerter, B.J., Williamson, V.M. and Baker, B. (2005). *EDS1* in tomato is required for resistance mediated by TIR-class R genes and the receptor-like *R* gene *Ve*. **Plant J.** 42:376-391.

Huang, H.B. (1980). Viticulture in China. **Hort. Sci.** 15:461-466.

Hueck, C.J. (1998). Type III protein secretion systems in bacterial pathogens of animals and plants. **Microbiol. Rev.** 62:379-433.

Huettel, B., Santra, D., Muehlbauer, J. and Kahl, G. (2002). Resistance gene analogues of chickpea (*Cicer arietinum* L.): isolation, genetic mapping and association with *Fusarium* resistance gene cluster. **Theor. Appl. Genet.** 105:479-490.

Hunger, S., Di Gaspero, G., Möhring, S., Bellin, D., Schäfer-Pregl, R., Borchardt, D.C., Durel, C.F., Weber, M., Weisshaar, B., Salamini, F. and Schneider, K. (2003). Isolation and linkage analysis of expressed disease-resistance gene analogues of sugar beet (*Beta vulgaris* L.). **Genome** 46:70-82.

Irigoyen, M.L., Ferrer, E. and Loarce, Y. (2006). Cloning and characterization of resistance gene analogs from *Avena* species. **Genome** 49:54-63.

Jabco, J.P., Nesbitt, W.B. and Werner, D.J. (1985). Resistance of various classes of grapes to the bunch and muscadine grape froms of black rot. **J. Am. Soc. Hort. Sci.** 110:762-765.

Jemstad, K.D., Sheppard, L.A., Kinloch, B.B., Delfino-Mix, A., Ersoz, E.S., Krutovsky, K.V. and Neale, D.B. (2006). Isolation of full-lenght CC-NBS-LRR resistance gene analog candidate from sugar pine showing low nucleotide diversity. **Tree Genet. Genomes** 2:76-85.

Johal, G.S. and Briggs, S.P. (1992). Reductase activity encoded by *HM1* disease resistance gene in maize. **Science** 258:985-987.

Jones, D.A., Thomas, C.M., Hammond-Kosack, K.E., Balint-Kurti, P.J. and Jones, J.D.G. (1994). Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. **Science** 266:789-793.

Jones, J.D.G., Thomas, C.M., Balint-Kurti, P.J. and Jones, D.A. (1995). Plant pathogen resistance gene and used thereof. *Patent*WO96/35790.

Jones, D.A. and Jones, J.D.G. (1997). The role of leucine-rich repeat proteins in plant defences. **Adv. Bot. Res.** 24:90-167.

Joobeur, T., King, J.J., Nolin, S.J., Thomas, C.E. and Dean, R.A. (2004). The fusarium wilt resistance locus *Fom*-2 of melon contains a single resistance gene with complex features. **Plant J.** 39:283-297.

Keen, N.T. (1990). Gene-for-gene complementarity in plant-pathogen interactions. **Annu. Rev. Genet.** 24:447-463.

Kentucky State University. (2005). KSU viticulture: The Genus Vitis. [On-line]. Available http://www.pawpaw.kysu.edu/viticulture/Information.

Knogge, W. (1996). Fungal infection of plants. **Plant Cell** 8:1711-1722.

Kobe, B. and Deisenhofer, J. (1994). The leucine-rich repeat: a versatile binding motif. **Trends Biochem. Sci.** 19:415-421.

Konieczny, A. and Ausubel, F.M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominants ecotype-specific PCR-based markers. **Plant J.** 4:403- 419.

Lalli, D.A., Decroocq, V., Blenda, A.V., Schurdi-Levraud, V., Garay, L., LeGall, O., Damsteegt, V., Reighard, G.L. and Abbott, A.G. (2005). Identification and mapping of resistance gene analogs (RGAs) in *Prunus:* a resistance map for *Prunus*. **Theor. Appl. Genet.** doi:10.1007/s00122-005-0079-z.

Lamboy, W.F. and Alpha, C.G. (1998). Using simple sequence repeats (SSRs) for DNA fingerprinting germplasm accessions of grape (*Vitis* spp. L.) species. **J. Am. Soc. Hort. Sci.** 123:182-188.

Langcake, P. and Lovell, P.A. (1980). Light and electron microscopical studies of the infection of *Vitis* spp. by *Plasmopara viticola,* the downy mildew pathogen. **Vitis** 19:321-337.

Landschulz, W.H., Johnson, P.F. and McKnight, S.L. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. **Science** 240:1759-1762.

Lawrence, G.J., Finnegan, E.J., Ayliffe, M.A. and Ellis, J.G. (1995). The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. **Plant Cell** 7:1195-1206.

Leister, D., Ballvora, A., Salamini, F. and Gebhardt, C. (1996). A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. **Nat. Genet.** 14:421-429.

Lider, L.A. (1954). Inheritance of resistance to a rootknot nematode (*Meloidogyne incognita* var. *acrita* Chitwood) in *Vitis* spp. **Proc. Helminthol. Soc. Wash. DC** 21:53-60.

Liebhard, R., Koller, B., Patocchi, A., Kellerhals, M., Pfammatter, W., Jermini, M. and Gessler, C. (2003). Mapping quantitative field resistance against apple scab in a 'Fiesta' x 'Discovery' progeny. **Phytopathol.** 93:493-501.

Liu, J.J. and Ekramoddoullah, A.K. (2003). Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. ex. D. Don.). **Mol. Genet. Gemomics** 270:432-441.

Long, S.R. and Staskawicz, B.J. (1993). Prokaryotic plant parasites. **Cell** 73:921-935.

Luderer, R. and Joosten, M.H.A.J. (2001). Avirulence proteins of plant pathogens:determinants of victory and defeat. **Mol. Plant Pathol.** 2:355-364.

Lupas, A. (1996). Coiled colis: New structures and new functions. **Trends Biochem. Sci.** 21:375-382.

May, A.P., Whiteheart, S.W. and Weis, W.I. (2001). Unraveling the mechanism of the vesicle transport ATPase NSF, the *N*-ethylmaleimide-sensitive factor. **J. Biol. Chem.** 276:21991-21994.

Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Frary, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D. (1993). Map-based cloning of a protein kinase gene conferring disease resistance in tomato. **Science** 262:1432-1436.

McDowell, J.M., Dhandaydham, M., Long, T.A., Aarts, M.G., Goff, S., Holub, E.B. and Dangl, J.L. (1998). Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. **Plant Cell** 10:1861-1874.

McGrew, J.R. (1976). Screening grape seedlings for black rot resistance. **Fruit Var. J.** 30:31-32.

Mclntyre, C.L., Casu, R.E., Drenth, J., Knight, D., Whan, V.A., Croft, B.J., Jordan, D.R. and Manners, J.M. (2005). Resistance gene analogues in sugarcane and sorghum and their association with quantitative trait loci for rust resistance. **Genome** 48:391-400.

Meyers, B.C., Shen, K.A., Rohani, P., Gaut, B.S. and Michelmore, R.W. (1998). Receptor-like genes in the major resistance locus lettuce are subject to divergent selection. **Plant Cell** 10:1833-1846.

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W. and Young, N.D. (1999). Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. **Plant J.** 20:317-332.

Meyers, B.C., Morgante, M. and Michelmore, R.W. (2002). TIR-X and TIT-NBS proteins: two new families related to disease resistance TIR-NBS-LRR proteins encoded in *Arabidopsis* and other plant species. **Plant J.** 32:77-92.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R.W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. **Plant Cell** 15:809-834.

Michelmore, R.W. and Meyers, B.C. (1998). Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. **Genome Res.** 8:1113-1130.

Miklas, P.N., Hu, J., Grunwald, N.J. and Larsen, K.M. (2006). Potential application of TRAP (targeted region amplified polymorphism) markers for mapping and tagging disease resistance traits in common bean. **Crop Science** 46:910-916.

Millam, S. and Spoor, W. (1999). Biotechnology and crop improvement. In N.W. Simmonds, J. Smartt (eds). **Principles of Crop Improvement**. Malden: Blackwell.

Mindrinos, M., Katagiri, F., Yu, G.L. and Ausubel, F.M. (1994). The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. **Cell** 78:1089-1099.

Moffett, P., Farnham, G., Peart, J. and Baulcombe, D.C. (2002). Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. **EMBO. J.** 17:4511-4519.

Mondragon-Palomino, M., Meyers, B.C., Michelmore, R.W. and Gaut, B.S. (2002). Patterns of positive selection in the complete NBS-LRR gene family of *Arabidopsis thaliana*. **Genome Res.** 12:1305-1315.

Mortensen, J.A., Stover, L.H. and Balerdi, C.F. (1977). Sources of resistance to Pierce's disease in *Vitis*. **J. Am. Soc. Hort. Sci.** 102:695-697.

Mortensen, J.A. (1981). Sources and inheritance of resistance to anthracnose in *Vitis*. **J. Hered.** 72:423-426.

Naik, S., Hampson, C., Gasic, K., Bakkeren, G. and Korban, S.S. (2006). Development and linkage mapping of E-STS and RGA markers for functional gene homologues in apple. **Genome** 49:959-968.

Natural Resources Conservation Service, United States Department of Agriculture. (2007). Plant data base [On-line]. Available: http://www.plants.usda.gov.

Navabi, A., Singh, R.P., Tewari, J.P. and Briggs, K.G. (2003). Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. **Amer. Phytopathol. Soc.** 87:1522-1529.

Noel, L., Moores, T.L., van der Biezen, E.A., Parniske, M., Daniels, M.J., Parker, J.E. and Jones, J.D.G. (1999). Pronounced intraspecific haplotype divergence at *RPP5* complex disease resistance locus of *Arabidopsis*. **Plant Cell** 11:2099-2111.

Noir, S., Combes, M.C., Anthony, F. and Lashermers, P. (2001). Origin, diversity and evolution of NBS-type disease-resistance gene homologues in coffee trees. **Mol. Genet. Genomics** 265:654-662.

Noutoshi, Y., Ito, T., Seki, M., Nakashita, H., Yoshida, S., Marco, Y., Shirasu, K. and Shinozaki, K. (2005). A single amino acid insertion in the WRKY domain of the *Arabidopsis* TIR-NBS-LRR-WRKY-type disease resistance protein SLH1 (sensitive to low humidity 1) causes activation of defense responses and hypersensitive cell death. **Plant J.** 43:873-888.

Oh, J.W., Kong, Q., Song, C., Carpenter, C.D. and Simon, A.E. (1995). Open reading frames of turnip crinkle virus involved in satellite symptom expression and incompatibility with *Arabidopsis thaliana* ecotype Dijon. **Mol. Plant-Microbe Interact.** 8:979-987.

Olmo, H.P. (1976). Grapes, *Vitis*, *Muscadinia* (Vitaceae). In N.W. Simmonds (ed.). **Evolution of Crop Plants**. London: Longman.

Owolade, O.F. (2006). Line x tester analysis for resistance to cassava anthracnose disease. **World J. Agric. Sci.** 2:109-114.

Owolade, O.F., Dixon, A.G.O. and Adeoti, A.Y.A. (2006). Diallel analysis of cassava genotypes to anthracnose disease. **World J. Agric. Sci.** 2:98-104.

Pan, Q., Wendel, J. and Fluhr, W. (2000). Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. **J. Mol. Evol.** 50:203-213.

Parker, J.E., Coleman, M., Szabo, V., Frost, L., Schmidet, R., Biezen, E.V.D., Moores, T., Dean, C. and Jones, J.D.G. (1997). The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the Toll and Interleukin-1 receptors with *N* and *L6*. **Plant Cell** 9:879-894.

Parniske, M., Hammond-Kosack, K.E., Golstein, C., Thomas, C.M., Jones, D.A., Harrison, K., Wulff, B.B.H. and Jones, J.D.G. (1997). Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf*-4/9 locus of tomato. **Cell** 91:821-832.

Paterniani, M.E.A.G.Z., Sawazaki, E., Dudienas, C., Duarte, A.P. and Gallo, P.B. (2000). Diallel crosses among maize lines with emphasis on resistance to foliar diseases. **Genet. Mol. Biol.** 23:381-385.

Pastuglia, M., Swarup, R., Rocher, A., Saindrenan, P., Roby, D., Dumas, C. and Cock, J.M. (2002). Comparison of the expression patterns of two small gene families of S gene family receptor kinase genes during the defence response in *Brassica oleracea* and *Arabidopsis thaliana*. **Gene** 282:215-225.

Pauquet, J., Bouquet, A., This, P. and Adam-Blondon, A.F. (2001). Establishment of local map of AFLP markers around the powdery mildew resistance gene *Run*1 in grapevine and assessment of their usefulness for marker assisted selection. **Theor. Appl. Genet.** 103:1201-1210.

Pearson, R.C. and Goheen, A.C. (1988). **Compendium of Grape Diseases.** St. Paul:APS.

Penuela, S., Danesh, D. and Young, N.D. (2002). Targeted isolation, sequence analysis, and physical mapping of nonTIR NBS-LRR genes in soybean. **Theor. Appl. Genet.** 104:261-272.

Peterlunger, E., Di Gaspero, G., Cipriani, G., Sivilotti, P., Zulini, L., Marrazzo, M.T., Andreetta., D. and Testolin, R. (2003). Breeding strategy for the introgression of disease resistance genes into European grapevine. **Acta. Hort.** 603:123-124.

Pflieger, S., Lefebvre, V. and Causse, M. (2001). The candidate gene approach in plant genetics: **a review Mol. Breed.** 7:275-291.

Pieniazek, S.A. (1976). Fruit production in China. In **Proc. 17th Int. Hort. Congress** (pp 427-452). Beijing, China.

Pierquet, P. and Stushnoff, C. (1978). Variation and breeding potential of some Northern clones of *Vitis riparia* Michx. **Fruit Var. J.** 32:74-84.

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B. and Wang, G. (2006). The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-luecine-rich repeat protein and is a member of a multigene family in rice. **Genetics** 172:1901-1914.

Quirino, B.F. and Bent, A.F. (2003). Deciphering host resistance and pathogen virulence: the *Arabidopsis*/*Pseudomonas* interaction as a model. **Mol. Plant Pathol.** 4:517-530.

Radwan, O., Mouzeyar, S., Nicolas, P. and Bouzidi, M.F. (2005). Induction of a sunflower CC-NBS-LRR resistance gene analogue during incompatible interaction with *Plasmopara halstedii*. **J. Exp. Bot.** 56:567-575.

Ren, T., Qu, F. and Morris, T.J. (2000). *HRT* gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. **Plant cell** 12:1917-1925.

Reisch, B.I., Goodman, R.N., Martens, M.H. and Weeden, N.F. (1993). The relationship between 'Norton' and 'Cynthiana', red wine cultivars derived from *Vitis aestivalis*. **Am. J. Enol. Vitic.** 44:441-444.

Reisch, B.I. and Pratt, C. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Reisch, B.I. (2003). Cornell grape breeding. [On-line]. Available http:// www.nysaes.edu/

Reynier, A. (1989). **Manual de Viticultura.** Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Riaz, S., Dangl, G.S., Edwards, K.J. and Meredith, C.P. (2004). A molecular marker based framework linkage map of *Vitis vinifera* L. **Theor. Appl. Genet.** 108:864-872.

Richter, T.E. and Ronald, P.C. (2000). The evolution of disease resistance genes. **Plant Mol. Biol.** 42:195-204.

Roine, E., Wei, W., Yuan, J., Nurmiaho-Lassila, E.J., Kalkinen, N., Romantschuk, M. and He, S.Y. (1997). Hrp pilus: an *hrp*-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. **Proc. Natl. Acad. Sci. USA** 94:3459-3464.

Rossetto, M., McNally, J. and Henry, R.J. (2002). Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the *Vitaceae*. **Theor. Appl. Genet.** 108:864-872.

Ryals, J.A., Neuenshwander, U.H., Willits, M.G., Molina, A., Steniner, H.Y. and Hunt, M.D. (1996). Systemic acquired resistance. **Plant Cell** 8:1809-1819.

Seah, S., Sivasithamparam, K., Karakousis, A. and Lagudah, E.S. (1998). Cloning and characterization of a family of disease resistance gene analogs from wheat and barley. **Theor. Appl. Genet.** 97:937-945.

Sefc, K.M., Regner, F., Turestchek, E., Glossl, J. and Steinkellner, H. (1999). Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. **Genome** 42:367-373.

Seglias, N.P. and Gessler, C. (1997). Genetics of apple powdery mildew resistance from *Malus zumi* (*Pl2*). Integrated control of pome fruit disease. IQBC/WDRS. Bulletin, 20:195-208.

Severson, D.W., Mori, A., Zhang, Y. and Christensen, B.M. (1993). Linkage map for Acedes aegypti using restriction fragment length polymorphisms. **J. Hered.** 84:241-247.

Shi, Z.X., Chen, X.M., Line, R.F., Leung, H. and Wellings, C.R. (2001). Development of resistance gene analog polymorphism markers for the *Yr*9 gene resistance to wheat strip rust. **Genome** 44:509-516.

Sinapidou, E., Williams, K., Nott, L., Bahkt, S., Tor, M., Crute, I., Bittner-Eddy, P and Beynon, J. (2004). Two TIR:NBS:LRR genes are required to specify resistance to *Peronospora parasitica* isolate Cala2 in *Arabidopsis*. **Plant J.** 38:898-909.

Small, J.K. (1903). Vitis and Muscadinia. In J.K. Small (ed) **Flora of the Southeastern United States**. New York: John Wiley and Sons.

Spellenberg, R. (1998). *Vitis cinerea* var Helleri [On-line]. Available http://nmareplants.unm.edu/

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. **Science** 270:1804-1806.

Soriano, J.M., Vilanova, S., Romero, C., Llacer, G. and Badenes, M.L. (2005). Characterization and mapping of NBS-LRR resistance gene analogs in apricot (*Prunus armeniaca* L.). **Theor. Appl. Genet.** 110:980-989.

Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G. and Jones, J.D.G. (1995). Molecular genetics of plant disease resistance. **Science** 268:661-667.

Stover, L.H. (1960). Progress in the development of grape varieties for Florida. **Proc. Fla. St. Hort. Soc.** 73:320-323.

Subudhi, P.K. and Nguyen, H.T. (2004). Genome mapping and genomic strategies for crop improvement. In H.T. Nguyen, A. Blum (eds.) **Physiology and Biotechnology Integration for Plant Breeding**. New York: Marcel Dekker.

Szegedi, E., Korbuly, J. and Koleda, I. (1984). Crown gall resistance in East-Asian *Vitis* species and in their *V. vinifera* hybrids. **Vitis** 23:21-26.

Taler, D., Galperin, M., Benjamin, I., Cohen, Y. and Kenigsbuch, D. (2004). Plant *eR* genes that encode photorespiratory enzyme confer resistance against disease. **Plant Cell** 16:172-184.

Toroser, D., Thormann, C.E., Osborn, T.C. and Mithen, R. (1995). RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L.). **Theor. Appl. Genet.** 91:802-808.

Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tirong, J.G. and Osborn, T.C. (1994). Comparison of RFLP and RAPD markers to estimating genetic relationship within and among cruciferous species. **Theor. Appl. Genet.** 88:973-980.

Toojinda, T., Broers, L.H., Chen, X.M., Hayes, P.M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R.F., Powell, W., Ramsay, L., Livar, H. and Waugh, R. (2000). Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). **Theor. Appl. Genet.** 101:580-589.

Traut, T.W. (1994). The functions and consensus motifs of nine types of peptide segments from different types of nucleotide-binding sites. **Eur. J. Biochem.** 222:9-19.

Van der Hoorn, R.A., Roth, R. and Joosten, M.H.A.J. (2001). Identification of distinct specificity determinants in resistance protein *Cf-4* allows construction of a *Cf-9* mutant that confers recognition of avirulence protein *AVR4*. **Plant Cell** 13:273-285.

Van Gijsegem, F., Genin, S. and Boucher, C. (1995). The *hrp* gene locus of *Pseudomonas solanacearum*, which controls the production of a type III secretion system, encodes eight proteins related to components of bacterial flagellar biogenesis complex. **Mol. Microbiol.** 15:1095-1114.

Vision, T., Brown, D.G. and Tanksley, S.D. (2000). The origins of genomic duplications in *Arabidopsis thaliana*. **Genome** 42:887-892.

Walker, M.A., Meredith, C.P. and Goheen, A.C. (1985). Sources of resistance to grapevine fanleaf virus (GFV) in *Vitis* species. **Vitis** 24:218-228.

Walker, M.A. and Meredith, C.P. (1990). The genetics of resistance to grapevine fanleaf virus in *Vitis vinifera*. In **Proc. 5th Int. Sym. on Grape Breeding** (pp 228-238). Pfalz, Germany.

Warren, R.F., Henk, A., Mowery, P., Holub, E. and Innes, R.W. (1998). A mutation within the leucine-rich repeat domain of *Arabidopsis* disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. **Plant Cell** 10:1439-1452.

Westman, A.L. and Kresovich, S. (1997). Use of molecular marker techniques for description of plant genetic variation. In J.A. Callow, B.V. Ford-Lloyd, H.J. Newbury (eds). **Biotechnology and Plant Genetic Resources Conservation and Use.** New York: Cab international.

Whitham, S., Dinesh-Kumar, S.P., Choi, D., Hehl, R., Corr, C. and Baker, B. (1994). The product of the tobacco mosaic virus resistance gene *N*: Similarity to Toll and the interleukin-1 receptor. **Cell** 78:1011-1015.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitorary primers are useful as genetic markers. **Nucleic Acid Res.** 18:6531-6535.

Wu, H.X. and Matheson, A.C. (2004). General and specific combining ability from partial diallels of radiate pine: implications for utility of SCA in breeding and deployment populations. **Theor. Appl. Genet** 108:1503-1512.

Wulff, B.B., Thomas, C.M., Smoker, M., Grant, M. and Jones, J.D. (2001). Domain swapping and gene shuffling identify sequences required for induction of an *Avr*-dependent hypersensitive response by the tomato *Cf-4* and *Cf-9* proteins. **Plant Cell** 13:255-272.

Yaish, M.W., Saenz de miera, L.E. and Perez de la Vega, M. (2004). Isolation of a family of resistance gene analogue sequences of the nucleotide binding site (NBS) type from *Lens* species. **Genome** 47:650-659.

Yan, G.P., Chen, X.M., Line, R.F. and Wellings, C.R. (2003). Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. **Theor. Appl. Genet.** 106:636-643.

Yamasaki, K., Kigawa, T. and Inoue, M. (2005). Solution structure of an *Arabidopsis* WRKY DNA binding domain. **Plant Cell** 17:944-956.

Young, N.D. (2000). The genetic architecture of resistance. **Curr. Opin. Plant Biol.** 3:285-290.

Yu, Y., Tomkin, J.P., Waugh, R., Frisch, D.A., Kudrna, D., Kleinhofs, A., Brueggeman, R.S., Muehlbauer, G.J., Wise, R.P. and Wing, R.A. (2000). A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. **Theor. Appl. Genet.** 101:1093-1099.

Zabeau, M. and Vos, P. (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. EPO Patent No. 0534858A1.

Zimmerman, H. and Becker, N.J. (1978). Selection of rootstocks based on *Vitis cinerea*. In **Proc. 2nd Int. Symp. Grapevine Genetics and Breeding** (pp 281-286). Paris, France.

Zhang, R., Cai, H., Fatima, N., Buczko, E. and Dufau, M.L. (1995). Functional glycosylation sited of the rat luteinizing hormone receptor required for ligand binding**. J. Biol. Chem.** 270:21722-217228.

Zhang, Y., Dorey, S., Swiderski, M. and Jones, J.D.G. (2004). Expression of *RPS4* in tobacco induces an AvrRps4-independent HR that requires *EDS1*, *SGT1* and *HSP90*. **Plant J.** 40:213-224.

Zhang, Y., Zhang, S., Qi, L., Liu, B., Xiong, B., Gao, J., Chen, X., Chen, C., Li, X. and Song, W. (2006). Cloning and characterization of disease resistance gene analogs from poplar (*Populus tremula*) chromosome 1. **Int. J. Plant Sci.** 167:403-412.

Zhou, T., Wang, Y., Chen, J.Q., Araki, H., Ping, Z., Jiang, K., Shen, J. and Tian, D. (2004). Genome-wide identification of NBS genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. **Mol. Genet. Genomics** 271:402-415.

Zhu, H., Cannon, S.B., Young, N.D. and Cook, D.R. (2002). Phylogeny and genomic organization of the TIR and non-TIR NBS-LRR resistance gene family in *Medicago truncatula*. **Mol. Plant-Microbe Interact.** 15:529-539.

CHAPTER III

RESISTANCE GENE ANALOGS FROM *Vitis cinerea, V. rupestris, V.* hybrid 'Horizon': CLONING AND DEVELOPMENT OF MOLECULAR MARKERS FOR DOWNY MILDEW RESISTANCE

3.1 Abstract

Resistance gene analogs (RGAs) characterized by the presence of nucleotide binding site (NBS) were cloned from *Vitis cinerea*, *V. rupestris* and *V.* hybrid 'Horizon'. The two degenerate PCR primer pairs were designed from conserved regions of NBS motifs within known resistance genes and used for PCR amplification of putative RGAs. A total of 122 putative RGA sequences were cloned from all three genotypes by P-loop/GLPLAL-1. Based on nucleic acid sequence-identity of 90% or greater, RGA clones were subdivided into eight, four and seven groups for *V. cinerea*, *V. rupestris* and Horizon, respectively. All of these clones showed similarity of nucleotide sequences to other R-genes or known NBS-type nucleotide sequences and seven clones showed high similarity sequences to RGA clones of *V. amurensis*. Thirty sequences were cloned from *V. cinerea* by P-loop/Rev loop and subdivided into four sequence groups, all of which had no similarity to nucleotide sequences of other R-genes. The nineteen representative RGA clones from P-loop/GLPLAL-1 were classified into thirteen TIR-NBS-LRR-like genes and six non-TIR-NBS-LRR-like genes based primarily on nucleotide sequences of kinase-2 motifs and phylogenetic analysis with known TIR or non-TIR proteins. The

seventeen STS markers were developed from *Vitis* RGA sequences. Those markers plus six STS and three CAPS that were previously developed from other grape species were evaluated for segregation among progenies from 'Horizon x Illinois 547-1 (Ill. 547-1)'. The 18 markers showed goodness of fit for 1:1 and 3:1 ratios using a chi-square test. These STS markers are currently being investigated for their potential use in molecular breeding for downy mildew resistance.

3.2 Introduction

The most widely cultivated grape species (*Vitis vinifera*) is highly susceptible to many diseases caused by fungi, bacteria and viruses. However, many North American grape species such as *V. riparia, V. rupestris* and *V. rotundifolia* are reported to be highly resistant to several important diseases of *V. vinifera* including downy mildew, anthracnose, Botrytis rot, black rot and powdery mildew (Eibach et al., 1989; Alleweldt et al., 1990).

Grape downy mildew, caused by the oomycete *Plasmopara viticola,* is one of the most economically important grape diseases worldwide. The disease can rapidly affects the whole vineyard, destroying 50 to 75% of a crop in one season (Müller and Sleumer, 1934; Agrios, 1997). Most European grapes (*V. vinifera*) are highly susceptible to downy mildew, young leaves and fruits are particularly susceptible (Kennelly et al., 2005).

The most commonly employed tactic for controlling grape downy mildew is the application of fungicides. Two types of fungicide are used for downy mildew control: preventative fungicides (e.g. copper, captan and mancozeb) and curative fungicides (e.g. metalaxyl and ridomil). However, the effective chemical control is expensive and may

have environmental consequences. Therefore, a grape cultivar highly resistant to downy mildew, without spraying, is a desirable alternative.

The development of molecular markers linked to disease resistance genes could provide a valuable tool for breeding programs employing marker assisted selection (MAS) or for map-based cloning efforts. Cloned resistance (R) genes obtained from a number of plant species have been shown to confer resistance to individual diseases caused by viruses, bacteria, fungi, oomycetes or nematodes (Hammond-Kosack and Jones, 2000; Taler et al., 2004). Indeed, some R genes encode proteins that act in the signaling process by interacting with pathogen *Avr* gene products, or, in other cases, R genes encode proteins involved in race-specific recognition or act as general elicitors (Keen, 1990; Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001). R genes have been previously cloned from plants such as tobacco (*N)*, flax (*L6)*, rice (*Xa21)*, tomato (*Cf)*, *Arabidopsis* (*RPS2* and *RPM1*), maize (*HM*) and sunflower (*Pl*; Martin et al., 1993; Bent et al., 1994; Whitham et al., 1994; Song et al., 1995; Dixon et al., 1998; Hulbert et al., 2001; Jones, 2001; Radwan et al., 2004; Qu et al., 2006).

The major class of R genes in plants is characterized by the presence of nucleotide binding site (NBS)-leucine-rich repeat (LRR) domains. LRR and NBS domains have a role in both cell surface recognition and intracellular signaling (Saraste et al., 1990; Parker et al., 1997; Falk et al., 1999; Zhu et al., 2002). The amino acid compositions of the N-terminus separate the NBS-LRR R proteins into two types. These include the Toll/Interleukin-1 receptor (TIR) that contains a domain resembling the TIR and the non-TIR which contains a coiled-coil (CC) or a leucine-zipper (LZ). The motif at N-terminus was frequently duplicated and/or reorganized at the NBS sequence (Meyers et al., 1999). The *L6* (Lawrence et al., 1995) and *N* (Whitham et al., 1994) are representative of TIR proteins, and possess resistance nucleotide binding site (RNBS)-A-

TIR (LQKKLLSKLL) and RNBS-D-TIR (FLHIACFF) motifs in the NBS domain. While genes belonging to non-TIR proteins such as *RPS2*, *RPS5* (Mindrinos et al., 1994; Warren et al., 1998) and *Mi* (Milligan et al., 1998; Meyers et al., 1999) encode resistance nucleotide binding site (RNBS)-A-non-TIR (FDLxAWVCVSQxF) and RNBS-D-non-TIR (CFLYCALFPED) motifs. Moreover, the final residue in Kinase-2 motif can be used to predict protein type at N-terminus because non-TIR genes are highly likely to encode tryptophan (W) in the Kinase-2 motif instead of aspartic acid (D) found frequently in TIR-NBS-LRR proteins (Parker et al., 1997; Meyers et al., 1999).

The sequences of NBS domains in general are highly divergent among members, however, short motifs such as P-loop, Kinase-2 and RNBS are conserved in both dicot and monocot plants (Donald et al., 2002). The sequence conservation of specific domains (e.g. the P-loop and GLPL domains) has facilitated the use of degenerate oligonucleotide primers to amplify and clone RGA sequences from genomic DNA of diverse genus, including *Glycine*, *Brassica*, *Hordeum*, *Arabidopsis*, *Beta*, *Helianthus*, *Malus*, *Pinus*, *Populus* and *Prunus* (Yu et al., 1996; Aarts et al., 1998; Joyeux et al., 1999; Gedil et al., 2001; Gowda et al., 2002; Hunger et al., 2003; Baldi et al., 2004; Soriano et al., 2005; Jemstad et al., 2006; Zhang et al., 2006). RGA sequences have been developed as molecular markers using the resistance gene analog polymorphism (RGAP) technique, which has allowed identification of markers linked to disease resistance genes in plants such as barley, rice, sunflower, wheat, common bean and *Avena* species (Chen et al., 1998; Toojinda et al., 2000; Dodds et al., 2001; Gedil et al., 2001; Pflieger et al., 2001; Yan et al., 2003; Irigoyen et al., 2006; Miklas et al., 2006). Due to the high probability of finding clustered RGAs in the plant genome, molecular markers developed from RGAs have great potential for co-localization with alleles for disease resistance on linkage maps. Yan et al. (2003) showed that six and eleven

markers from RGA primers were co-segregating or tightly linked, respectively, to the *YR5* locus conferring stripe rust resistance in wheat.

Di Gaspero and Cipriani (2002) cloned RGAs from *V. amurensis* and *V. riparia* by degenerate primers of the P-loop and GLPL domains, revealing twelve RGA groups with at least 40% identity to known R- genes such as *Arabidopsis RPS5* and tobacco *N*. The major groups of RGAs cloned in this study were used to distinguish three disease resistant varieties and six susceptible grape varieties (Di Gaspero and Cipriani, 2002). In addition, 45 sequence tagged site (STS) markers were developed from RGA sequences that showed polymorphism among 20 *Vitis* species (Di Gaspero and Cipriani, 2003). RGAs have also been isolated from *V. rotundifolia* and converted to 20 RGA markers co-segregating with the *Run1* locus that controls powdery mildew resistance (Baker et al., 2005).

In previous work, the interspecific hybrid population, 'Horizon ('Seyval' x 'Schuyler') x Illinois 547-1 (Ill. 547-1; *V. rupestris* x *V. cinerea*)' was used for evaluating the inheritance of powdery mildew resistance and identifying quantitative trait loci (QTL) linked to powdery mildew resistance (Dalbo et al., 2000). Ill. 547-1 has been shown previously to be highly resistant to several fungal diseases, including downy mildew and powdery mildew (Dalbo et al., 2000). One hundred and fifty-three markers including random amplified polymorphic DNA (RAPD), cleaved amplified polymorphism sequences (CAPS), amplification fragment length polymorphism (AFLP), simple sequence repeat (SSR) and isozymes markers were mapped onto Horizon covering 1199 cM, whereas the Ill. 547-1 map had 179 markers including RAPD, AFLP, SSR and CAPS markers covering 1470 cM (Dalbo et al., 2000). A single marker (CS25b) was found to be associated with a major QTL (LOD score 6.56) from Ill. 547-1, which accounted for 41% of the phenotypic variation of powdery mildew resistance.

Interestingly, the allele of this marker associated with resistance was also present in *V. cinerea* B9, one of the parents of Ill. 547-1 (Dalbo et al., 2001).

The RGA sequences were cloned from the disease resistant genotypes *V. cinerea* B9, *V. rupestris* B38 and Horizon. These sequences were classified based on variation present in the NBS domain. The RGA sequences were then used to develop STS markers for placement on the 'Horizon x Ill. 547-1' genetic map. The mapping will be used to identify marker(s) co-segregating with downy mildew resistance gene(s) which can improve the efficacy of MAS for disease resistance traits in grape. This also will facilitate the identification and cloning of disease resistance genes in the future.

3.3 Materials and Methods

Plant materials and DNA extraction

The lines *V. cinerea* B9, *V. rupestris* B38 and Horizon ('Seyval' x 'Schuyler'; Appendix Figure 1A) were used as template for PCR-based cloning of NBS sequences. DNA was extracted from 2 g of young leaves using the method of Lodhi et al. (1994) with the following modification to the CTAB (cetyltrimethylammonium bromide) extraction buffer: 3% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 0.1 M Tris HCl, pH 8.0, 2% (w/v) polyvinylpolypyrrolidone and 0.2% (v/v) ß-mercaptoethanol. The DNA pellets were dissolved in double distilled water (ddH_2O) and, after RNase A treatment, DNA concentrations were calculated from absorbance values measured at 260 nm using a spectrophotometer.

Amplification and cloning of NBS-LRR genes by degenerate primers

Two oligonucleotide primers, P-loop (5'GGIGGIGTIGGIAAIACIAC 3') and GLPLAL-1 (5' IAGIGCIAGIGGIAGICC 3') were modified from Hunger et al. (2003), having been designed from the most conserved domains within the NBS P-loop and

GLPL motifs from the *N, RPS2, L6* and *N, RPS2, RPM1, L6* genes, respectively. The other degenerate primer, Rev loop (5'GTIGTITTICCIACICCICC 3'; modified from Hunger et al., 2003), was derived from the *N, RPS2* and *L6* genes. The P-loop/GLPLAL-1 and P-loop/Rev loop primer pairs were used to amplify RGA fragments from *V. cinerea* B9. NBS regions of *V. rupestris* B38 and Horizon were amplified using only the P-loop/GLPLAL-1 primer pair. PCR amplifications were performed in a reaction volume of 20 μL containing 1x PCR buffer, 0.1 mM dNTPs, 2.5 mM $MgCl_2$, 2 μM of each primer, 30 ng of DNA and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI, USA). The initial step of the amplification reaction was denaturation at 95^{o}C for 4 min; followed by 35 cycles of 95^{o}C for 45 sec, 50^{o}C for 1 min and 72^{o}C for 1 min; and a final extension at 72^{o}C for 10 min.

Cloning and sequencing of RGAs

PCR products for cloning were fractionated on 0.8% agarose gel and stained with SYBR Green. Fragments of the expected size were excised from the agarose gel and purified using the QIAquick Gel Extraction Kit (Promega, Madison, WI, USA). The PCR products were then cloned into the pGEM-T Easy plasmid vector (Promega, Madison, WI, USA) and transformed into competent *Escherichia coli* Top-10 cells (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The transformation reaction was plated on selective media containing 20% (w/v) X-gal / 2% (w/v) IPTG for blue/white screening of plasmids with inserts (Appendix Figure 2A).

Plasmid DNA from single white colonies was extracted; briefly white colony was cultured in liquid LB medium with 100 mg/L ampicilin and shake at 250 rpm at 37^{o}C overnight. The bacterial pellet was resuspended in 200 μL of GTE buffer (50 mM glucose, 10 mM EDTA, pH 8.0, 25 mM Tris-HCl, pH 8.0). The 300 μL of 0.2 N

NaOH/1% (w/v) SDS was added and incubated on ice for 5 min. Cellular debris and denatured chromosomal DNA were removed by centrifugation at 5635 x g for 10 min, and the supernatant was then transferred to a clean tube. Seven hundred and fifty microlitter of chloroform was added and mixed by inverted the tubes then centrifuged at 5635 x g for 1 min. Plasmid DNA was precipitated by adding 1 V of 100% (v/v) isopropanol and 1/10 volume of 3 M sodium acetate (NaOAc), incubating at -20^{o}C for 30 min and centrifuging at 5635 x g for 10 min. DNA pellet was washed with 500 μL of 70% (v/v) ethanol. The pellet was dissolved in 32 μL of ddH_2O, and the plasmid DNA was precipitated by adding 8.0 μL of 4 M NaCl, and 40 μL of 13% (w/v) PEG8000. The sample was incubated on ice for 20 min. Plasmid DNA was collected by centrifuging at 22539 x g for 15 min at 4^{o}C. Pellet was rinsed with 500 μL of 70% (v/v) ethanol and resuspended in 20 μL of ddH_2O. Plasmid DNA was examined by *Eco*RI restriction analysis (1 unit *Eco*RI, 200 ng of plasmid DNA, 37^{o}C, 3 h) to determine the size of the inserted fragments.

Plasmid DNA with inserted fragments was sequenced. DNA sequence analysis was performed using the Applied Biosystems Automated 3730 DNA Analyzer at the Biotechnology Resource Center (Cornell University, Ithaca, NY, USA). Sequencher 4.2 (Genecodes Corp. Ann Arbor, MI, USA) was used to trim the sequence of pGEM-T Easy plasmid vector out of inserted sequences and to select representative clones.

Sequence analysis

Identification of clones showing significant homology to known RGA sequences and resistance proteins in GenBank was performed by Nucleotide-Nucleotide Basic local alignment search tool (BLASTn) and translated query vs. Protein database (BLASTx) software (www.ncbi.nlm.nih.gov/Blast). Nucleotide sequences of RGAs cloned from

the P-loop/GLPLAL-1 primer pair were translated into amino acid sequences by Translate, a program provided by Göteborg University, Sweden (http://bio.lundberg.gu.se/edu/translat).

Amino acid sequences from known resistance genes already classified as TIR or non-TIR proteins were searched using GenBank Entrez (www.ncbi.nlm.nih.gov). Phylogenetic tree and alignment of cloned RGA amino acid sequences were performed by ClustalW-XXL with neighbor-joining method (http://clustalW.genome.jp; www.ch.embnet.org). In addition, selected TIR-NBS-LRR genes (*L6* and *M* from flax, *N* from tobacco and *RPS4* from *Arabidopsis)* and nonTIR-NBS-LRR genes (*RPS2* and *RPS5* from *Arabidopsis*, *Xa1* from rice and *I2* from tobacco) were added to the alignment. Motif structures present in RGA clones were analyzed by the MEME program (http://meme.nbcr.net; Bailey and Elkan, 1994).

RGA-STS marker

Young leaves of seedling from a cross 'Horizon x Ill. 547-1' were collected for DNA extraction. The DNA extraction was as modified by Owens (2003). Briefly, the CTAB extraction buffer (3% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 0.1 M Tris HCl, pH 8.0, 2% (w/v) polyvinylpolypyrrolidone and 0.2% (v/v) ß-mercaptoethanol) was added into ground leaf tissues and incubated at 60^{o}C for 30 min. One V of 24:1 chloroform:isoamyl alcohol was added and the mixture was then spinned at 5635 x g for 15 min. Supernatant was transferred to new tubes and added with 0.5 V of 5 M NaCl. DNA was precipitated by adding 1 V of isopropanol and incubated at -20^{o}C for 20 min. DNA pellet was rinsed with 70% and 95% (v/v) ethanol and resuspended in 200 μL ddH_2O. After RNase A treatment, DNA concentrations were calculated from absorbance values measured at 260 nm using a spectrophotometer.

Table 1. Specific primers, annealing temperatures and sizes of PCR product for STS and CAPS markers.

Name[a]	Forward primer (5'—3')	Reverse primer (5'—3')	Anneal. Temp.	size	Enzyme	Exp. Digest Frag.[b]
rgVrip064[c]	GACTACTATTGCCAAGGCTGTTT	AATCTACTGCTTGGTAGGAGAG	58	467	*Eco*R I	
rgVamu085[c]	GACGACCCTCTTGACCAGGAT	TGAGAATTTATAGTGTCTTCTCCTACA	58	435	*Sau* 3AI	
stkVa011[c]	GAAGGCACTTTGAGCAATGG	AACCATTCGGGAGCCAAG	57	479	*EcoR* V	
rgVrip145[c]	GCCAGACTTGCTTATAACGATGA	CGCACTTTTCCACAATCTTCTT	58	475	*Alu* I	
GLPL6-1[c]	GCATATGCTACAAACTCCATTCA	CAATTTCTTCTAGTTCTGGGATG	58	206	*Hinf* I	
rgVrip158[c]	CCAGTTGATATACAGGGACGATG	GATCCTTGTATCAAGCAATCTCA	58	463	*Mnl* I	
rgVamu100[c]	CATCAATATGATGGTAGTAGCTTTCTT	GAGCTTAGACACCTCTTTATCACACT	58	164		
rgVamu092[c]	AACTCACATCAATTTGAGAGTAGAATC	TGATTTGAGAGGTCAACATAGTCA	58	431	*Alu* I	
rgVamu111[c]	ACCAGAGAGTGGTGGGACAC	CCTTTTATCTTGTAAATACTGCCTGA	58	194		
rgVcin109	GGAAGACGACAATTGCCAAA	GCATCGACTCCAAGCACAT	56	358	*Alu* I	84,274
rgVcin111	ATGGTGTCATGAAGGGAAAAA	AGACCAAACCAACCATGCTC	57	164	*Xba* I	31,133
rgVcin123	GATGGGATGGAGTCAAAGGA	CACTCACTCCATGGCACATT	58	217	*aTaq* I	43,174
rgVcin125	GTCCAGGAAACCGTTCTCAA	CCTTGGTCCGAAACAAAGAA	54	304	*Hinf* I	144,160
rgVcin127	GATGGGATGGAGTCAAAGGA	GGGGAGGCCTTTAGCATAAT	54	352	*Mnl* I	134,218
rgVcin139	TGACGTGGATGATTTGATGC	GGGGAGGCCTTTAGCATAAT	58	259	*Alu* I	62,197
rgVcin165	CATGGTATCCTGAGGGGAAA	GGAGGCCATCAGCATAATCT	58	361	*Mae* II	163,198
rgVrup103	CATGGTATCCTGAGGGGAAA	GGCCATCAGCGTAATCTATGA	56	358	*Rsa* I	169,189
rgVrup119	GGTGCAAATGCTCACAGAGA	CTCCCAAACAAGGTCCAAGA	58	383	*Eco*R I	152,231
rgVrup124	AATGGAGGCTCGTTTTGAGA	GCCGATGTGTTCTCTCTTCC	58	323	*Mnl* I	153,170
rgVrup126	GGTCCAGGAAACCATTCTCA	CCTTGGTCCGAAACAAAGAA	54	304	*Hinf* I	143,161
rgVhyb101	GGGGTGGGGAAGACAACTAT	CCTACTTCTTGGACCAAACCA	50	306	*Aci* I	133,173
rgVhyb102	CACAAATGCAATTTGCCCTA	GCTGGAGGAGGTTGGTGTTA	58	329	*Eco*R I	133,196
rgVhyb110	ATCCAGGGTTGAGTTTGACG	CAATGCCCTTAGCTCCCATA	58	317	*Dpn* II	111,206
rgVhyb121	AATTCGATTGAGGGCAAGTG	GTGAGGATGAAAGGGCAGAA	58	347	*Nco* I	128,219
rgVhyb127	TGATCGTGGTGTGCTTCAAT	TTCCGTAGCTTGCTTGTGTG	54	310	*Nco* I	59,251
rgVhyb149	GATTGGTTTGGTCGAGGAAG	CGGCAGACCTTGAGGATAAA	46	212	*Eco*R I	63,149

[a]The primers were named by the names of RGAs cloned

[b]The expected digest fragment (bp) from restriction enzyme

[c]The markers that were developed by Di Gaspero and Cipriani (2003)

PCR primers specific to the 19 cloned *Vitis* RGAs were designed using Primer 3 (Table 1; http://frodo.wi.mit.edu/primer3/primer3_www.cgi). Six additional STS primers and 3 CAPS markers developed by Di Gaspero and Cipriani (2003) were also used (Table 1). PCR amplifications were performed in a reaction volume of 20 μL containing 1x PCR buffer, 0.1 mM dNTPs, 2.5 mM $MgCl_2$, 2 μM of each primer, 30 ng of DNA and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI, USA). The initial step of the amplification reaction was denaturation at 94^{o}C for 1 min; followed by 25 cycles of 92^{o}C for 50 sec, 46-58^{o}C (variable by primer; Table 1) for 50 sec and 72^{o}C for 1 min; and a final extension at 72^{o}C for 10 min. The corresponding restriction enzymes were selected by Sequencher 4.2 for fragments that are more than 200 bp (Genecodes Corp. Ann Arbor, MI, USA; Table 1). Amplified DNA fragments (ca 200 ng) were cut by 1 unit restriction enzyme and incubated for 3 hr at the appropriate temperature.

CAPS analysis was performed on 2% agarose gels and stained with SYBR Green. single strand conformation polymorphism (SSCP) analysis was performed using polyacrylamide gels (8% (v/v) Acrylamide/Bis, 2% (v/v) glycerol, 1x TBE, 0.10% (v/v) TEMED and 0.01% (w/v) ammonium persulfate). Briefly, the polyacrylamide gel was pre-run at 200 V, 10 W for 15 min at 4^{o}C to clean and pre-cool gel. Five μL of original amplified fragments or restriction-enzyme-digested fragments were added into 5 μL of 3X SSCP loading dye (95% (v/v) formamide, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue and 20 mM EDTA, pH 8.0). Loading samples were denatured for 5 min at 95^{o}C on thermocycler and immediately placed on ice until ready to be loaded. Ten μL of loading samples were loaded into each wells. The gel was run at 200-230 V, 0.06 A, 12-13 W at 4^{o}C until the dark blue dye reaches the end of the gel. Gels were stained with silver nitrate. Briefly, the gels were fixed with fixing solution (10% (v/v) acetic acid) for 30 min and then rinsed twice with ddH_2O for 5 min. Stain solution (0.2% (w/v)

silver nitrate [$AgNO_3$]) was prepared and used to stain the gel for 30 min. The gels were rinsed twice with ddH_2O for 5 min. The bands were developed by developer solution (1.5% (w/v) sodium hydroxide [NaOH] plus 1% (v/v) formaldehyde) for 30 min, and then the gels were fixed with fixing solution.

The statistical analyses of correlation among markers segregation were performed by SAS 9.1.2 (SAS Institute Inc., Cary, NC, USA). The linkage analysis was performed using the program MAPMAKER 3.0 (Lander et al., 1987; UNIX version/EXP 3.0 b). The linkage analysis of Ill. 547-1 map was performed by bands heterozygous in male including rgVamu085, GLPL6-1, rgVcin125, rgVcin127, rgVcin139, rgVrup126, rgVhyb102 and rgVhyb110. While, 'Horizon' map was performed by bands heterozygous in female including rgVamu100, rgVrup119, rgVhyb101, rgVhyb121 and rgVhyb127.

Plasmopara viticola inoculation

Sporangia of *P. viticola* were harvested from sporulating leaves of *V.* hybrid cv. 'Delaware' into distilled water using a spray bottle. The spore concentration of collected sporangial suspension was estimated using a hemacytometer and adjusted to 10^5 sporangia per mL. The segregating progenies were developed by the grape breeding program at Cornell University, Geneva, New York (Appendix Figure 3A). Leaf node 1, being the first expanded leaf (Appendix Figure 4A), leaf nodes 5, 6 and 7 of 179 'Horizon x Ill. 547-1' progenies were used for inoculation. The inoculated leaves were placed abaxial surface up onto moist filter paper in Petri dishes. The sporangial suspensions were sprayed onto the abaxial leaf surface until wet and covered all surfaces. Petri dishes were held at 22^oC, 18 h photoperiod for 8 days. A second set of the experiments was incubated for 10 days. Infected leaves from two experiments were

placed in 5 mL of double distilled water in a 50-mL tube and then shaken for 3 min. The total number of spores produced per leaf was determined by counting the number of spores in 5 μL under a microscope. The length and width of infected leaves were measured. The area of ten different leaves was measured using a leaf area meter. The regression equation between ten leaf areas calculated from the length and width and areas measured by a leaf area meter was used to convert leaf length and width to leaf area (Appendix Figure 5A). The number of spores per leaf was converted to number of spores/25 cm^2 leaf area by following a formula; number of spores/25 cm^2 leaf area = (number of spores x 25 cm^2 leaf area)/actual leaf areas. Resistance levels were based on average spore production from two sets of experiments. The six-point disease resistance classification was defined as: 0 is 0 - 5 spores/25 cm^2, highly resistant; 1 is >5 – 10 spores/25 cm^2, resistant; 2 is >10 – 15 spores/25 cm^2, moderate or intermediate; 3 is >15 – 25 spores/25 cm^2, moderately susceptible; 4 is >25 – 40 spores/25 cm^2, susceptible and 5 is >40 spores/25 cm^2, highly susceptible (D.M. Gadoury, personal communication). The statistical analyses of correlation between phenotypic data and marker segregation were performed by SAS 9.1.2 (SAS Institute Inc., Cary, NC, USA).

3.4 Results

Cloning RGA sequences

Genomic DNA of *V. cinerea* B9, a genotype resistant to multiple diseases including downy mildew, was amplified using two degenerate primer pairs, P-loop/GLPLAL-1 and P-loop/Rev loop, producing approximately 500- and 850-bp PCR products, respectively (Figure 1).

Complete nucleotide sequences were obtained from 78 of 100 clones. Based on 90% minimum overlap and 90% minimum identity, 48 clones from P-loop/GLPLAL-1

primers were subdivided into eight unique groups (Table 2; Figure 2; Appendix Table 1A). In contrast, 52 clones from P-loop/Rev loop primers were sequenced but unambiguous nucleotide sequences were obtained for only 30 clones. These 30 clones were subdivided into four representative groups (Table 2; Figure 2).

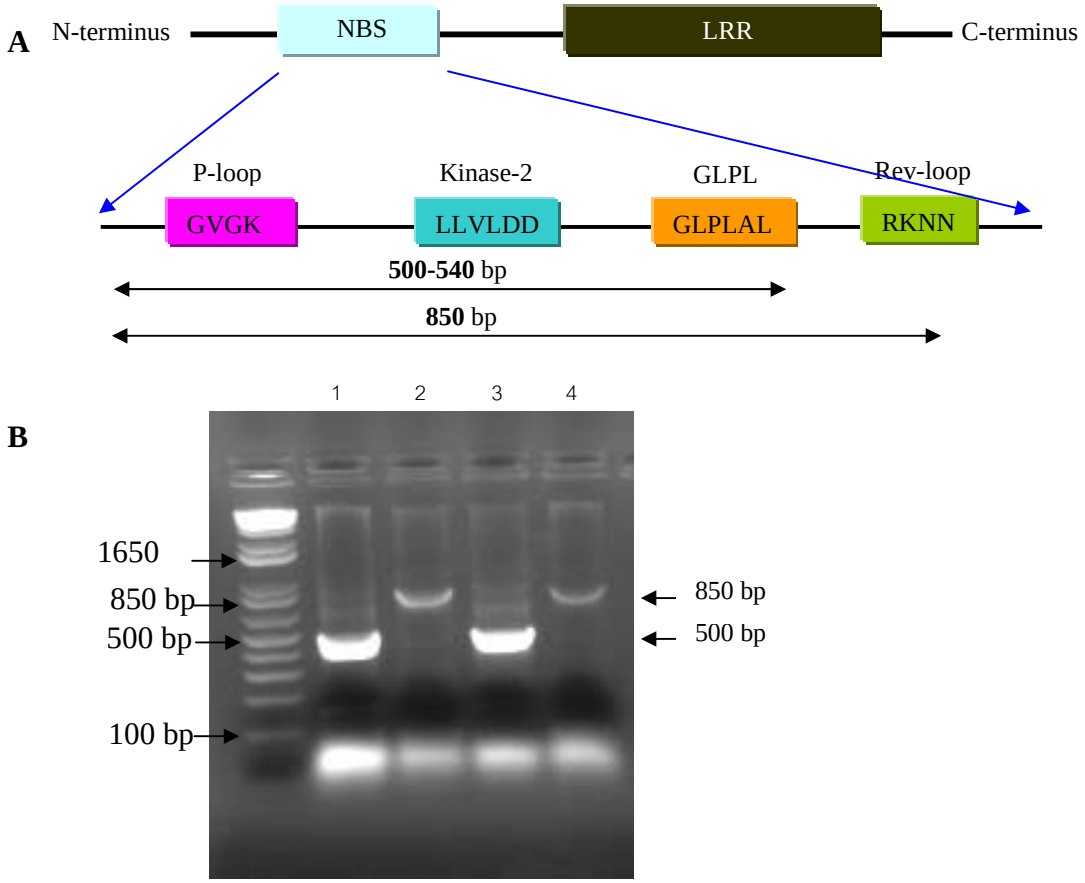


Figure 1. RGAs (A) Model of the structure of NBS-LRR type resistance genes; (B) PCR products amplified with two degenerate primer pairs from *V. cinerea* B9. The expected sizes of amplified DNA bands are 500 bp for the P-loop/GLPLAL-1 primer pair (samples 1, 3) and 850 bp for the P-loop/Rev loop primer pair (samples 2, 4). Marker DNA (1 kb) is shown in the lane on the left.

With the finding that clones generated from *V. cinerea* B9 using the P-loop/GLPLAL-1 oligonucleotide primer pairs were highly conserved at the NBS domain, this primer set was considered to have more potential for marker development than the P-loop/Rev loop primers. Therefore, only P-loop/GLPLAL-1 primers were used to clone RGA sequences from *V. rupestris* B38 and Horizon. Twenty seven and 47 sequences were cloned from *V. rupestris* B38 and Horizon, respectively. RGAs cloned from *V. rupestris* B38 were separated into four unique groups, while 47 clones from Horizon were subdivided into seven unique groups (Table 2; Appendix Table 1A).

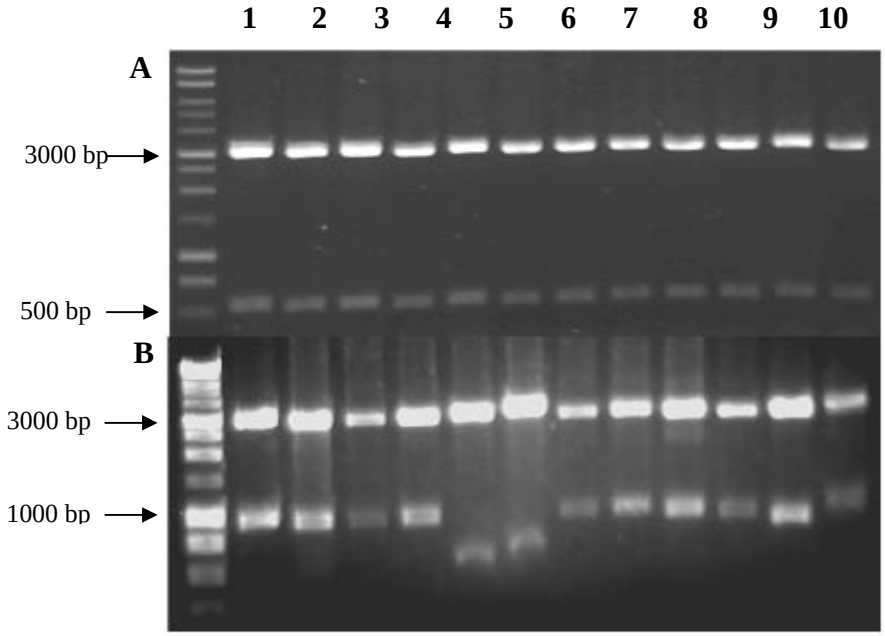


Figure 2. Selected colonies from *V. cinerea* B9 were analyzed for inserted fragments by *Eco*RI. Marker DNA (1 kb) is shown on the left lane;
(A) P-oop/GLPLAL-1 primer pair with 500 bp inserted DNA fragment;
(B) P-loop/Rev loop primer with 850 bp inserted DNA fragment
(except lanes 5 and 6).

Table 2. Number of RGA clones, number of unique clones, representative RGA clones and number of clones per group from three grape genotypes.

Genotype/ Primer Pairs	Number of RGA clones	Number of unique clones	Representative RGA clone/frequency
V. cinerea B9 P-loop/GLPLAL-1	48	8	rgVcin 109/3
			rgVcin 111/5
			rgVcin 123/6
			rgVcin 125/4
			rgVcin 127/13
			rgVcin 139/6
			rgVcin 152/8
			rgVcin 165/3
V. cinerea B9 P-loop/Rev loop	30	4	rgVcin 209/4
			rgVcin 210/6
			rgVcin 254/13
			rgVcin 269/7
V. rupestris B38 P-loop/GLPLAL-1	27	4	rgVrup 103/4
			rgVrup 119/6
			rgVrup 124/10
			rgVrup 126/7
Horizon P-loop/GLPLAL-1	47	7	rgVhyb 101/11
			rgVhyb 102/9
			rgVhyb 110/4
			rgVhyb 121/3
			rgVhyb 127/7
			rgVhyb 139/5
			rgVhyb 149/8

Sequence analysis of RGA clones

Nucleotide sequences from eight of the twelve unique groups from *V. cinerea* B9 had significant BLAST hits to RGAs in GenBank (Table 3). All of these were generated

with the P-loop/GLPLAL-1 primer pair whereas the four RGA clones from P-loop/Rev loop primers were not similar to any RGA clones in GenBank. Seven showed similarity to RGA clones that were isolated from *V. amurensis* (Di Gaspero and Cipriani, 2003). Moreover, nucleotide sequences of rgVcin109, rgVcin125, rgVcin139 and rgVcin165 were nearly or completely similar to RGA clones from *V. amurensis* (E-value = 0). Only one clone (rgVcin152) showed sequence similarity to a NBS-LRR-like gene from a non-*Vitis* species (*Oryza sativa).*

Similarly, BLASTx analysis showed that ten of twelve representative clones from *V. cinerea* B9 had amino acid sequence similarity to resistant protein candidates in GenBank (Table 3). As with the nucleotide sequences, amino acid sequences of most RGA clones were similar to resistance protein candidates from *V. amurensis*. Two exceptions among those derived from the P-loop/GLPLAL-1 primers were rgVcin123 and rgVcin152, which showed similarity with resistance protein candidates and NBS-type resistance proteins from *Gossypium barbadense* and *Mentha longifolia*, respectively.

In addition, the rgVcin254 and rgVcin269 from P-loop/Rev loop primers were similar to resistance protein candidates from *Manihot esculenta* and *V. amurensis*, respectively; however, these were not as strongly matched as the RGA clones from P-loop/GLPLAL-1 primers (Table 3).

Two out of four RGA nucleotide sequences from *V. rupestris* B38 (rgVrup103 and rgVrup126) and one out of seven sequences from Horizon (rgVhyb101) showed high similarity to nucleotide sequences of RGA clones isolated from *V. amurensis* as found in *V. cinerea* B9 (E-values = 0; Table 3). In addition, several RGA amino acid sequences from *V. rupestris* B38 (two clones) and Horizon (five clones) were similar to resistance protein candidates from *V. amurensis* and *V. riparia*, while the other clones

had similar sequences to putative disease resistance proteins from *Malus prunifolia*, *Arabidopsis thaliana* and *Theobroma cacao* (Table 3).

NBS-LRR domain

Nineteen RGAs amplified by P-loop/GLPLAL-1 primers were analyzed for the presence of conserved amino acid motifs. As expected, P-loop and GLPL motifs were present in the first seven and last six amino acids of all RGAs cloned, except rgVhyb121. Those amino acids correspond to oligonucleotide primers derived from P-loop and GLPL motifs (modified from Hunger et al., 2003). RNBS-A, kinase-2, RNBS-B and RNBS-C motifs also appeared in all RGAs cloned (Figure 3). The RNBS-A motifs could not be identified by MEME analysis because they were diffuse and poorly conserved. However, these motifs were found and verified by visual inspection of alignments. As previously suggested, kinase-2 is useful to distinguish between TIR and non-TIR proteins (Meyers et al., 1999). The presence of tryptophan in the kinase-2 motif is predictive of non-TIR proteins (e.g. *RPS2*, *RPS5*, *I2* and *Xa1*). On the other hand, *L6* and *N* from flax and *M* from tobacco have aspartic acid in the kinase-2 motif, which is typical of TIR proteins (Figure 3). The amino acid sequence of the kinase-2 motif classified rgVcin125, rgVcin152, rgVrup119, rgVrup126 and rgVhyb110 as well as *RPS2*, *RPS5*, *I2* and *Xa1*, into non-TIR proteins. While *L6*, *N*, rgVcin109, rgVcin139, rgVcin165, rgVrup103 and rgVhyb101, were classified into TIR proteins (Figure 3). Although, no aspartic acid was found in kinase-2 motif of rgVcin111, rgVcin123, rgVcin127, rgVhyb127 and rgVhyb149, but the aspartic acid was found in other translated protein frames (data not shown). These groups also appeared in the same branch with known TIR proteins as described by Figure 4. Therefore, these five RGAs cloned were classified into TIR proteins as well.

Table 3. Results of the search for similarity between *Vitis* RGA sequences with nucleotide and amino acid GenBank accessions carried out using the BLASTn and BLASTx programs.

Representative RGA clone	GenBank nucleotide accession showing the highest similarity					
	Nucleotide	(bit)	E[a]	Amino acid	(bit)	E[a]
rgVcin109/3 (DQ885292)[b]	*V. amurensis* isolate rgVamu090 gi/38045679/gb/AY427105.1/	724	0.0	Resistance protein candidate (*V. amurensis*)	267	1e-70
rgVcin111/5 (DQ885293)[b]	*V. amurensis* isolate rgVamu092	502	1e-139	Resistance protein candidate (*V. amurensis*)	254	6e-67
rgVcin123/6 (DQ885294)[b]	*V. amurensis* isolate rgVamu090	86	1e-13	NBS-type resistance protein (*Gossypium barbadense*)	115	8e-25
rgVcin125/4 (DQ885295)[b]	*V. amurensis* isolate rgVamu151 gi/38045730/gb/AY427133.1/	894	0.0	Resistance protein candidate (*V. amurensis*)	306	2e-82
rgVcin127/13 (DQ885296)[b]	*V. amurensis* isolate rgVamu092	80	7e-12	Resistance protein candidate (*V. amurensis*)	127	2e-82
rgVcin139/6 (DQ885297)[b]	*V. amurensis* isolate rgVamu053 gi/38045673/gb/AY427102.1/	876	0.0	Resistance protein candidate (*V. amurensis*)	272	3e-72
rgVcin152/8 (DQ885298)[b]	*O. sativa* clone sk98 NBS-LRR like gene	74	4e-10	Disease resistance-like protein 585-8 (*M. longifolia)*	118	8e-26
rgVcin165/3 (DQ885299)[b]	*V. amurensis* isolate rgVamu094 gi/38045681/gb/AY427106.1/	718	0.0	Resistance protein candidate (*V. amurensis*)	295	5e-79
rgVcin209/4	No significant similarity found			No significant similarity found		
rgVcin210/6	No significant similarity found			No significant similarity found		
rgVcin254/13	No significant similarity found			RCa10.6 NBS type resistance protein (*M. esculenta*)	140	5e-32
rgVcin269/7	No significant similarity found			Resistance protein candidate (*V. amurensis*)	52	3

[a]Expected (E) value refers to the number of matches expected by chance alone. The lower the E value, the more strongly supported the match is;
[b]GenBank accessions number

Table 3. Results of the search for similarity between *Vitis* RGA sequences with nucleotide and amino acid GenBank accessions carried out using the BLASTn and BLASTx program (continued).

Representative RGA clone	GenBank nucleotide accession showing the highest similarity					
	Nucleotide	(bit)	E[a]	Amino acid	(bit)	E [a]
rgVrup103 (DQ885300) [b]	*V. amurensis* isolate rgVamu094	712	0.0	Resistance protein candidate (*V. amurensis*)	292	3e-78
rgVrup119 (DQ885301) [b]	*O. sativa* clone sk98 NBS-LRR like gene	87.7	3e-14	Putative disease resistance gene analog (*Malus prunifolia*)	143	3e-33
rgVrup124 (DQ885302) [b]	*V. riparia* isolate rgVrip148	446	3e-122	Putative disease resistance gene analog (*A. thaliana*)	228	1e-58
rgVrup126 (DQ885303) [b]	*V. amurensis* isolate rgVamu151	900	0.0	Resistance protein candidate (*V. amurensis*)	195	6e-49
rgVhyb101 (DQ885304) [b]	*V. amurensis* isolate rgVamu050 gi/38045671/gb/AY427101.1/	860	0.0	Resistance protein candidate (*V. amurensis*)	248	4e-63
rgVhyb102 (DQ885305) [b]	*O. sativa* clone sk98 NBS-LRR like gene	65.9	1e-07	Probable methyeletrahydrofolate red	65	2e-04
rgVhyb110 (DQ885306) [b]	*V. riparia* isolate rgVrip068	628	4e-177	Resistance protein candidate (*V. riparia*)	248	6e-65
rgVhyb121 (DQ885307) [b]	*V. amurensis* isolate rgVamu035	261	9e-69	Resistance protein candidate (*V. amurensis*)	261	9e-69
rgVhyb127 (DQ885308) [b]	*V. riparia* isolate rgVrip004	173	3e-42	Resistance protein candidate (*V. amurensis*)	173	2e-42
rgVhyb139 (DQ885309) [b]	*O. sativa* clone sk50 NBS-LRR like gene	78.9	8e-12	NBS/LRR resistance protein-like (*Theobroma cacao*)	43.1	0.004
rgVhyb149 (DQ885310) [b]	*M. prunifolia* putative disease	67.9	3e-08	Resistance protein candidate (*V. amurensis*)	53.5	3e-06

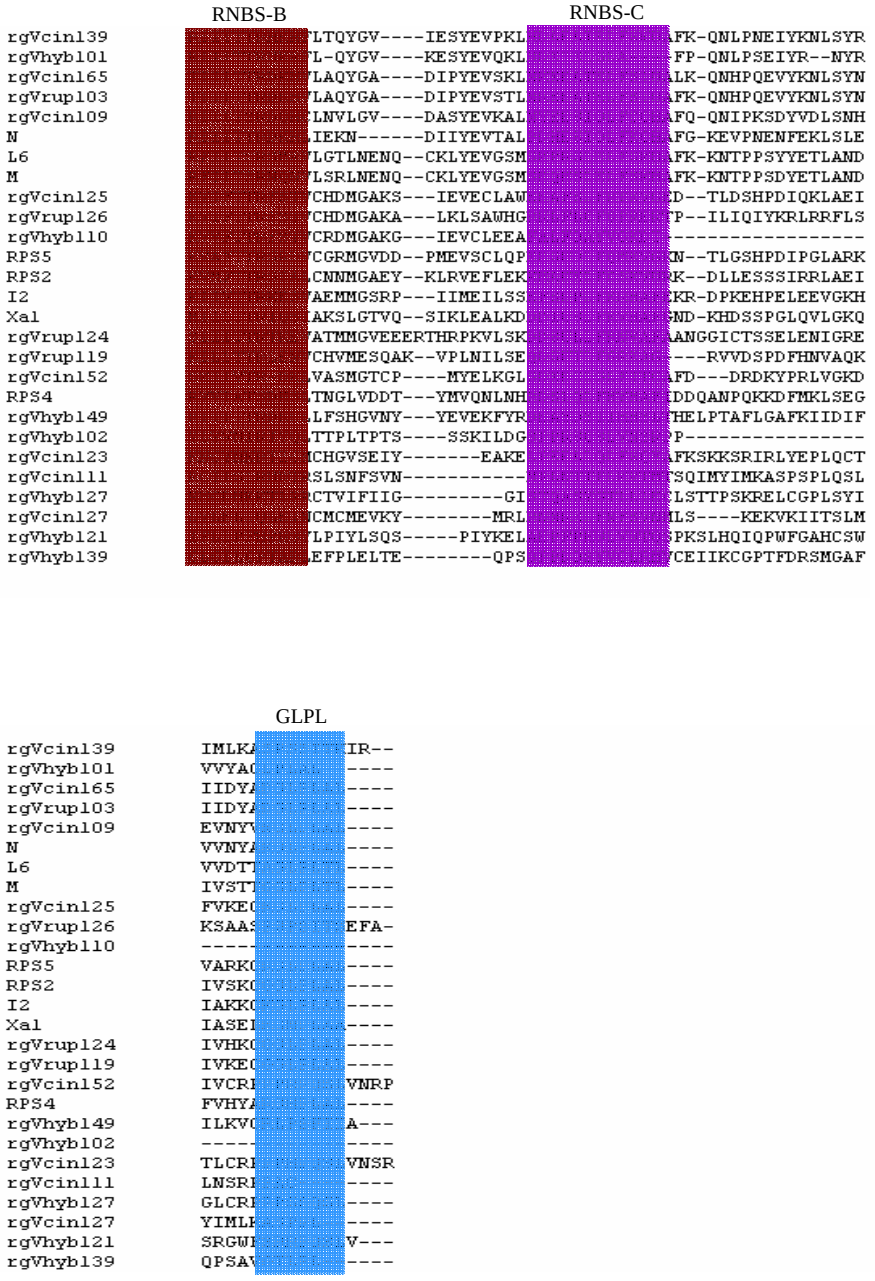
[a]Expected (E) value refers to the number of matches expected by chance alone. The lower the E value, the more strongly supported the match is;
[b]GenBank accessions number

Using the amino acid change in the kinase-2 motif to classify proteins, four out of nineteen RGAs including rgVrup124, rgVhyb102, rgVhyb121 and rgVhyb139, could be classified as neither TIR nor non-TIR types. This may be caused by the lack of some sequences in conserved domains for these clones. Therefore, phylogenetic analysis was used to verify the overall sequence similarity to other R-genes representative of the two subclasses. The unclassified RGA, rgVhyb124, can be found in the major branches along with *Xa1* from rice *(Oryza sativa)*, and *I2* from tomato *(Lycopersicon esculentum)*, as well as *Vitis* non-TIR proteins, suggesting that this clone is more closely related to non-TIR proteins than TIR proteins, respectively (Figure 4). In addition, the rgVhyb102, rgVhyb121, and rgVhyb139 were clustered in the same branch with *RPS4* and *N*, TIR proteins cloned from *Arabidopsis* and tobacco, respectively (Figure 4). Therefore, these clones are likely more closely related to TIR proteins than to non-TIR proteins. In total, nineteen RGA clones were classified into thirteen TIR-NBS-LRR-like genes and six non-TIR-NBS-LRR-like genes.

Plasmopara viticola inoculation

The parents, Horizon and Ill. 547-1, have been reported as moderately and highly resistant to downy mildew, respectively (Reisch et al., 1982; Dalbo et al., 2000). The detached leaf assay confirmed this observation. Horizon and Ill. 547-1 had 15.11 and 2.18 spores/25 cm^2, respectively (Appendix Table 2A). The progeny population segregating for resistance showed the number of downy mildew spores/25 cm^2 ranging from 2.17 to 122.11 (Figure 5; Appendix Table 2A). Eighty-seven seedlings or 48.6% were grouped into the intermediate to downy mildew (classes 2 and 3). Resistant (classes 0 and 1) and susceptible (classes 4 and 5) individuals comprised 32.4 and 19.0% of the population, respectively.

Figure 3. Multiple alignments of representative amino acid sequences of nineteen RGA clones and eight known R-genes based on ClustalW analysis. The P-loop and GLPL motifs corresponding to primer sequences are shown in the first seven and the last six amino acids, respectively. Aspartic acid (D) and tryptophan (W; underline) in the kinase-2 motif are characteristic of TIR and non-TIR proteins, respectively.



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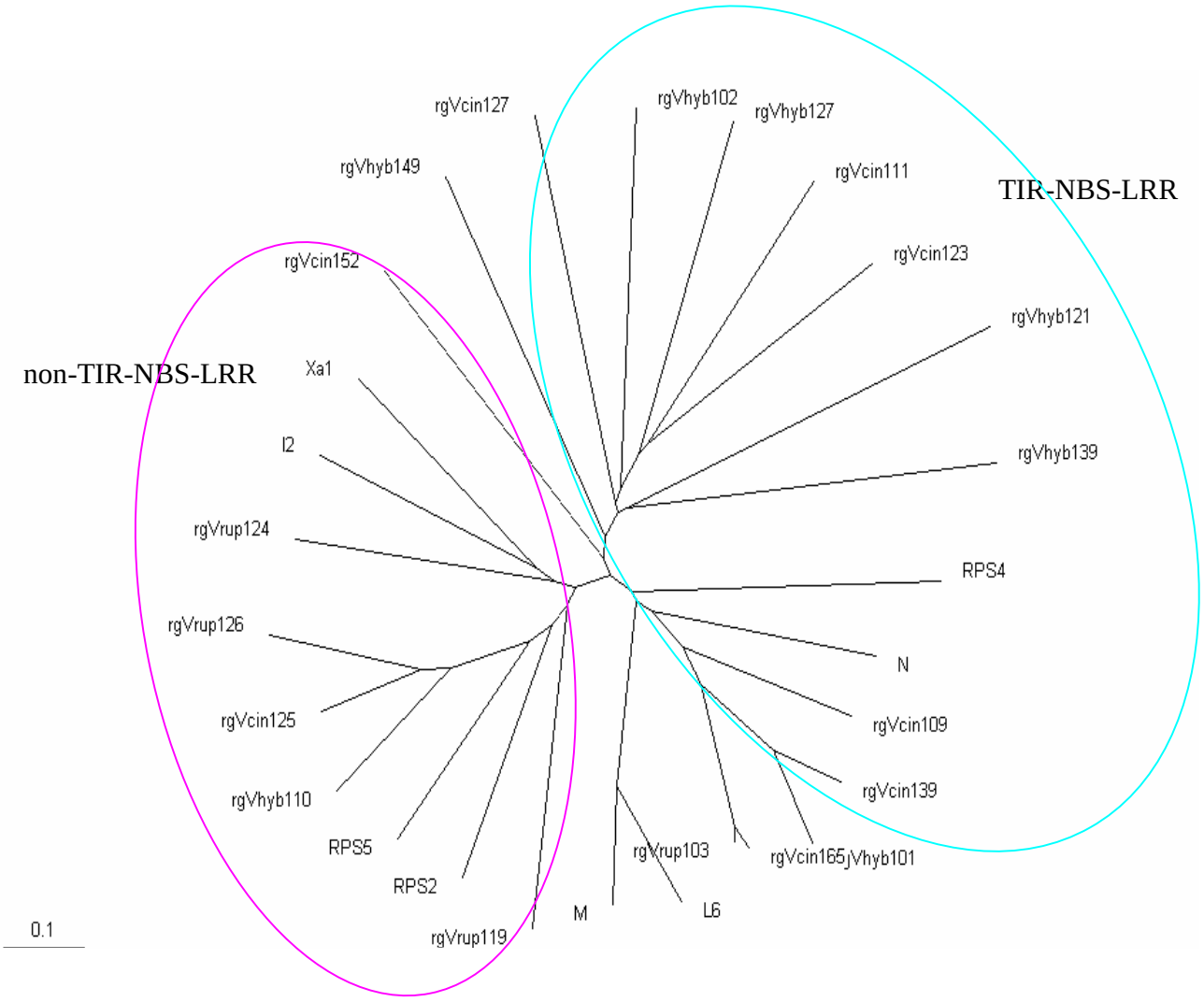


Figure 4. N-J clustering of nineteen *Vitis* RGA sequences and eight known TIR or non-TIR proteins. The scale bar in the bottom left corner displays a distance corresponding to 10% amino acid substitution per site.

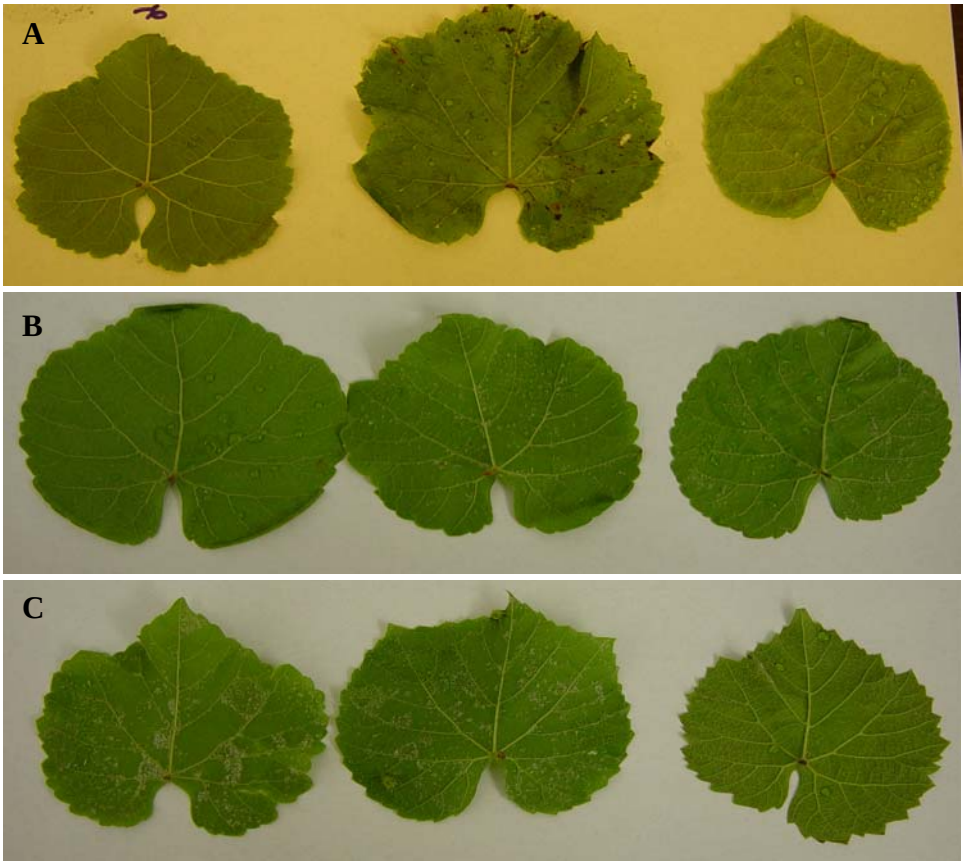


Figure 5. Downy mildew evaluation of progenies from cross ‘Horizon x Ill. 547-1’;

A) From left to right genotypes 361095 node 6, 361077 node 6 and 361025 node 5 which are resistant to downy mildew (rating score = 1);

B) From left to right genotypes 365032 node 6, 365074 node 7 and 365086 node 5 which are intermediate (rating score = 3);

C) From left to right genotypes 365065 node 5, 365077 node 7 and 361051 node 5 which are susceptible to downy mildew (rating score = 5).

RGA-STS marker

Twenty-three STS and three CAPS primer pairs were used to amplify the parents and the 179 progenies of the 'Horizon x Ill. 547-1' cross. Nine (six STS and three CAPS) out of these 26 markers were developed by Di Gaspero and Cipriani (2003), based on RGA sequences from *V. amurensis* and *V. riparia*. Surprisingly, at least four-five primer pairs from two clones, rgVcin152 and rgVhyb139, were developed but no PCR product could be amplified. Therefore, a total of 17 STS markers were developed from the 19 RGA nucleotide sequences in the present study (from *V. cinerea*, *V. rupestris* and Horizon). All of these 26 primers pairs produced polymorphic markers among 179 progenies (Figure 6).

From segregation analysis, eight STS markers including rgVamu085, GLPL6-1, rgVcin125, rgVcin127, rgVcin139, rgVrup126, rgVhyb102 and rgVhyb110 were present in the male parent (Ill. 547-1) but absent in the female parent (Horizon; Appendix Table 3A). Five STS markers (rgVamu100, rgVrup119, rgVhyb101, rgVhyb121 and rgVhyb127) were present in the female but absent in the male parent (Appendix Table 3A). The rest of the STS markers were either present or absent in both parents (Appendix Table 3A).

The chi-square goodness-of-fit test was used to check conformity of marker segregation with the expected ratio. Nine of the thirteen markers that were present in one parent but absent in the other segregated at a 1:1 ratio including GLPL6-1, rgVcin125, rgVcin127, rgVcin139, rgVrup119, rgVhyb101, rgVhyb110, rgVhyb121 and rgVhyb127 (Appendix Table 4A). Each of these markers was amplified at the locus that is heterozygous only in one parent and homozygous null in the other as described by the presence of DNA band in only one parent. These markers will be used for the analysis

of initial linkage of each parent. In contrast, thirteen markers that were either present or absent in both parents were tested for the 3:1 segregation ratio. Nine of these markers showed a 3:1 segregation ratio including rgVrip064, rgVrip145, stkVa011, rgVamu092, rgVcin109, rgVcin111, rgVcin123, rgVrup103 and rgVrup124 (Appendix Table 4A). These markers reflect heterozygosity at the locus in both parents and will not be considered in the initial linkage map construction, but will be useful for the construction of consensus map, using inter-parental phase data or bridge between parental maps in the future.

Eight markers including rgVamu085, rgVamu100, rgVamu111, rgVrip158, rgVcin165, rgVrup126, rgVhyb102 and rgVhyb149 were identified as segregation distorted because they did not follow Mendelian segregation (1:1 or 3:1 segregation). However, these markers will be mapped on parental or consensus maps unless the marker(s) show low quality or affect the order of their neighbor according to the linkage analyses performed by Adam-Blondon et al. (2004).

Significant correlation of segregation among markers was found. Based on correlation analysis among CAPS and STS markers, three groups of markers emerged (Table 4). As described above, some markers were developed by Di Gaspero and Cipriani (2003), and these have already been located on the *Vitis* map. For example, rgVamu092 is located on linkage group 13, rgVrip064 on linkage group 18 and rgVamu085 on linkage group 19 (Di Gaspero et al., 2007; linkage groups are numbered according to standards set by Riaz et al. (2004) and Doligez et al. (2006)). These data suggest that markers with a significant correlation of their segregation in the same populations are probably located on the same linkage group (Table 4). However, when the maps of Ill. 547-1 and Horizon were analyzed by MAPMAKER 3.0, there was no linkage among these markers found in both maps, except linkage between rgVcin125

and rgVhyb110 (36.2 cM and LOD score 3) in Ill. 547-1 map. Nevertheless, these genetic data were not sufficient to create a map. More genetic data from other markers such as AFLP and SSR will be required to fill in the complete map. Note that these two markers (rgVcin125 and rgVhyb110) had the strongest correlation among all markers (r = 0.604; Table 4). Therefore, the other markers with much lower correlation coefficients (0.163 – 0.315) may not necessarily be located in the expected linkage group. The placement of these markers on fine *Vitis* map is needed to verify these linkages.

Interestingly, significant correlation was found between segregation for number of spores (index of downy mildew resistance) and segregation of three markers, rgVamu085, stkVa011 and rgVcin165 (r = -0.173, 0.152 and 0.151, respectively; Appendix Table 5A). Nevertheless, correlation coefficients of these three markers were quite low, suggesting that these markers may not necessarily be located near resistance allele(s). In addition, among the three markers only stkVa011 segregated at a ratio of 3:1, while rgVamu085 and rgVcin165 exhibited distorted segregation. The stkVa011 and rgVcin165 also had DNA bands present in both resistant (male) and susceptible (female) parents, complicating the association of DNA bands with either resistance or susceptible allele(s). In rgVamu085 which showed DNA polymorphism between parents (the DNA band was present in Ill. 547-1 but absent in Horizon), only 15.5% of the 58 resistant progenies expected to possess the DNA band showed no band, suggesting that this marker might be located at the same linkage group as the resistance allele(s) but may not be tightly linked. Even though, the mismatch of rgVamu085 was greater than 5% and the correlation coefficients of these three markers were low, they might be useful in future mapping attempt when more markers were utilized. The markers that co-segregate with a gene are usually useful for MAS when the mismatch is about 5% or lower.

Table 4. STS markers on three expected linkage groups and correlation analysis for marker segregation within each group.

STS marker	Expected LGs[b]	Correlation within the group	
		Marker	r[c]
rgVamu092[a]	13	rgVamu092 -rgVcin109	0.248**
rgVcin109[a]		rgVamu092 -rgVcin165	0.163*
rgVcin123		rgVcin109 -rgVcin165	0.240**
rgVcin165		rgVcin109 -rgVhyb149	0.258**
rgVhyb121		rgVcin109 -rgVhyb121	0.288**
rgVhyb149		rgVhyb121 -rgVamu092	0.174*
		rgVhyb121 -rgVcin165	0.261**
		rgVhyb121 -rgVcin123	0.266**
		rgVcin123 -rgVamu092	0.188*
rgVrip064[a]	18	rgVrip064 -rgVhyb101	-0.128**
rgVcin139[a]		rgVcin139 -rgVhyb101	0.315**
rgVhyb101[a]			
rgVamu085[a]	19	rgVamu085 -rgVcin125	-0.254**
rgVcin111		rgVamu085 -rgVhyb110	-0.301**
rgVcin125[a]		rgVcin125 -rgVhyb110	0.604**
rgVrup126		rgVcin125 -rgVcin111	-0.201*
rgVhyb110[a]		rgVhyb110 -rgVrup126	0.282**

[a]closely related original nucleotide sequences in the group
[b]lingkage groups; [c] correlation coefficient;
[*]significant at $P < 0.05$; [**]significant at $P < 0.01$

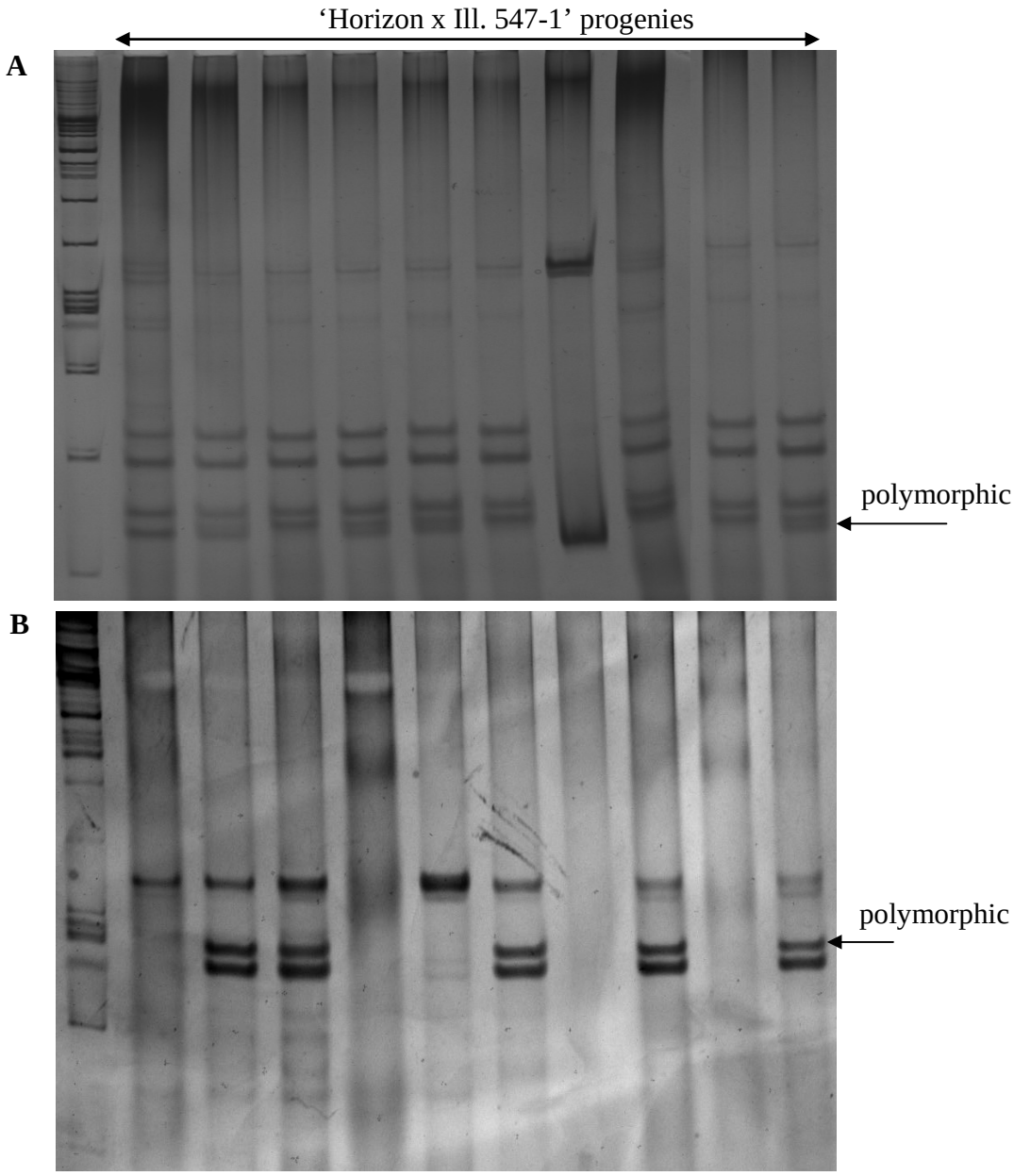


Figure 6. STS markers were applied to progenies of the 'Horizon x Ill. 547-1' cross:

A) rgVcin125

B) rgVrup 119.

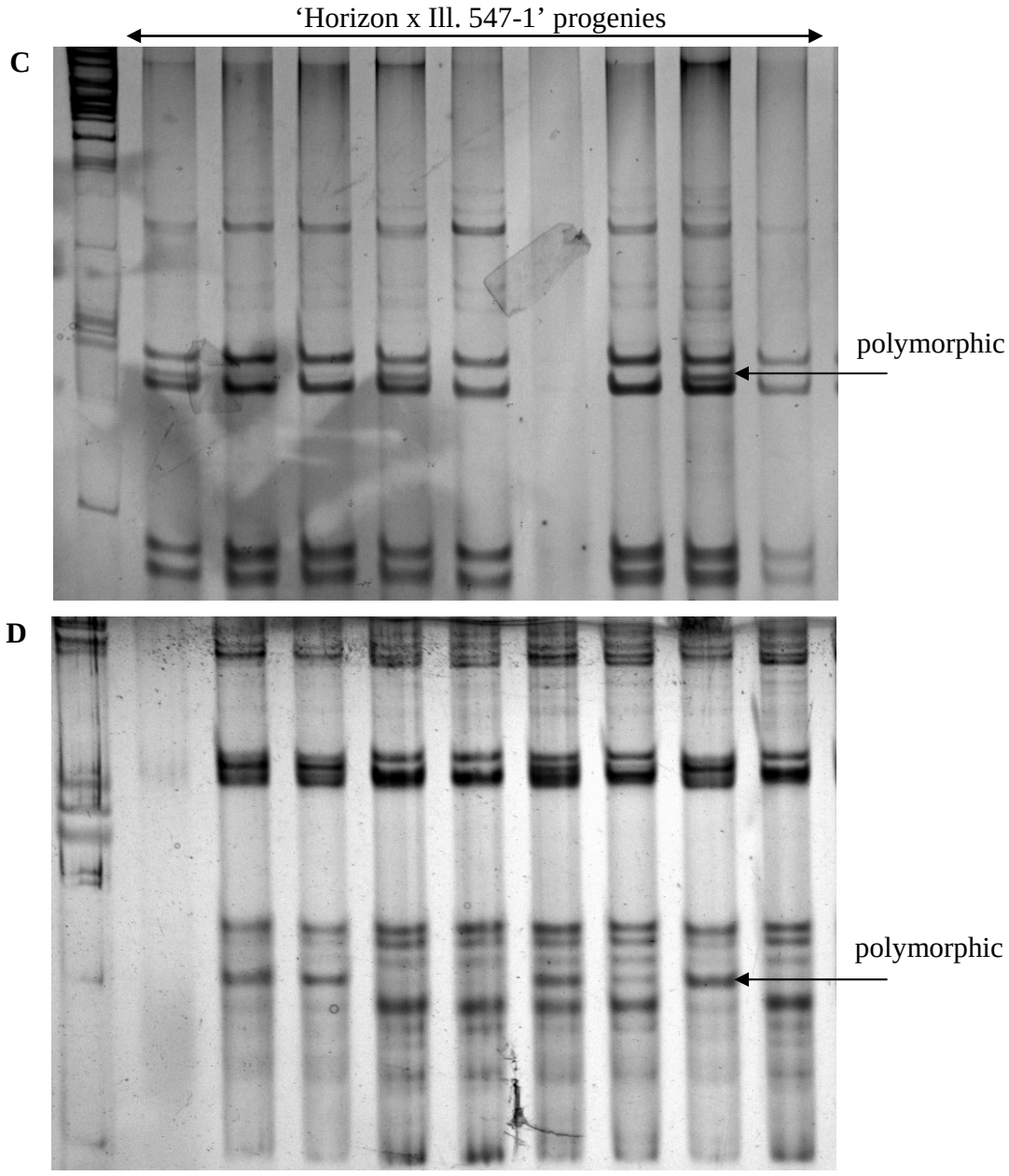


Figure 6. STS markers were applied to progenies of the 'Horizon x Ill. 547 -1' cross (continued):

C) rgVhyb 127

D) rgVrip145.

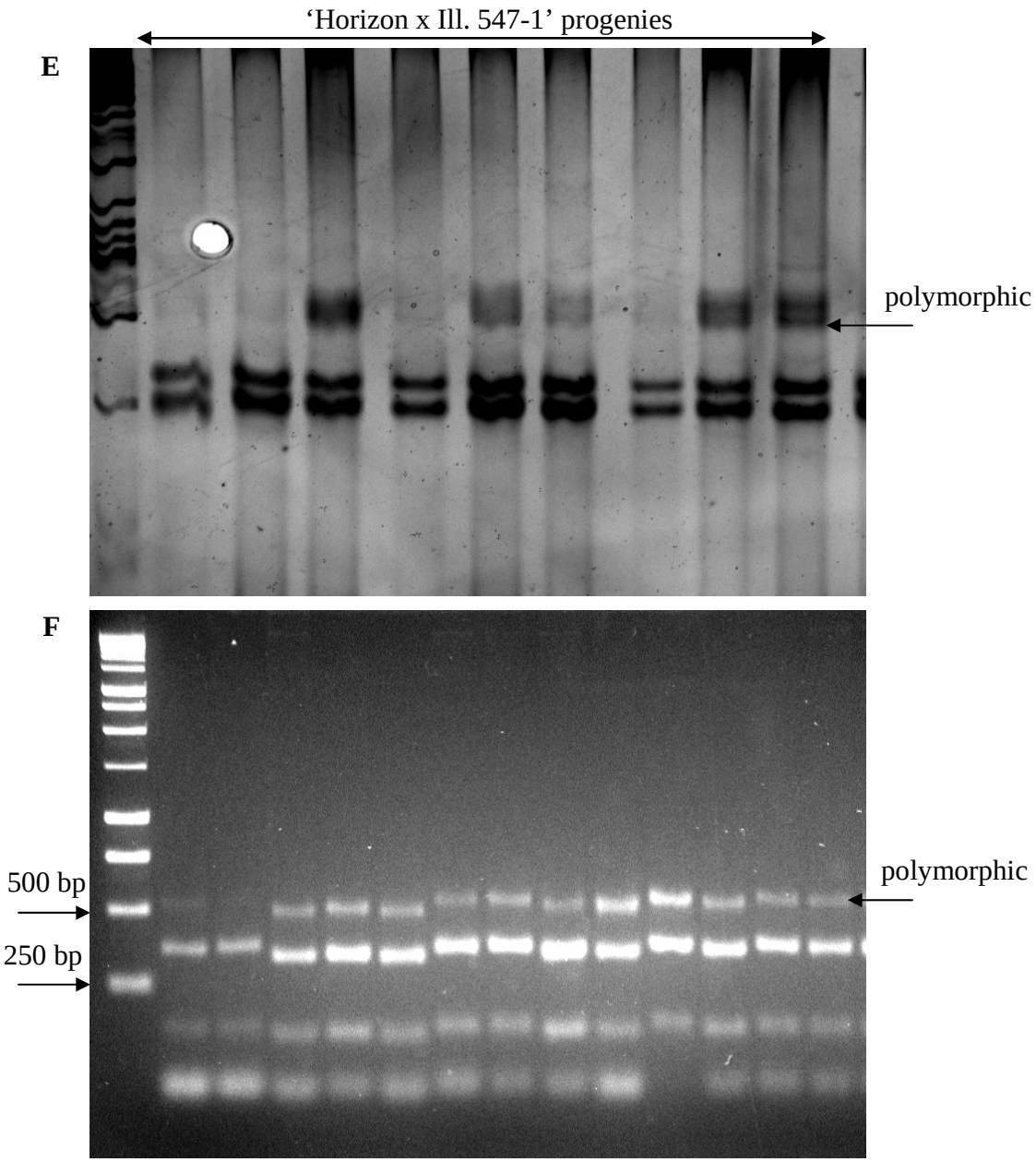


Figure 6. STS markers were applied to progenies of the 'Horizon x Ill. 547-1' cross

(continued):

E) rgVamu 111

F) stkVa011.

3.5 Discussion

Nineteen unique groups of RGA sequences from *V. cinerea* B9, *V. rupestris* B38 and Horizon have been cloned from P-loop/GLPLAL-1 PCR, and four unique groups were derived from *V. cinerea* B9 by P-loop/Rev loop PCR. The P-loop/Rev loop primers produced sequence data with a low percent match with known RGAs in GenBank. These data suggest that the P-loop/GLPLAL-1 primer pair used is highly conserved at the NBS domain and have greater potential for cloning RGAs from grape, compared with P-loop/Rev loop primer pair.

Most of the RGA clones from the three grape genotypes used here displayed similarity with RGA clones from *V. amurensis*, especially seven RGA clones that showed complete similarity to RGAs cloned from *V. amurensis*. The similarity with RGAs cloned from *V. riparia* was also found. Interestingly, *V. amurensis*, *V. cinerea*, *V. riparia* and *V. rupestris* have been reported as being highly resistant to downy mildew and powdery mildew (He and Wang, 1986; Eibach et al., 1989; Alleweldt et al., 1990). Moreover, none of the nineteen *Vitis* RGA clones showed similarity to sequences from susceptible species such as *V. vinifera*.

Di Gaspero and Cipriani (2002) presented evidence that RGA sequences from *V. amurensis* and *V. riparia* had a high probability of linkage with disease resistance genes in *Vitis* germplasm. Since these RGAs were found in several grape species which are resistant to diseases such as downy mildew and powdery mildew, they could possibly be linked to or be candidate genes for disease resistance (Di Gaspero and Cipriani, 2002). The nineteen *Vitis* RGA sequences from resistant genotypes may also be linked to disease resistance loci in *Vitis*, but further segregation studies will be needed to verify this.

NBS-LRRs domain

The NBS-LRR protein is the largest class of R proteins. To date at least 40 known R proteins from several plants are in the NBS-LRR family (Qu et al., 2006). This family is abundant in plants, but absent from organism in other kingdoms.

The LRR domain is comprised of a repeat of approximately 25-38 amino acids of leucine and other hydrophobic residues (Dixon et al., 1998). The LRR domain is involved in both downstream signal pathway and defense signal transduction in plants by recognition of the pathogen ligand (Aarts et al., 1998; Falk et al., 1999; Parker et al., 1997). The NBS-domain also has an important role in activating downstream signal transduction pathway and specific recognition for pathogen. This domain mediates activation of the N-terminal signaling domain (TIR and/or nonTIR). The N-terminus of the NBS domain functions in signal transduction by mediating homotypic or heterotypic protein-protein interactions and/or interacts with downstream signaling components (Koop and Modzihitov, 1999; Feys and Parker, 2000; Yu et al., 1996).

As described above, the N-terminal domain can be divided into two subclasses, TIR and non-TIR proteins. TIR-containing proteins are mostly found in dicot plants and are related to proteins that participate in signal transduction pathways (Meyers et al., 1999; Goff et al., 2002). Sixty-three percent of NBS-LRR genes in *Arabidopsis* are classified into TIR group. The non-TIR-NBS-LRR genes are less frequent in plants and are reported as containing CC motifs (Meyers et al., 2003). The CC motif is a bundle of two to five helices with two interacting hydrophobic amino acids at the helix-helix interface and functions in downstream signaling (Lupas, 1996; Century et al., 1997; Parker et al., 1997). The non-TIR-NBS-LRR and TIR-NBS-LRR genes have shown differences in their reduced defense response to pathogens. TIR and non-TIR-NBS-LRR genes have shown that TIR sequences operate through an *eds-1* dependent pathway,

while some of non-TIR sequences operate through the *ard-1* pathway (Aarts et al., 1998). These data suggest that amino-terminal TIR or CC motifs play a role in the bifurcation of the signal transduction pathway.

Interestingly, the amino acids in the conserved motif of the NBS domain can predict the structure at the N-terminal domain. Meyers et al. (2003) found that trytophan or aspartic acid in the Kinase-2 motif had been strongly correlated with the type of protein in the N-terminal domain (Meyers et al., 1999). Six RGA clones were classified as non-TIR by the amino acid sequence of Kinase-2 motif. These clones were also classified as non-TIR when comparing amino acid sequence identity with other R genes by clustalW (Figure 4). This result supports the high degree of accuracy (90%) to classify sequences as either TIR or non-TIR by the amino acids in kinase-2 motif, as has been previously suggested by Meyers et al. (2003).

Resistance gene evolution

Plants respond to pathogens by direct and/or indirect interaction between plant R genes and *avr* genes in the pathogen (Van der Biezen and Jones, 1998; Dangl and Jones, 2001; Luderer and Joosten, 2001). Gene for gene interactions may increase resistance to pathogens if R genes increase the ability to recognize the pathogen. The LRR and TIR/non-TIR have a role in pathogen recognition and also signal transduction, therefore the unbalance selections were needed to recognize rare *avr* gene product in pathogen population (Zhou et al., 2004). The mechanisms including chromosome breaking, rearrangement, pre-existing divergent duplication, unequal crossing over, gene conversion and diversifying selection have been proposed to generate diversity in LRR and TIR/non-TIR domains (Michelmore and Meyers, 1998; Ellis et al., 2000; Richter and Ronald, 2000; Young, 2000).

The diversifying selection is the potential of genomic instability mediated by unequal crossing over in meiosis (Parniske et al., 1997; Richter and Ronald, 2000). Interestingly, diversifying selection has been found in non-TIR-NBS-LRR rather than TIR-NBS-LRR genes. As shown in rice, there is no significant increase in number of similar or almost identical genes within non-TIR-NBS-LRR whereas copies of similar TIR-NBS-LRR genes were duplicated (Zhou et al., 2004). Their data support the hypothesis that non-TIR-NBS-LRR genes are more highly variable than TIR-NBS-LRR. The difference in diversity between TIR and non-TIR domains was also present in the NBS-LRR domain of the three grape genotypes in the present study. The DNA sequences among the TIR-NBS-LRR group displayed high similarity, ranging from 73.5% to 97.4% (for example, rgVcin111/rgVcin123, *L*6/*M* and rgVcin165/rgVrup103 have 73.5, 78.2 and 97.4% identity, respectively). On the other hand, the amino acid sequence in the non-TIR-NBS-LRR group showed more variation within groups, with similarities ranging from 43.0 to 97.4% (for example, *RPS*2/*RPS*5, rgVhyb110/rgVrup119 and rgVhyb110/rgVcin125 have 43.0, 57.5, 77.1% identity, respectively). The shorter branch lengths among TIR-NBS-LRR groups as compared to non-TIR-NBS-LRR groups support the hypothesis that these proteins are more highly conserved (Figure 4). Therefore, in terms of gene diversification, non-TIR-NBS-LRR might be more adaptively responsive and selection acting on this fluidity could lead to the more rapid development of pathogen recognition.

Potential of *Vitis* RGAs as molecular markers

Molecular markers based on RGAs have been developed from conserved domains of diverse plant species (Michelmore, 1996; Baker et al., 1997; Hammond-Kosack and Jones, 1997). The RGA sequences cloned in the present study from highly

conserved domains of three disease resistant grape genotypes will hopefully be useful for the development of markers linked to disease resistance. Due to their complete association with resistance-like genes, RGA markers may have the potential to improve the efficacy of MAS for disease resistance traits. In total 26 STS markers were developed; eight STS markers had a non-normal segregation ratio. These eight markers will be excluded from mapping program because their allele frequencies are not in Hardy-Weinberg equilibrium. Therefore, only eighteen STS markers from this study will be used to create grape linkage map. The five STS markers (GLPL6-1, rgVcin125, rgVcin127, rgVcin139 and rgVhyb110) will be used for the analysis of initial linkage of Ill. 547-1, and four STS makers (rgVrup119, rgVhyb101, rgVhby121 and rgVhyb127) for the Horizon map. On the other hand, nine STS markers that showed a 3:1 segregation ratio including rgVrip064, rgVrip145, stkVa011, rgVamu092, rgVcin109, rgVcin111, rgVcin123, rgVrup103 and rgVrup124 will be used to create a consensus map. Many of these RGA-STS markers are expected to map to linkage groups 13, 18 and 19 due to correlation coefficients among group as shown on Table 4. Interestingly, RGA-STS markers from Di Gaspero and Cipriani (2003) also showed high numbers on these three linkage groups from Cabernet Sauvignon, Bianca and Chardonnay maps (Di Gaspero et al., 2007). This is suggestive that RGAs cluster on linkage groups 13, 18 and 19 of the *Vitis* genome. However, further proof of this is required through final map placement of the RGA-STS markers identified here. Future work will also be able to determine whether RGAs in grapevine are responsible for disease resistance, and to relate each RGA to the disease it affects.

3.6 References

Aarts, M.G.M., te Lintel Hekkert, B., Holub, E.B., Beynon, J.L., Stiekema, W.J. and Pereira, A. (1998). Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. **Mol. Plant-Microbe Interact.** 11:251-258.

Adam-Blondon, A.F., Roux, C., Claux, D., Butterlin, G., Merdinoglu, D. and This, P. (2004). Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. **Theor. Appl. Genet.** 109:1017-1027.

Agrios, G.N. (1997). **Plant Pathology.** New York: Academic.

Alleweldt, G., Spiegel-Roy, P. and Reisch, B. (1990). Grape (*Vitis*). Quoted in J.N. Moore and J.R. Ballington. (1996). Genetic resources of temperate fruit and nut crops. **Acta. Hort.** 290:291-337.

Bailey, T.L. and Elkan, C. (1994). Fitting a mixture model by expectation maximization to discover motifs in biopolymers. In **Proc. of 2nd Int. Conf. on Intelligent Systems for Molecular Biology** (pp 28-36). Menlo Park, USA.

Baker, B., Zambryski, P., Stazkawicz, B. and Dinesh-Kumar, S.P. (1997). Signaling in plant-microbe interactions. **Science** 276:726-733.

Baker, C.L., Donald, T.M., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., Thomas, M.R. and Dry, I.B. (2005). Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*,using a bacterial artificial chromosome library. **Theor. Appl. Genet.** 111:370-377.

Baldi, P., Patocchi, A., Zini, E., Toller, C., Velasco, R. and Komjanc, M. (2004). Cloning and linkage mapping of resistance gene homologues in apple. **Theor. Appl. Genet.** 109:231-239.

Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J. and Staskawicz, B.J. (1994). *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. **Science** 265:1856-1860.

Century, K.S., Shapiro, A.D., Repetti, P.P., Dahlbeck, D., Holub E. and Staskawicz, B.J. (1997). *NDR1*, a pathogen-induced component required for *Arabidopsis* disease resistance. **Science** 278:1963-1965.

Chen, M., SanMiguel, P. and Bennetzen, J.L. (1998). Sequence organization and conservation in *sh2/a1*-homologous regions of sorghum and rice. **Genetics** 148:435- 443.

Dalbo, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M. and Reisch, B.I. (2000). A gene controlling sex in grapevines placed on a molecular marker-based genetic map. **Genome** 43:333-340.

Dalbo, M.A., Ye, G.N., Weeden, N.F., Wilcox, W.F. and Reisch, B.I. (2001). Marker-assisted selection for powdery mildew resistance in grapes. **J. Amer. Soc. Hort. Sci.** 126:83-89.

Dangl, J.L. and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. **Nature** 411:826-833.

Di Gaspero, G. and Cipriani, G. (2002). Resistance gene analogs are candidate markers for disease-resistance genes in grape (*Vitis* spp.). **Theor. Appl. Genet.** 106:163-172.

Di Gaspero, G. and Cipriani, G. (2003). Nucleotide binding site/leucine-rich repeats, Pto-like and receptor-like kinases related to disease resistance in grapevine. **Mol. Gen. Genomics** 269:612-623.

Di Gaspero, G., Cipriani, G., Adam-Blondon, A.F. and Testolin, R. (2007). Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for R-gene candidates. **Theor. Appl. Genet.** 114:1249-1263.

Dixon, M.S., Hatzixanthis, K., Jones, D.A., Harrison, K. and Jones, J.D.G. (1998). The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. **Plant Cell** 10:1915-1925.

Dodds, P.N., Lawrence, G.J. and Ellis, J.G. (2001). Six amino acid changes confined to The LRR beta-strand/beta-turn motif determine the difference between the *P* and *Ps* rust resistance specificities in flax. **Plant Cell** 13:163-178.

Doligez, A., Adam-Blondon, A.F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglud, D., Meridith, C.P., Riaz, S., Roux, C. and This, P. (2006). An integrated SSR map of grapevine based on five mapping populations. **Theor. Appl. Genet.** 113:369-382.

Donald, T.M., Pellerone, F., Adam-Blondon, A.F., Bouquet, A., Thomas, M.R. and Dry, I.B. (2002). Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. **Theor. Appl. Genet.** 104:610-618.

Eibach, R., Diehl, H. and Alleweldt, G. (1989). Untersuchungen zur Vererbung von Resistenzeigenschaften bei Reben gegen *Oidium tuckeri*, *Plasmopara viticola* und *Botrytis cinerea*. **Vitis** 28:209-228. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Ellis, J., Dodds, P. and Pryor, T. (2000). Structure, function and evolution of plant disease resistance genes. **Curr. Opin. Plant Biol.** 3:278-284.

Falk, A., Feys, B.J., Frost, L.N., Jones, J.D.G., Daniels, M.J. and Parker, J.E. (1999). *EDS1*, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. **Proc. Natl. Acad. Sci. USA** 96:3292-3297.

Feys, B.J. and Parker, J.E. (2000). Interplay of signaling pathways in plant disease resistance. **Trends Genet.** 16:449-455.

Goff, S.A., Ricke, D. and Lan, T.H. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). **Science** 296:92-100.

Gedil, M.A., Slabaugh, M.B., Berry, S., Johnson, R., Michelmore, R., Miller, J., Gulya, T. and Knapp, S.J. (2001). Candidate disease resistance genes in sunflower cloned using conserved nucleotide-binding site motifs: Genetic mapping and linkage to the downy mildew resistance gene *PI*1. **Genome** 44:205-212.

Gowda, B.S., Miller, J.L., Rubin, S.S., Sharma, D.R. and Timko, M.P. (2002). Isolation, sequence analysis, and linkage mapping of resistance-gene analogs in cowpea (*Vigna unguiculata* L. Walp.). **Euphytica** 126:365-377.

Hammond-Kosack, K.E. and Jones, J.D. (1997). Plant disease resistance genes. **Annu. Rev. Plant Physiol. Mol. Biol.** 48:575-607.

Hammond-Kosack, K.E. and Jones, J.D. (2000). Response to plant pathogens. In B.B. Buchanan, W. Gruissem, R.L. Jones (eds.) **Biochemistry and Molecular Biology of Plants**. Rockville: American Society of Plant Physiologists.

He, P. and Wang, G. (1986). Studies on the resistance of wild *Vitis* species native to China to downy mildew, *Plasmopara viticola* (Berk. et Curtis) Berl. et de Toni. **Acta. Hort. Sinica** 13:17-24.

Hulbert, S.H., Webb, C.A., Smith, S.M. and Sun, Q. (2001). Resistance gene complexes: evolution and utilization. **Annu. Rev. Phytopathol.** 39:285-312.

Hunger, S., Di Gaspero, G., Möhring, S., Bellin, D., Schäfer-Pregl, R., Borchardt, D.C., Durel, C.F., Weber, M., Weisshaar, B., Salamini, F. and Schneider, K. (2003). Isolation and linkage analysis of expressed disease-resistance gene analogues of sugar beet (*Beta vulgaris* L.). **Genome** 46:70-82.

Irigoyen, M.L., Ferrer, E. and Loarce, Y. (2006). Cloning and characterization of resistance gene analogs from *Avena* species. **Genome** 49:54-63.

Jemstad, K.D., Sheppard, L.A., Kinloch, B.B., Delfino-Mix, A., Ersoz, E.S., Krutovsky, K.V. and Neale, D.B. (2006). Isolation of full-lenght CC-NBS-LRR resistance gene analog candidate from sugar pine showing low nucleotide diversity. **Tree Genet. Genomes** 2:76-85.

Jones, J.D.G. (2001). Putting knowledge of plant disease resistance genes to work. **Curr. Opin. Plant Biol.** 4:281-287.

Joyeux, A., Fortin, M.G., Mayerhofer, R. and Good, A.G. (1999). Genetic mapping of plant disease resistance gene homologues using a minimal *Brassica napus* L. population. **Genome** 42:735-743.

Keen, N.T. (1990). Gene-for-gene complementarity in plant-pathogen interactions. **Annu. Rev. Genet.** 24:447-463.

Kennelly, M.M., Gadoury, D.M., Wilcox, W.F., Magarey, P.A. and Seem, R.C. (2005). Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. **Phytopathol.** 95:1445-1452.

Koop, E.B. and Modzihitov, R. (1999). The Toll-receptor family and control of innate immunity. **Curr. Opin. Immunol.** 11:13-18.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, J.M., Lincoln, S.E. and Newberg, L. (1978). Mapmaker : an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. **Genomics** 1:174-181.

Lawrence, G.J., Finnegan, E.J., Ayliffe, M.A. and Ellis, J.G. (1995). The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. **Plant Cell** 7:1195-1206.

Lodhi, M.A., Ye, G.N., Weeden, N.F. and Reisch, B.I. (1994). A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. **Plant Mol. Biol. Rep.** 12:6-13.

Luderer, R. and Joosten, M.H.A.J. (2001). Avirulence proteins of plant pathogens: determinants of victory and defeat. **Mol. Plant Pathol.** 2:355-364.

Lupas, A. (1996). Coiled colis: New structures and new functions. **Trends Biochem. Sci.** 21:375-382.

Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Frary, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D. (1993). Map-based cloning of a protein kinase gene conferring disease resistance in tomato. **Science** 262:1432-1436.

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W. and Young, N.D. (1999). Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. **Plant J.** 20:317-332.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R.W. (2003). Genome- wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. **Plant Cell** 15:809-834.

Michelmore, R. (1996). Flood warning – resistance genes unleashed. **Nature Genet.** 14:376-378.

Michelmore, R.W. and Meyers, B.C. (1998). Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. **Genome Res.** 8:1113-1130.

Miklas, P.L., Hu, J., Grunwald, N.J. and Larsen, K.M. (2006). Potential application of TRAP (targeted region amplified polymorphism) markers for mapping and tagging disease resistance traits in common bean. **Crop Sci.** 46:910-916.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M. (1998). The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. **Plant Cell** 10:1307-1319.

Mindrinos, M., Katagiri, F., Yu, G.L., Ausubel, F.M., Brown, K.L., Giraudat, R., Leung, J. and Staskawicz, B.J. (1994). The *Arabidopsis thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. **Cell** 78:1089-1099.

Müller, K. and Sleumer, H. (1934). Biologische Untersuchungen über die Peronosporakrankheit des Weinstocks mit besonderer Berücksichtigung ihrer Bekäampfung nach Inkubationsmethode. **Z. Wiss. Landwirtsch** 79:509-576. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Owens, C.L. (2003). SNP detection and genotyping in *Vitis*. **Acta. Hort.** 603:139-140.

Parker, J.E., Coleman, M., Szabo, V., Frost, L., Schmidet, R., Biezen, E.V.D., Moores, T., Dean, C. and Jones, J.D.G. (1997). The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the Toll and Interleukin-1 receptors with *N* and *L6*. **Plant Cell** 9:879-894.

Parniske, M., Hammond-Kosack, K.E., Golstein, C., Thomas, C.M., Jones, D.A., Harrison, K., Wulff, B.B.H. and Jones, J.D.G. (1997). Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf*-4/9 locus of tomato. **Cell** 91:821-832.

Pflieger, S., Lefebvre, V. and Causse, M. (2001). The candidate gene approach in plant genetics: **a review Mol. Breed.** 7:275-291.

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B. and Wang, G. (2006). The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-luecine-rich repeat protein and is a member of a multigene family in rice. **Genetics** 172:1901-1914.

Radwan, O., Bouzidi, M.F., Nicolas, P. and Mouzeyar, S. (2004). Development of PCR markers for the *Pl5*/*Pl8* locus for resistance to *Plasmopara halstedii* in sunflower, *Helianthus annuus* L., from complete CC-NBS-LRR sequences. **Theor. Appl. Genet.** 109:176-185.

Riaz, S., Dangl, G.S., Edwards, K.J. and Meredith, C.P. (2004). A molecular marker based framework linkage map of *Vitis vinifera* L. **Theor. Appl. Genet.** 108:864-872.

Richter, T.E. and Ronald, P.C. (2000). The evolution of disease resistance genes. **Plant Mol. Biol.** 42:195-204.

Reisch, B.I., Robinson, W.B., Kimball, K., Pool, R. and Watson, J. (1982). 'Horizon' grape. **New York's Food and Life Sciences Bulletin** 96:1-2.

Saraste, M., Sibbald, P.R. and Wittinghofer, A. (1990). The P-loop – a common motif in ATP- and GTP- binding protein. **Trends Biochem. Sci.** 15:430-434.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. **Science** 270:1804-1806.

Soriano, J.M., Vilanova, S., Romero, C., Llacer, G. and Badenes, M.L. (2005). Characterization and mapping of NBS-LRR resistance gene analogs in apricot (*Prunus armenia*ca L.). **Theor. Appl. Genet.** 110:980-989.

Taler, D., Galperin, M., Benjamin, I., Cohen, Y. and Kenigsbuch, D. (2004). Plant *eR* genes that encode photorespiratory enzyme confer resistance against disease. **Plant Cell** 16:172-184.

Toojinda, T., Broers, L.H., Chen, X.M., Hayes, P.M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R.F., Powell, W., Ramsay, L., Livar, H. and Waugh, R. (2000). Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). **Theor. Appl. Genet.** 101:580-589.

Van der Biezen, E.A. and Jones, J.D.G. (1998). Plant disease resistance proteins and the gene-for- gene concept. **Trends Biochem. Sci.** 23:454-456.

Warren, R.F., Henk, A., Mowery, P., Holub, E. and Innes, R.W. (1998). A mutation within the leucine-rich repeat domain of *Arabidopsis* disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. **Plant Cell** 10:1439-1452.

Whitham, S., Dinesh-Kumar, S.P., Choi, D., Hehl, R., Corr, C. and Baker, B. (1994). The product of the tobacco mosaic virus resistance gene *N*: similarity to Toll and the interleukin-1 receptor. **Cell** 78:1101-1115.

Yan, G.P., Chen, X.M., Line, R.F. and Wellings, C.R. (2003). Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. **Theor. Appl. Genet.** 106:636-643.

Young, N.D. (2000). The genetic architecture of resistance. **Curr. Opin. Plant Biol.** 3:285-290.

Yu, Y.G., Buss, G.R. and SaghaiMaroof, M.A. (1996). Isolation of superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. **Proc. Natl. Acad. Sci. USA** 93:11751-11756.

Zhang, Y., Dorey, S., Swiderski, M. and Jones, J.D.G. (2004). Expression of *RPS4* in tobacco induces an AvrRps4-independent HR that requires *EDS1*, *SGT1* and *HSP90*. **Plant J.** 40:213-224.

Zhang, Y., Zhang, S., Qi, L., Liu, B., Xiong, B., Gao, J., Chen, X., Chen, C., Li, X. and Song, W. (2006). Cloning and characterization of disease resistance gene analogs from poplar (*Populus tremula*) chromosome 1. **Int. J. Plant Sci.** 167(3):403-412.

Zhou, T., Wang, Y., Chen, J.Q., Araki, H., Ping, Z., Jiang, K., Shen, J. and Tian, D. (2004). Genome-wide identification of NBS genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. **Mol. Gen. Genomics** 271:402-415.

Zhu, H., Cannon, S.B., Young, N.D. and Cook, D.R. (2002). Phylogeny and genomic organization of the TIR and non-TIR NBS-LRR resistance gene family in *Medicago truncatula*. **MPMI.** 15:529-539.

CHAPTER IV

INHERITANCE OF DOWNY MILDEW (*Plasmopara viticola*) RESISTANCE IN TABLE GRAPE (*Vitis spp.*)

4.1 Abstract

Downy mildew caused by *Plasmopara viticola* has been recognized as one of the major grape diseases worldwide including Thailand. Nine factorial crosses between three resistant genotypes and three susceptible cultivars were made in year 2004 to study for gene action. Resistant genotypes, NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05, were used as male parents and three susceptible cultivars of *Vitis vinifera* L., Black Queen, Carolina Black Rose and Italia, were used as female parents. A total of 120 seedlings were obtained from all crosses, but only 102 survived in year 2006. Eighty-three of healthy seedlings were evaluated for downy mildew resistance by detached leaf assay based on number of spores per 25 cm^2 leaf area in year 2006. The female parents were categorized as either highly susceptible (Black Queen, rating score = 4.15) or susceptible (Carolina Black Rose and Italia, rating scores = 3.64 and 3.87, respectively). On the other hand, the male parents, NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05, with the rating scores of 0.48, 0.96 and 0.46, respectively were resistant or highly resistant to downy mildew. Among all crosses, approximately 56.6% of the F_1 hybrids were shown to be susceptible to downy mildew, while 25.3% were resistant and 18.1% were intermediate. The cross 'Carolina Black Rose x NY 65.0550.04' gave the highest proportion of resistant seedlings (75.0%) and the lowest number of susceptible

seedlings (12.5%). The gca variance in male parents was highly significant (5.30; $P < 0.01$). The variances of gca in female and sca were not significant (being 0.56 and 1.85, respectively), indicating that additive gene action is important for downy mildew resistance. And the male parents contributed more additive gene effects to the inheritance of this trait than female parents. The estimated narrow sense heritability of downy mildew resistance was 60.91%. The results also indicated that the additive gene action was prevalent over the non-additive gene action for downy mildew resistance character. Moreover, the gca variance was approximately six times higher than the sca variance. These results suggested that the selection of parents based on gca value was effective in this population. The significant sca effects were also found in the crosses 'Carolina Black Rose x NY 65.0550.04' (-1.09; $P < 0.01$), 'Italia x NY 88.0517.01' (-0.45; $P < 0.05$) and 'Italia x NY 65.0551.05' (-0.66; $P < 0.01$). However, Italia crosses generally had lower percentages of pollinated berries and very low numbers of surviving seedlings (40.6 and 10.0%, respectively) and may not be practical for the breeding programs. Therefore, the 'Carolina Black Rose x NY 65.0550.04' cross is strongly recommended for future use in grape breeding programs for downy mildew resistance.

4.2 Introduction

Downy mildew caused by *Plasmopara viticola* is one of the diseases responsible for economic loss of the grape production especially in the humid areas of the world. At high humidity and warm temperature, zoospores are produced from oospores. Free water and wind are the carriers of flagellate zoospores to healthy leaves nearby; zoospores emerge through the stomata of the lower surface. When climatic condition is favorable, sexual propagation cycle of *P. viticola* can be accelerated to as short as four days leading to a rapid spread of the disease over the entire vineyard (Vercesi et al.,

1999). The effects of downy mildew are variable depending on the tissues and the period of infection. Shoots, leaves or berries will become distorted, defoliated, thickened and may die from downy mildew infection at the early stage of growth or fruit set. The disease might not cause yield loss of the mature berries directly because they are more resistant than the young berries. However, the quality may decline since only 25-50% of the sugar remains in the infected berries (Agrios, 1997).

Even though *Vitis vinifera* L. is highly susceptible to several diseases including downy mildew (Föex, 1981; Reynier, 1989), many American and Asian *Vitis* species are reported as resistant to *P. viticola*. *V. rupestris* and *V. amurensis* are moderately resistant, while *V. cinerea*, *V. riparia*, *V. rubra*, *V. candicans* and *V. rotundifolia* are highly resistant (Boubals, 1959; Langcake and Lovell, 1980; He and Wang, 1986; Eibach et al., 1989; Alleweldt et al., 1990). The exact mechanism of downy mildew resistance is unknown, but the hypersensitive response has been implicated (Boubals, 1959; Langcake and Lovell, 1980). Two genetic systems are suggested to confer downy mildew resistance. Firstly, a single gene controls a block of initial penetration at the time of infection. The resistant species especially American species are homozygous dominant in resistance gene and responsible for the hypersensitive response, while the susceptible species e.g. *V. vinifera* is homozygous recessive. Secondly, the multiple genes confer the inhibition of mycelial growth in the host by controlling inter- and intracellular development of mycelia (Boubals, 1959; Coutinho, 1963; Denzer et al., 1995).

Eibach et al. (1989) estimated the narrow sense and broad sense heritabilities for downy mildew resistance at 0.26 to 0.39 and 0.83 to 0.94, respectively. These results indicated that the resistance characteristics were only slightly influenced by the environment. In addition, the maternal inheritance of downy mildew resistance was not

found to be important (Becker and Zimmermann, 1978; Doazan and Kim, 1978; Brown et al., 1999a).

Downy mildew becomes increasingly important grape disease in Thailand due to the tropical climate which is favorable to the fungus. In Thailand, the planting and pruning of the crop could be done at anytime of the year. Consequently, two to three crops can be harvested from individual vine in a year. This practice leads to no dormant phase to break the disease cycle in the vineyard and thus promotes the rapid spread of the disease that causes substantial yield losses. Approximately 90% of the cultivars now grown in the world grape cultivated areas including Thailand are *V. vinifera.* In the twentieth century, Italia (Pirovaho 65), Cardinal, Perlette and Flame Seedless were the most commercially important table grapes among 180 table and raisin grape cultivars developed by 37 plant breeding programs (Wagner and Truel, 1988). In Thailand, the main cultivars for table grapes are White Malaga and Cardinal (Department of Agricultural Extension, 1998). Other cultivars such as Carolina Black Rose, Early Muscat, Italia and Black Queen are also cultivated in Thailand because of their high fruit quality (Nilnond, 2001). However, these cultivars are extremely susceptible to downy mildew that affects both yield and quality. Several applications of fungicides are usually needed to protect grapevine throughout its life cycle. But, chemical control increases the cost and has negative effects on human and the environment. Therefore, most grape breeding programs frequently aim at developing the new disease resistant cultivars by combining the disease resistant characteristics but low fruit quality from the American and Asian species with the high fruit quality of *V. vinifera*.

Expressions of quantitative characters are influenced by gene actions. Thus, the study of gene action is primarily important for any breeding programs. Hayman (1958) developed a model to separate additive, dominance and epistatic effects. With this

model various gene effects are equated to the means of F_1, F_2, backcross and other generations derived from inbred lines. Further, Gamble (1962) used the method similar to those described by Hayman (1958) to obtain parameters for various gene effects for the yield of corn. Previously, Spraque and Tatum (1942) developed diallel cross method to obtain estimates of general and specific combining ability in this crop. They were the first to define general combining ability (gca) as the average performance of lines in hybrid combinations and specific combining ability (sca) as the deviation of certain crosses from the performance of lines. Hayman (1954) and Griffing (1956) independently developed the methods to analyze the gene actions. Hayman's analysis has been criticized on the ground that the analysis appeared to have been calculated using a progressive fitting of unknown parameters. Later, Griffing (1956) suggested four methods to analyze the gene actions. For each method, the basis for sampling the experiments gave rise to two models, I and II. In these analyses, the gca variance components may reflect the additive effects and additive interactions, while sca variance components may reflect dominance effects and dominance epistasis plus components of additive epistasis. Moreover, the size of gca and sca variances are also influenced by several factors e.g. gene distribution (i.e. linkage-disequilibrium [LD]), existence of epistasis, environmental effect and maternal effect (Pswarayi, 1993; Dieters et al., 1995; King and Johnson, 1998). The observation in corn by Hallauer and Miranda (1988) was that the populations may differ greatly in gene distribution, level of dominance and epistasis, all of which influence the value of sca.

Mating North Carolina (NC) design I, II and III proposed by Comstock and Robinson (1948, 1952) have been used extensively in estimating the kinds and relative importance of genetic variation in specified populations. The use of these designs involves the mating of plants to form groups of relatives. Covariances among relatives

have mean square expectations in terms of additive, dominance and epistatic genetic variances. Thus, the gca and sca effects provide information for selecting the best parents, crosses and strategies for plant breeding programs (Dabholkar, 1992). For example, in radiata pine (*Pinus radiata*), four combinations between parents selected based on higher gca provided increase in yield up to 52.9% (Wu and Matheson, 2004). The heritability provides information on contribution of additive variance and influence of environment to the studied characters. High narrow sense heritability value indicates the high percentage of success of a breeding program to obtain desirable characters. This guideline will be useful for plant breeders to improve efficacy of breeding programs.

The objective of this study was to determine the gca, sca and heritability of downy mildew resistance of grape using NC II design. The gca and sca analysis of this study will benefit the grape breeding program in Thailand as a guideline for efficient selection of parents and strategies for incorporating the resistance genes from resistant genotypes into high fruit quality *V. vinifera*.

4.3 Materials and Methods

Production of crosses

Six grape genotypes were used as parental lines in a 3 x 3 NC II mating design to generate F_1 hybrids. The female parents were *V. vinifera* which have high fruit quality but are susceptible to downy mildew including Black Queen, Carolina Black Rose and Italia (Appendix Figure 1B). The resistant male parents were developed by the grape breeding program at Cornell University, Geneva, New York. These resistant genotypes, NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05, were selected on the basis of observations made in field screens from 1992 through 2002 at New York State

Agricultural Experiment Station (NYSAES), Cornell University (Table 1; Appendix Figure 2B). These genotypes had variable levels of genetic composition from several American species such as *V. labrusca*, *V. riparia*, *V. rupestris*, *V. lincecumil* and *V. vinifera* in their pedigrees as shown in Appendix Figure 3B.

Table 1. Average leaf disease rating of male parents for 1992 – 2002 (modified from Reisch, 2003).

Genotype	Parent/Source	Downy mildew[a]	Years of data
NY 88.0517.01	JS 23-416 x Ill 547-2	1.5	4
NY 65.0550.04	Ill 796-1 x Ill 271-1	1.5	8
NY 65.0551.05	Ill 796-1 x Ill 182-1	1.9	7

[a]Rating scales: percentage of leaf area infected; 1 = 0 – 3%; 2 = 3 – 12%; 3 = 12 – 25%; 4 = 25 – 50%; 5 = >50%

The pollen of the male parents was collected from NYSAES, Cornell University in 2003. The opened flowers of resistant genotypes which were at least 60% open on the primary shoot and 5 – 10% open on the secondary shoot were collected in a paper bag. Anthers were passed through a metal sieve and then dried in a desiccator at room temperature overnight. The pollen was stored in the tubes that contained a small amount of desiccant held in place by a cotton plug. The tubes were kept at -20^{o}C until use. The pollen was examined for viability before being used for pollination of female flowers in 2004 with a method modified from Mulugeta et al. (1994). Briefly, 1,2,3-triphenyl tetrazolium chloride (TTC; 1.0% (w/v) in 50% (w/v) sucrose) was prepared and used to stain pollen of the resistant genotypes. Grape pollen was also heat-killed at 70^{o}C for 48 h for use as negative control. Collected and heat-killed pollen was dusted onto the glass

microscopic slides on which three drops of stain were added. Pollen viability was observed and calculated by the number of light pink or dark red pollen under compound microscope at 40x magnification after three h of staining.

Nine crosses were made in 2004 at the Suranaree University of Technology (SUT) farm following Reisch and Pratt (1996). Briefly, anthers and calyptra were removed from flowers by fine-tipped forceps. The emasculated clusters were covered with paper bags overnight. Pollen was examined for viability before pollination. Forty to fifty percent of the pollen from each male parent stained light pink or dark red with TTC, indicating viability of 40 – 50%. No pink or red pollen was observed in the heat-killed pollen (negative control). This pollen was brushed onto the stigmas of emasculated clusters by a soft paint brush. The pollinated clusters were covered with paper bags until berries were formed. Four weeks after pollination, the paper bags were removed and the clusters were labeled with parent names and treated with 3 g/l Bordeaux-mixture (cupric sulphate and bisdithio carbamate) every week to protect against diseases. Berries were harvested when fully ripe. The seeds were extracted, thoroughly rinsed with water and the cleaned seeds were dried at room temperature for 24 h before storage at 4^{o}C.

The F_1 seeds were treated with gibberellic acid (GA) for seed stratification. Germination boxes were used to humidify the seeds at 90% RH overnight before soaking them in 1.5% (w/v) hydrogen peroxide (H_2O_2) at room temperature for 24 h. After decanting off the H_2O_2, seeds were rinsed with distilled water three times and with 70% (v/v) ethanol one time. A solution of 1000 ppm GA was used to soak seeds for 24 h. The seeds were rinsed with distilled water for three times and dried at room temperature. Seeds were pre-chilled at 5^{o}C for 21 days. After stratification, the seeds were sown in sand in plastic flats and allowed to germinate and grow for 6 weeks. Seedlings were then transplanted to pots containing 1 part peat moss, 1 part soil, ½ part burnt rice-chaff,

1 part perlite, 1 part vermiculite, ¾ part sand, and 1 g of 40-0-0 NPK fertilizer per a six-inch pot. The 120 F_1 hybrid plants from all crosses were grown in a nursery in 2004 and treated with muck fertilizer and 10 ml/L of 11-8-6 foliar fertilizer every two weeks. The fungicide, 2 g/L mancozeb (manganese ethylenebis [dithiocarbamate]), was monthly applied to protect against downy mildew. And 0.6 g/L triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl) butanone) was also applied to protect against rust (*Phakopsora euvitis*) every two weeks.

Disease evaluation

F_1 hybrids and the parents were evaluated for resistance to downy mildew by detached leaf assay in 2006. Five or six plants of each parent were randomly chosen for downy mildew resistance evaluation. To prepare the inoculum, *V. vinifera* leaves infected with downy mildew were collected from SUT farm. Distilled water was sprayed onto the abaxial surface of infected leaves until moist and incubated at 22^{o}C overnight. The sporangia of *P. viticola* were washed from the abaxial surface using a spray bottle. The collected sporangia suspensions were counted in a hemacytometer and adjusted to 10^5 sporangia per mL. Leaves from nodes 4, 5, 6 and 7 (node 1 being the first expanded leaf) of the F_1 hybrids and parents were used for inoculation. The sporangia suspensions were sprayed onto the abaxial surface of the inoculated leaves and leaves were then placed onto moist filter paper in Petri plates. Sterile water was sprayed onto control (uninoculated) leaves of each genotype. The inoculated Petri plates were placed the bottom up and held at 22^{o}C with 18 h photoperiod for 7 days. After the incubation, the number of total spores was determined to categorize resistance levels. The inoculated leaves were washed by placing them into 50 mL tubes, adding 5 mL of distilled water and followed by 3-min shaking. The spores were counted from 5 μL of the washed water to determine the total number of spores

produced per leaf. The length and width of the inoculated leaves were measured and used to calculate the leaf areas by length x width. To convert these calculated leaf areas to actual leaf areas, the leaf areas of ten random leaves of all genotypes were also measured using leaf area meter. The regression equation between calculated leaf areas and actual leaf areas was estimated and used to convert the calculated leaf areas of all inoculated leaves to actual leaf areas (Appendix Figure 4B). The number of total spores per leaf was converted to number of spores/25 cm^2 leaf area using the following formula; number of spores/25 cm^2 leaf area = (number of spores x 25 cm^2 leaf area)/actual leaf areas. Resistance levels were classified into 6 classes based on spore production; 0 = 0 - 5 spores/25 cm^2, highly resistant; 1 = >5 – 10 spores/25 cm^2, resistant; 2 = >10 – 15 spores/25 cm^2, moderate or intermediate; 3 = >15 – 25 spores/25 cm^2, moderately susceptible; 4 = >25 – 40 spores/25 cm^2, susceptible; 5 = >40 spores/25 cm^2, highly susceptible (D.M. Gadoury, personal communication).

Statistical analysis

Data recorded for disease reaction were transformed using $X' = (X+1)^{1/2}$ and first analyzed using completely randomized design (CRD) model:

$$X_{ij} = m + A_{i(j)} + e_{ij}$$

where X_{ij} = observed reaction on the i^{th} plant in j^{th} environment

m = grand mean

$A_{i(j)}$ = effect of reaction on the i^{th} plant

e_{ij} = the error associated with the i^{th} plant

The reaction of individuals was further separated to verify effects as:

$$A_{ij} = A_i + B_j + A \text{ vs } B$$

where A = effects of parents

B = effects of hybrids

A vs B = heterotic effects of hybrids

Hybrids were then analyzed for combining ability using the model:

$B_{ij} = G_i + G_j + S_{ij}$

where B_{ij} = cross effect for cross between the j^{th} female and i^{th} male parents

G_i = the average effect of the i^{th} male parent on its crosses, i = 1, 2, …, m; m = 3

G_j = the average effect of the j^{th} female parent on its crosses, j = 1, 2,…, f; f = 3

S_{ij} = the deviation of the ij^{th} cross from the expected performance based on the parents' average effects

Mean squares for the male parents as well as the female parents are both independent estimates of gca effects. The male x female interaction mean square is an estimate of sca.

Means of all observations were back-transformed and estimates of gca and sca effects were calculated by using the following equations:

$G_i = (\overline{X}_{i.} - \overline{X}_{..})$

$G_j = (\overline{X}_{.j} - \overline{X}_{..})$

$S_{ij} = (\overline{X}_{ij} - \overline{X}_{i.} - \overline{X}_{.j} + \overline{X}_{..})$

Where G_i and G_j are gca effects of the i^{th} male and j^{th} female, respectively. S_{ij} is the interaction between the i^{th} male and j^{th} female and is used as a measure of the sca effects. $\overline{X}_{i.}$, $\overline{X}_{.j}$ and $\overline{X}_{..}$ are the means of hybrids having the i^{th} male and j^{th} female as used as parents and the grand mean, respectively. The estimates were considered significant if they were greater than two times of their respective standard error of $[MSE*(1/2(1/N))]^{1/2}$.

Expected mean squares for the analysis of variance given in Table 7 can be expressed in terms of combining variances:

$$\sigma^2_m = \sigma^2_{gca\ (male)} = 1/4\ \sigma^2_A$$

$$\sigma^2_f = \sigma^2_{gca\ (female)} = 1/4\ \sigma^2_A$$

$$\sigma^2_{mf} = \sigma^2_{sca} = 1/4\ \sigma^2_D$$

The total of σ^2_A is the pooled of σ^2_A for male and female = $\sigma^2_A = 2(\sigma^2_m + \sigma^2_f)$. Heritability in narrow sense was estimated using the following formula:

$$\text{Heritability (\%)} = [\sigma^2_A/(\sigma^2_A + \sigma^2_D + \sigma^2_E)] \times 100.$$

4.4 Results

Production of crosses

Forty-five clusters, fifteen clusters per female parent, were made at SUT farm in 2004. Only 36 pollinated clusters gave high berry setting (50 – 70%; Table 2; Figure 1). Nine pollinated clusters mostly from Italia crosses (only one from Carolina Black Rose) had a lower percentage of berry set (5 – 10%) and were excluded from the experiment (Table 2; Figure 1). The fully ripen clusters (Appendix Figure 5B) were harvested and seeds were extracted and germinated. A total of 120 seedlings were obtained from all nine crosses and transplanted into a nursery in 2004 (Appendix Figure 6B). The number of survived and healthy plants for each cross which were further used for evaluation of downy mildew resistance levels is shown on Table 3. The 47.0%, 37.3% and 15.7% of seedlings from Black Queen, Carolina Black Rose and Italia progenies survived were used for disease screening, respectively.

Despite its extreme susceptibility to downy mildew, Black Queen has excellent vegetative and reproductive growth. This character was also found in its progenies. High vigor and excellent vegetative growth appeared in the progenies with Black Queen

as a female parent. Progenies of Carolina Black Rose also showed high germination percentage or seed size as good as Black Queen progenies. However, the vegetative and reproductive growth might be slightly lower than the Black Queen progenies. Italia is found to be poor female parent in this program. It was found that the progenies from Italia crosses with three male parents had low percentage of pollinated berries, berry setting and low seedling survival (40.6%, 25.0% and 10.0%, respectively).

Table 2. Number of pollinated clusters with high berry setting from nine crosses.

Male / Female	NY 88.0517.01	NY 65.0550.04	NY 65.0551.05
Black Queen	5	5	5
Carolina Black Rose	5	5	4
Italia	2	3	2

Table 3. Number of survived and healthy plants used for disease screening.

Cross	No. of plants survived	No. of plants used for disease screening
Black Queen x NY 88.0517.01	17	14
Black Queen x NY 65.0550.04	10	6
Black Queen x NY 65.0551.05	21	19
Carolina Black Rose x NY 88.0517.01	13	10
Carolina Black Rose x NY 65.0550.04	9	8
Carolina Black Rose x NY 65.0551.05	15	13
Italia x NY 88.0517.01	2	2
Italia x NY 65.0550.04	9	7
Italia x NY 65.0551.05	6	4
Total	102	83

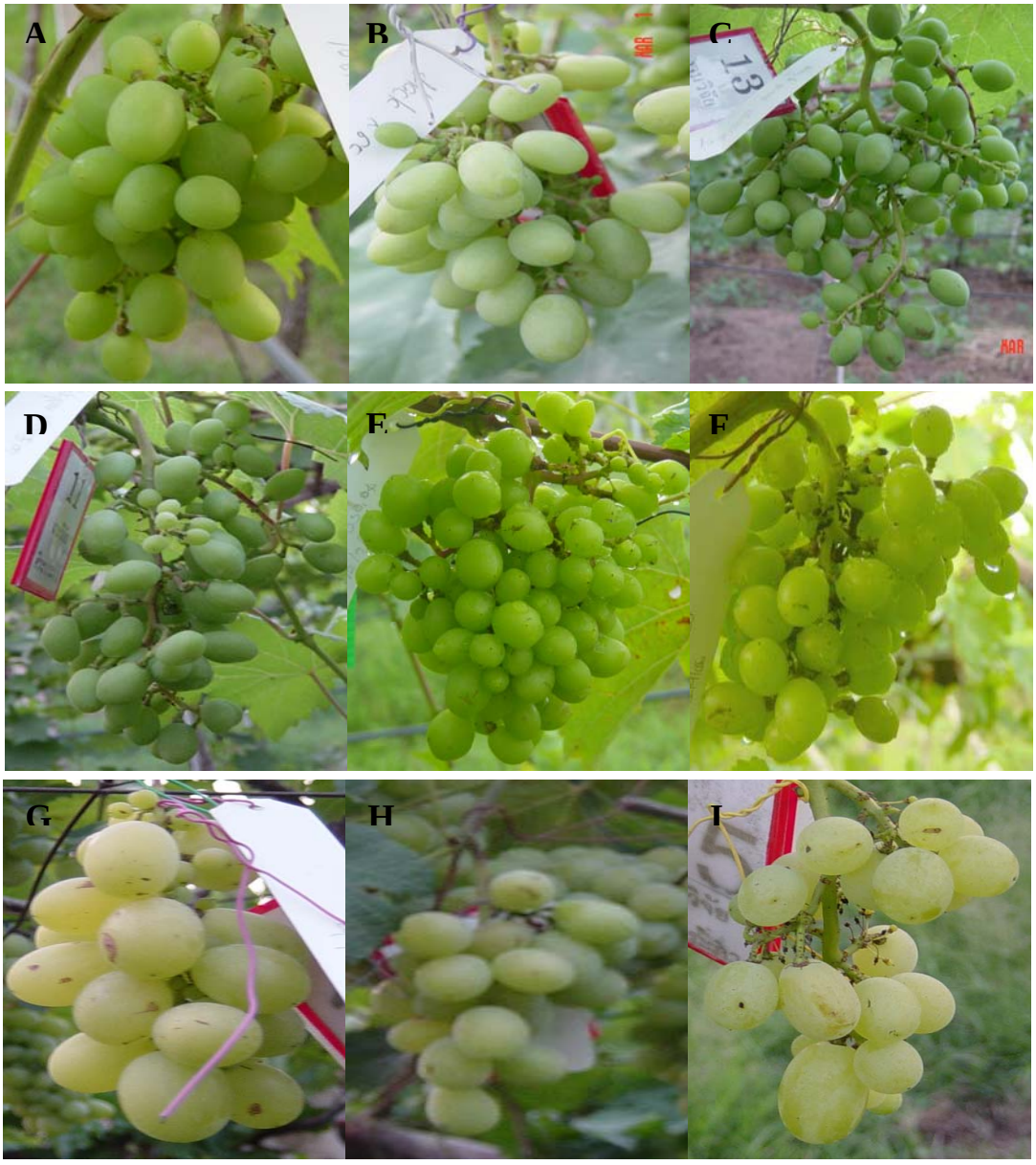


Figure 1. Pollinated clusters from nine crosses:

A) Black Queen x NY 88.0517.01; B) Black Queen x NY 65.0550.04

C) Black Queen x NY 65.0551.05; D) Carolina Black Rose x NY 88.0517.01

E) Carolina Black Rose x 65.0550.04; F) Carolina Black Rose x NY 65.0551.05

G) Italia x NY 88.0517.01; H) Italia x NY 65.0550.04; I) Italia x NY 65.0551.05.

Downy mildew evaluation

The data from the parents and hybrids were analyzed in a CRD manner and mean squares from analysis of variance for the disease reaction are presented in Table 4. Highly significant differences among entries were obtained. The mean squares derived by partitioning the entries sum of squares were tested against their error. The contrasted comparison of hybrids vs. parents was significant at $P < 0.05$ and can be interpreted as a measure of heterosis. Highly significant differences were found among parents and among hybrids, indicating high genetic variability within each group.

Table 4. Mean squares from analysis of variance for disease reaction.

Sources	df	SS	MS	F (test)	F (table)	
					5%	1%
Treatments	14	14.31	1.02	6.27**	1.79	2.26
Parents vs hybrids	1	1.02	1.02	6.26*	3.94	6.90
Parents	5	7.24	1.45	8.88**	2.30	3.20
Hybrids	8	6.05	0.76	4.64**	2.03	2.69
Error	100	16.32	0.16			
Total	114	30.63				
CV (%)	21.06%					

* $P < 0.05$; ** $P < 0.01$

Parents were significantly different ($P < 0.01$) in their mean disease ratings. Table 5 shows the means of disease ratings of all parents. Among female parents, Black Queen was highly susceptible (Table 5; Appendix Figure 7B; Appendix Table 1B). The number of spores in Black Queen was as high as 178.5 spores/25 cm^2 leaf area (Appendix Table 1B), while the highest number of spores in Carolina Black Rose and Italia were only 86.3 and 90.0 spores/25 cm^2 leaf area, respectively (Appendix Table

1B). Among male parents, NY 88.0517.01 and NY 65.0551.05 showed higher resistant scores than NY 65.0550.04 (Table 5; Appendix Figure 8B; Appendix Table 2B). The resistance of NY 88.0517.01 was also reflected by the single crosses that have this parent as the male counterpart (Table 6). The highest resistant score was shown by the single cross between NY 65.0550.04 and Carolina Black Rose, but NY 65.0550.04 itself did not show the highest resistant score. This reflected the importance of sca. The resistant scores of single crosses of 'Carolina Black Rose x NY 88.0517.01' and 'Italia x NY 88.0517.01' were also high (Table 6).

Table 5. Downy mildew evaluation of parents by detached leaf assay.

Genotype	Downy mildew (rating score)[a]	Phenotype
Black Queen (female)	4.15	highly susceptible
Carolina Black Rose (female)	3.64	susceptible
Italia (female)	3.87	susceptible
NY 88.0517.01 (male)	0.48	highly resistant
NY 65.0550.04 (male)	0.96	resistant
NY 65.0551.05 (male)	0.46	highly resistant

[a]Rating scores: number of spores/25 cm^2; 0 = 0 - 5 spores/25 cm^2, highly resistant; 1 = >5 – 10 spores/25 cm^2, resistant; 2 = >10 – 15 spores/25 cm^2, moderate or intermediate; 3 = >15 – 25 spores/25 cm^2, moderately susceptible; 4 = >25 – 40 spores/25 cm^2, susceptible; 5 = >40 spores/25 cm^2, highly susceptible

The progenies of all resistant x susceptible crosses showed variation in downy mildew resistance ranging from highly resistant (rating score = 0) to highly susceptible (rating score = 5; Appendix Figure 9B; Appendix Table 3B). Resistant levels of the F_1 hybrids as classified into three groups; resistance (rating scores = 0 and 1), intermediate (rating scores = 2 and 3) and susceptible (rating scores = 4 and 5), showed that the

majority of the seedlings were susceptible (56.6%). The remaining 25.3% were resistant and 18.1% intermediate (Table 6).

Table 6. Downy mildew ratings of F_1 hybrids from nine crosses.

Cross	Disease evaluation (No. of plants (percentage))[a]		
	Resistance (score = 0,1)	Intermediate (score = 2,3)	Susceptible (score = 4,5)
Black Queen x NY 88.0517.01	3 (21.4%)	5 (35.7%)	6 (42.9%)
Black Queen x NY 65.0550.04	1 (16.7%)	2 (33.3%)	3 (50.0%)
Black Queen x NY 65.0551.05	1 (5.3%)	3 (15.8%)	15 (78.9%)
Carolina Black Rose x NY 88.0517.01	5 (50.0%)	-	5 (50.0%)
Carolina Black Rose x NY 65.0550.04	6 (75.0%)	1 (12.5%)	1 (12.5%)
Carolina Black Rose x NY 65.0551.05	1 (7.7%)	1 (7.7%)	11 (84.6%)
Italia x NY 88.0517.01	1 (50.0%)	1 (50.0%)	-
Italia x NY 65.0550.04	2 (28.6%)	1 (14.3%)	4 (57.1%)
Italia x NY 65.0551.05	1 (25.0%)	1 (25.0%)	2 (50.0%)
Total	21 (25.3%)	15 (18.1%)	47 (56.6%)

[a]Rating scores: number of spores/25 cm^2; 0 = 0 – 5 spores/25 cm^2; 1 = >5 – 10 spores/25 cm^2; 2 = >10 – 15 spores/25 cm^2; 3 = >15 – 25 spores/25 cm^2; 4 = >25 – 40 spores/25 cm^2; 5 = >40 spores/25 cm^2

Certain crosses including ‘Black Queen x NY 88.0517.01’, ‘Black Queen x NY 65.0550.04’, ‘Black Queen x NY 65.0551.05’, ‘Carolina Black Rose x NY 65.0551.05’, ‘Italia x NY 65.0550.04’ and ‘Italia x NY 65.0551.05’ gave lower numbers of resistant seedlings ranging from 5.3 – 28.6% (Table 6). At least half of the seedlings from all crosses, except ‘Black Queen x NY 88.0517.01’ and ‘Carolina Black Rose x NY 65.0550.04’, were susceptible as shown in Table 6. These data suggest that the

dominant gene(s) controlling downy mildew resistance in resistant parents are in heterozygous condition. Moreover, the segregation of progenies from 'Black Queen x NY 88.0517.01' and 'Black Queen x NY 65.0551.05' crosses on downy mildew resistance level suggested that at least two gene(s) controlled downy mildew resistance in this population (Appendix Figure 10B, 11B). However, further study using a larger population is needed to accurately estimate the number of gene involved.

The combination between NY 65.0551.05 with Black Queen or Carolina Black Rose gave the lowest number of resistant seedlings (5.3% and 7.7%) and the highest number of susceptible seedlings (78.9% and 84.6%; Table 6). On the other hand, 'Carolina Black Rose x NY 65.0550.04' gave the highest number of resistant seedlings (75.0%) and the lowest number of susceptible seedlings (12.5%). It is obvious in this experiment that 'Carolina Black Rose x NY 65.0550.04' is the best candidate parental combination to develop disease resistant hybrid in grape breeding program.

Combining ability analysis

Combining ability analysis mean squares for gca and sca effects are presented in Table 7. Differences among the hybrids were highly significant ($P < 0.01$). The mean squares attributable to male and female percentage of the hybrids provide the measurements of gca effects for these parental groups. The data indicated that the gca effect was highly significant for males but not females. The interaction between male and female effects provides a measure of the sca effect. The source of variation was not significant for this character. The results of nine crosses between resistant and susceptible parents indicated that additive gene effects were important for downy mildew resistance in this population.

The relative importance of the mean squares for gca and sca were 9.27:1, 5.23:1 and 0.56:1 for gca(males):gca(females), gca(males):sca and gca(females):sca,

respectively. These results indicated that the male parents used in this experiment contributed more additive gene effects to the inheritance of this trait than female parents.

Table 7. Mean squares for combining ability analysis of variance for disease reaction.

Sources	df	SS	MS	F (test)	F (table)		EMS
					5%	1%	
Hybrids	8	6.05	0.75	3.64**	2.07	2.77	
gca (female)	2	0.43	0.22	0.56	3.13	4.92	$\sigma^2_w + n\sigma^2_{mf} + nm\sigma^2_f$
gca (male)	2	4.08	2.04	5.30**	3.13	4.92	$\sigma^2_w + n\sigma^2_{mf} + nf\sigma^2_m$
sca	4	1.54	0.39	1.85	2.50	3.60	$\sigma^2_w + n\sigma^2_{mf}$
Error	74	15.42	0.21				σ^2_w
Total	82	21.47					
CV (%)	23.26 %						

* $P < 0.05$; ** $P < 0.01$

Due to the fact that the mean squares of the gca were significant, therefore the gca and sca effects were calculated from means of the disease reaction in each parent (Table 8) and presented in Table 9. The downy mildew rating score of zero represented highly resistant and the rating score of 5 represented highly susceptible. Therefore, negative values of gca and sca effects indicated contribution toward the resistance against downy mildew, while positive values indicated susceptibility. Significant negative gca effects were found in NY 88.0517.01 and NY 65.0550.04, respectively (Table 9). The potential to transmit the disease resistant character from the parents to the progenies is suggested by a negative gca effect. Therefore, NY 65.0551.05 which showed positive gca effects is not desirable. The significant gca effects of NY 88.0517.01 and NY 65.0550.04 (-0.70 and -0.25), suggested that these genotypes may be

the effective parents in the breeding for downy mildew resistance program. On the other hand, gca effects of the female parents were not significantly different. Black Queen which was determined as the most susceptible female parent also showed high positive gca effects while the Carolina Black rose and Italia showed negative gca effects (Table 9).

Five out of nine crosses showed negative sca effects including 'Black Queen x NY 88.0517.01', 'Black Queen x 65.0550.04', 'Carolina Black Rose x NY 65.0550.04', 'Italia x NY 88.0517.01' and 'Italia x NY 65.0551.05' as presented in Table 9. However, only three combinations including 'Carolina Black Rose x NY 65.0550.04', 'Italia x NY 88.0517.01' and 'Italia x NY 65.0551.05' gave significant sca effects (-1.09 in 'Carolina Black Rose x NY 65.0550.04'; ($P < 0.01$), -0.45 in 'Italia x NY 88.0517.01'; ($P < 0.05$) and -0.66 in 'Italia x NY 65.0551.05'; ($P < 0.01$)) and should be selected for further use because the high level of downy mildew resistance in the seedlings should be found from these combinations.

Table 8. Means of disease reaction.

Male	Female			Average
	Black Queen	Carolina Black Rose	Italia	
NY88.0517.01	2.50	2.46	1.50	2.15a
NY65.0550.04	2.95	1.35	3.51	2.60a
NY65.0551.05	4.22	4.26	2.95	3.81b
Average	3.22b	2.69a	2.65a	2.85

[a]Rating scores: number of spores/25 cm^2; 0 = 0 - 5 spores/25 cm^2; 1 = >5 – 10 spores/25 cm^2; 2 = >10 – 15 spores/25 cm^2; 3 = >15 – 25 spores/25 cm^2; 4 = >25 – 40 spores/25 cm^2; 5 = >40 spores/25 cm^2

Mean followed by a common letter are not significantly different at the 5% level by DMRT

Table 9. Estimates of general and specific combining ability effect.

	sca			gca (male)
	Black Queen	Carolina Black Rose	Italia	
NY88.0517.01	-0.02	0.47	-0.45*	-0.70**
NY65.0550.04	-0.02	-1.09**	1.11	-0.25*
NY65.0551.05	0.04	0.61	-0.66**	0.97
gca (female)	0.36	-0.16	-0.20	

$P < 0.05$; ** $P < 0.01$

The estimated narrow sense heritability of downy mildew resistance was 60.91%. The high percentage of heritability may result from the prevalent additive gene action over the non-additive gene action as described by significant gca variance and non-significant sca variances.

4.5 Discussion

Production of hybrids

A large number of F_1 hybrids were obtained from crosses that used Black Queen as the female parent. However, the lowest number of resistant (12.8%) and highest number of susceptible (61.5%) seedlings was found when Black Queen was crossed with all resistant male parents. Therefore, it is recommended that Black Queen not be used as the female parent in the future disease resistance breeding program. Italia was found not suitable to be used as a female parent in disease resistant breeding program. This was due to the low percentage of pollinated berries, berry setting and low seedling survival (40.6%, 25.0% and 10.0%, respectively) of crosses using Italia as the female parent. On the other hand, Carolina Black Rose was found to be the most effective female parent for a downy mildew resistant breeding program. This evidence was shown by the highest

numbers of resistant seedlings (38.7%) obtained from progenies of Carolina Black Rose crossed with all resistant genotypes.

Downy mildew evaluation

Among female parents, Black Queen was found to be the most susceptible to downy mildew based on detached leaf assay, as it showed the highest rating score (Table 5). Moreover, the field observations also showed that Black Queen was the most susceptible cultivar among female parents. The infection of downy mildew and other diseases found on Black Queen was more severe than on other cultivars, even though the pollinated clusters of this cultivar were sprayed with Bordeaux-mixture every two days. In contrast, Carolina Black Rose which was planted next to Black Queen needed only weekly disease protection. These evidences suggested that the disease resistance evaluation by detached leaf assay correlated with field observation. Similarly, Brown et al. (1999b) found high correlation of disease evaluation based on downy mildew sporulation among leaf disk assay, greenhouse evaluation and field observation. Eibach et al. (1989) also reported that leaf disk evaluation was significantly correlated with field results (r = 0.98). Therefore, the leaf disk assay appeared to be the appropriate procedure for downy mildew evaluation on a large population even more so than a greenhouse method (Brown et al., 1999b). In addition, the detached leaf assay in this study used field isolates for inoculation. The single spore isolates of *P. viticola* maybe needed for downy mildew evaluation of all progenies in the future.

Combining ability analysis

Results from analysis of variance for combining ability showed that most of the genetic variation for disease reaction was associated with significant gca effects of male

parents. The mean square for gca (males) was larger than gca (females) for this trait, indicating greater genetic diversity among the male parents. These results were not unexpected since the male parents are genotypes with accumulation of resistance gene(s), while the female parents are cultivars with susceptible gene(s). The mean squares for gca effects, particularly that of male parents were larger than the means squares for sca which suggested that the additive gene effects were relatively more important than other types of the gene action in the variation expressed by this character. The difficulty in this analysis was that the sca effect was not significant, whereas the parent vs. hybrid mean square was significant (Table 4 and 7). Both were attributable entirely to non-additive gene effects.

The greater influence of additive gene action on downy mildew resistance character than non-additive gene action was also supported by the high percentage of heritability (60.91%). It is apparent that 60.91% of the variation in resistance levels in this study resulted from additive gene effect. Similarly, Brown et al. (1999a) also reported the high percentage of heritability (88.00%) of this character in their experiment. They also reported that the additive gene action played a major role in downy mildew resistance character in grape.

The genotypes which give negative and significant gca effects are appropriate to be used in downy mildew resistance breeding program. NY 88.0517.01 and NY 65.0550.04 were found to have highly significant gca effects (-0.70 and -0.25; Table 9). Therefore, these are the effective male parents for the disease resistant breeding program. The most susceptible cultivar, Black Queen, showed positive gca effects and therefore should be discarded. This suggestion is supported by non-significant sca effects found in crosses that involved Black Queen as a female parent (Table 9).

Crosses with Italia as a female parent gave low percentage of pollinated berries, berry setting and low seedling survival. Therefore, the crosses 'Italia x NY 88.0517.01' and 'Italia x NY 65.0551.05' were not recommended for future breeding program due to their low vegetative growth. It was found that the highest sca effect of all crosses, -1.09, was obtained from a cross 'Carolina Black Rose x NY 65.0550.04'. Therefore, Carolina Black Rose should be selected as an effective female parent and the 'Carolina Black Rose x NY 65.0550.04' cross is the best combination to be used in selection on cultivar development. There is a great opportunity to develop the resistant cultivars or genotypes that are resistant to downy mildew with high fruit quality, which are highly desirable in world grape production including Thailand.

4.7 References

Agrios, G.N. (1997). **Plant Pathology.** New York: Academic.

Alleweldt, G., Spiegel-Roy, P. and Reisch, B. (1990). Grape (*Vitis*). Quoted in J.N. Moore and J.R. Ballington. (1996). Genetic resources of temperate fruit and nut crops. **Acta. Hort.** 290:291-337.

Becker, N.J. and Zimmermann, H. (1978). Breeding of wine varieties resistant to downy mildew. In **Grapevine Genetics and Breeding, II Sym. Int. Sur I'Amelioration de la Vigne** (pp 209-214). Bordeaux, France.

Boubals, D. (1959). Contribution a l'etude des causes de la resistance des Vitacees au Mildiou de la Vigne et de leur mode de transmission rereditaire. **Ann. Amelior. Plantes** 9:5-233. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Son.

Brown, M.V., Moore, J.N., McNew, R.W. and Fenn, P. (1999a). Inheritance of downy mildew resistance in table grapes. **J. Amer. Soc. Hort. Sci.** 124:262-267.

Brown, M.V., Moore, J.N. and McNew, R.W. (1999b). Comparison of leaf disk, greenhouse, and field screening procedures for evaluation of grape seedling for downy mildew resistance. **Hort. Sci.** 34:331-333.

Comstock, R.E. and Robinson, H.F. (1948). The components of genetic variance in populations of biparental progenies and their use in estimating average degree of dominance. **Biometrics** 4:254-266.

Comstock, R.E. and Robinson, H.F. (1952). Estimation of average dominance of genes. In G.W. Gowen (ed.). **Heterosis**. Iowa: Iowa State College.

Coutinho, M.P. (1963). Notes on the genetical aspect to the resistance of vines to *Plasmopara viticola*. **Plant Breeding Abstr.** 37:5033.

Dabholkar, A.R. (1992). **Elements of Biometrical Genetics.** New Delhi: Ashok Kumar Mittal Concept.

Denzer, H., Staudt, G. and Schlosser, E. (1995). Host settlement of *Plasmopara viticola* on different susceptible hosts. **Vitis** 34:45-49.

Department of Agricultural Extension. (1998). Quoted in S. Nilnond. (2001). Grape production in Thailand. In **Proc. of Grape Production in the Asia-Pacific Region** (pp 70-79). Bangkok, Thailand.

Dieters, M.J., White,T.L. and Hodge, G.R. (1995). Genetic parameters estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). **Can. J. For. Res.** 25:1397-1408.

Doazan, J.P. and Kim, S.K. (1978). Recherche de genotypes resistants au mildou dans des croisements interspecifiques. In **Grapevine Genetics and Breeding, II Sym. Int. Sur I'Amelioration de la Vigne**. Bordeaux, France. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Eibach, R., Diehl, H. and Alleweldt, G. (1989). Untersuchungen zur vererbung von resistenzeigenschaften bei reben gegen *Oidium tuckeri*, *Plasmopara viticola* und *Botrytis cinerea*. **Vitis** 28:209-228. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Föex, G. (1981). **Cours complet de Viticulture**. Paris. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Gamble, E.E. (1962). Gene effects in corn (*Zea mays* L.). I. Separation and relative importance of gene effects for yield. **Can. J. Plant Sci.** 42:339-348.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. **Aust. J. Biol. Sci.** 9:463-493.

Hallauer, A.R. and Miranda, J.B. (1988). **Quantitative Genetics in Maize Breeding.** Iowa: Iowa State University Ames Press.

Hayman, B.I. (1954). The theory and analysis of diallel-cross. **Genetics** 39:789-809.

Hayman, B.I. (1958). The separation of epistatic from additive and dominance variation in generation means. **Heredity** 12:371-390.

He, P. and Wang, G. (1986). Studies on the resistance of wild *Vitis* species native to China to downy mildew, *Plasmopara viticola* (Berk. et Curtis) Berl. et de Toni. **Acta. Hort. Sinica** 13:17-24.

King, J.N. and Johnson, G.R. (1998). Analysis of disconnected diallel mating design II results from a third generation progeny test of the New Zealand radiata pine improvement programme. **Silvae. Genet.** 47:80-87.

Langcake, P. and Lovell, P.A. (1980). Light and electron microscopical studies of the infection of *Vitis* spp. by *Plasmopara viticola,* the downy mildew pathogen. **Vitis** 19:321-337.

Mulugeta, D., Maxwell, B.D., Fay, P.K. and Dyer, W.E. (1994). Kochia (*Kochia scoparia*) pollen dispersion, viability and germination. **Weed Sci.** 42:548-522.

Nilnond, S. (2001). Grape production in Thailand. In **Proc. of Grape Production in the Asia-Pacific Region** (pp 70-79). Bangkok, Thailand.

Pswarayi, I.Z. (1993). **Genetic parameters and selection indices for a population of *Pinus elliottii* Engelm. var *elliottii***. PhD thesis, Linacre College, Oxford University, Oxford.

Reisch, B.I. and Pratt, C. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Reisch, B.I. (2003). Cornell grape breeding. [On-line]. Available http://www.nysaes.edu/

Reynier, A. (1989). **Manual de Viticultura.** Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Spraque, G.E. and Tatum, L.A. (1942). General vs specific combining ability in single cross of corn. **J. Amer. Soc. Agron.** 34:923-932.

Vercesi, A., Tornaghi, R., Burruano, S.S. and Faoro, F. (1999). A cytological and ultrastructural study on the maturation and germination of oospores of *Plasmopara viticola* from overwintering vine leaves. **Mycol. Res.** 103:193-202.

Wagner, R. and Truel, P. (1988). Nouvelles varieties de raisins de table et de raisins secs. In **Office International de la vigne et du Vin**, Paris. Quoted in B.I. Reisch and C. Pratt. (1996). **Fruit Breeding, Volume II: Vine and Small Fruit Crops.** New York: John Wiley and Sons.

Wu, H.X. and Matheson, A.C. (2004). General and specific combining ability from partial diallels of radiata pine: implications for utility of SCA in breeding and deployment populations. **Theor. Appl. Genet.** 108:1503-1512.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion and Recommendation

Downy mildew may cause severe losses in yield and reductions in fruit quality of susceptible grape cultivars. *V. vinifera* is the primary scion species grown around the world, and is highly susceptible to downy mildew. Resistance genes are accessible in American and Asiatic species of *Vitis*, which hybridize readily with *V. vinifera*, and have been used extensively in breeding programs to create resistant cultivars. To improve the efficiency of grape breeding, an important goal is to locate molecular markers linked to alleles responsible for disease resistance.

RGAs should have very good potential for use as molecular markers for disease resistance traits due to their known associations with disease resistance in plants. RGA sequences derived from three downy mildew resistant genotypes have a high degree of similarity with RGAs cloned from *V. amurensis* and *V. riparia*, two species that have also been shown to harbor downy mildew resistance. There is a possibility that the RGA sequences characterized in the present work may confer functional resistance to downy mildew, but this requires further work to confirm. The precise linkage map locations of the most promising markers identified from *V. cinerea*, *V. rupestris* and Horizon will need to be identified in future work. Indications at this time are that some of these RGAs are located on linkage groups already known to be associated with resistance loci.

The interspecific hybridization crosses between American and European species gave the parental germplasm for downy mildew resistance breeding program. The

resistance gene(s) from resistant genotypes were introduced into *V. vinifera* as described in the chapters IV by the presence of high downy mildew resistance levels in some seedlings resulting from this breeding program. In the future, field evaluation for downy mildew resistance in all seedlings is required. The backcross program between resistant seedlings to the female *V. vinifera* parents may also be needed in order to increase the proportion of *V. vinifera* genes while retaining the wild species resistance gene(s).

Presently, numerous *Vitis* genetic maps have been created, and QTL analyses have been used to locate markers with strong associations to disease resistance. Breeders are actively trying to incorporate various molecular markers into their programs. Even though, marker association with downy mildew resistance genes in grape has not been published yet, if markers have been observed to link with downy mildew resistance genes, their association with resistance genes will improve the efficiency of disease resistance breeding program.

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer.

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVcin109	GGGANTCCCTTTGGGGGGGTGGGGAAGACGACAATTGCCAAAGTTG TATATAATAATATCTCGCATCAATTTGAGAGTAGAATCTTTCTTGAA AATGTTAGAGAAAGATCCAAGGACCAATCAAGTCTACTTCAATTAC AGAAAGAACTTCTTAATGGTGTTGTGAAGGGAAAAAATCTAGAAAT AAGTAATGTTCATGAAGGGATTGATGTGATAAGAAACAGGTTTAAC TCTAAAAAGGTTCTTCTTATTCTTGATGATGTAGACAATTTGAAGCA ATTAAAATTCTTAGCTGGAGGGCATGGTTGGTTTGGTCCTAGAAGTA GAATCATCATAACCTCTAGAGATCAACATTGTCTAAATGTGCTTGGA GTCGATGCATCATATGAAGTTAAGGCACTAAATTATGAGGAGTCTA TTCAACTTTTCTGTCAACATGCTTTTCAACAAAACATTCCTAAAAGT GACTATGTAGACCTCTCAAATCATGAAGTAAATTATGTGAATGGCC TCCCCCTCGCCCTCAATCACTAGTGAATTCGCGGC	G G V G K T T I A K V V Y N N I S H Q F E S R I F L E N V R E R S K D Q S S L L Q L Q K E L L N G V V K G K N L E I S N V H E G I D V I R N R F N S K K V L L I L D D V D N L K Q L K F L A G G H G W F G P R S R I I I T S R D Q H C L N V L G V D A S Y E V K A L N Y E E S I Q L F C Q H A F Q Q N I P K S D Y V D L S N H E V N Y V N G L P L A L
rgVcin111	AATTCCATTGGGGGGGTGGGGAAGACGACAACTTGCCAAAG:TTGT ATATAATAATATCTCACATCAATTTGAGAGTAGAATCTTTCTTGAAA ATGTTAGAGAAAGATCCAAAGACTACTCAAGTCTACTTCAATTACA AAAAGAACTTCTTAATGGTGTCATGAAGGGAAAAAATAAAAAAAT AAGTAATGTTCATGAANGGATTAATGTGATAAGAAACAGGTTTCAC TCAAAAAAGGTTCTTCTTATTCTTGATGATGTAGACAATTTGAAGCT ATTACAATTCTTAGCTGGA:GAGCATGGTTGGTTTGGTCTTAAAAGT AGAATCATCATAACCTCTAGAGATCGACATTG:TTTAAATGTGCATG GAGTCGGTGCATCATATAAAGTTTGAGGCACTAAG:ATACTAGGAG: TCTATCCAAC:TTTTCTGTCAACATGCCTTTAAACACAACATTCCTAA AAGTGACTATGTAAACCTCTCAGATCATGTATTAAATTATGTGAAA GGCCTCCCCCTCGCCC:C:TCCAATCACTAT	G G V G K T T T C Q S C I * * Y L T S I * E * N L S * K C * R K I Q R L L K S T S I T K R T S * W C H E G K K * K N K * C S * ? D * C D K K Q V S L K K G S S Y S * * C R Q F E A I T I L S W R A W L V W S * K * N H H N L * R S T L F K C A W S R C I I * S L R H * D T R S L S N F S V N M P L N T T F L K V T M * T S Q I M Y * I M * K A S P S P L Q S L L N S R P P A C

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVcin123	CGGGAAATTCCCATTGGGGGGGTGGGGAAGACGACTTATAGCCAAA GTTGTTTATAATCTAATCTCTAGTCAATTTGAGGGCATTAGCTTCCTT GCTAATATTAGAGAAGTCTCCAAAAACTGTGGTTTGCTTCCATTACA GAAACAACTTCTAGGTGATATTTTGATGGGATGGAGTCAAAGGATA AGCAATGTCGATGAATGAATCAATGTGCTAATGGACAGACTTCACT CTAAAAAGGTTCTTATTATTCTTGATGACGTGGATGATTTGAATCAA TTACAATCCTTAGCTGGAAATGTTGATTGGTTTGGTATTGGAAGTAG AATTGTTATAACAACTAGAGATAAACCATCTGCTAAATGTGCCATG GAGTGAGTGAAATATATGAGGCTAAGGAATTANAACCAGAGGAAG CTCTTCAACTTTTCAGTCAATATGCTTTCAAAAGTAAAAAGTCCAGA ATAAGATTATATGAACCTCTCTGACAATGTACTACATTATGCTGAAG GCCTCCCCCTCACCCTCAATCACTAGTGAATTCGCGGC	G G V G K T T Y S Q S C L * S N L * S I * G H * L P C * Y * R S L Q K L W F A S I T E T T S R * Y F D G M E S K D K Q C R * M N Q C A N G Q T S L * K G S Y Y S * * R G * F E S I T I L S W K C * L V W Y W K * N C Y N N * R * T I C * M C H G V S E I Y E A K E L ? P E E A L Q L F S Q Y A F K S K K S R I R L Y E P L * Q C T T L C * R P P P H P Q S L V N S R
rgVcin125	GGAATTCCNTTGGGGGGGTGGGGAAGACGACCCTCTTGAAGAGAAT CGACAACGATTTCCTCCAAACAGGCTACGAAGTCGATGTAGTCATT TGGGTTGTTGTGTCCCAACAAGGGAACGTGGAAAAGGTCCAGGAAA CCGTTCTCAATAAGTTGGAGATTGCTGAATACAAATGGAAAGATAG GAGCGTGCATGAAAGGGCTGAAGAAATATTCAGTGTCTTGCAAACA AAGAAATTTGTGCTCTTGTTAGATGATATATGGAAGCAGCTTGATCT TTTGGAAGTGGGGATTCCTCCTTTGAATGATCAAAAAAAGTCCAAA GTAATATTTACAACACGGTTTTCAACTGTGTGCCACGACATGGGAG CTAAAAGCATTGAAGTTGAGTGCTTGGCATGGGAGGAAGCTTTTTC TTTGTTTCGGACCAAGGTAGGAGAAGACACCTTAGATTCTCATCCA GATATACAAAAGCTTGCGGAGATTTTTGTCAAAGAGTGCAAAGGCC TCCCCCTCGCCCTCAATCACTAGTGAATTCGCG	G G V G K T T L L K R I D N D F L Q T G Y E V D V V I W V V V S Q Q G N V E K V Q E T V L N K L E I A E Y K W K D R S V H E R A E E I F S V L Q T K K F V L L L D D I W K Q L D L L E V G I P P L N D Q K K S K V I F T T R F S T V C H D M G A K S I E V E C L A W E E A F S L F R T K V G E D T L D S H P D I Q K L A E I F V K E C K G L P L A L

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVcin127	GCGGCCGCGGGAACTTCGCCCTTGGGGGGGTGGGGAAGACGACTAT AGCCAANGTTTGTTTTATAATCCAATCTCTAGTCAATTTGAGGGCAT TAGCTTCCTTGCAAATATTAGAGAAGTCTCCAAAAACTGTGGTTTGC TTCCATTACAGAAACAACTTCTAGGTGATATTTTGATGGGATGGAGT CAAAGGATAAGCANTGTCGATGAAGGAATCAATGTGCTAATGGACA GACTTCACTCTAAAAAGGTTCTTATTATTCTTGATGACGTGGATGAT TTGAATCAATTANAATCCTTAGCTGGAAATGTTGATTGGTTTGGTAT TGGAAGNANAATTGTTATAACAACTAGAGATAAACNTCTGCTAAAT GTGCATGGAGNGAGTGAAATATATGAGGCTAAGGAATTNGAACCA NAGGAAGCTCTTCAACTTTTCAGTCAATATGCTTTCAAAAGAAAAA GTCCNNANAAAGATTATATGAACCTCTCTGACNATGTAGTACATTA TGCTAAAGGCCTCCCCCTCNCCCCTCAATCACTAGTGAATTCNCGGC CG	G G V G K T T I A ? V C F I I Q S L V N L R A L A S L Q I L E K S P K T V V C F H Y R N N F * V I F * W D G V K G * A ? S M K E S M C * W T D F T L K R F L L F L M T W M I * I N ? N P *L E M L I G L V L E ? ? L L * Q L E I N ? C * M C M E ? V K Y M R L R N ? N ? R K L F N F S V N M L S K E K V ? ? K I I * T S L ? M * Y I M L K A S P ? P L
rgVcin139	GGGTAATTCCATTGGGGGGGTGGGGAAGACGACTATCACCAAGGCG GTTTATAATGATATCTCATGTCAATTTGATGGCAGTAGTTTTCTTAA CAATGTTAGAGAAAGATCCAAAGACAATGCACTTCAATTACAACAA GAACTACTTCATGGTTCCTTAAAGGGAAAAAGTCTAAAAGTAAGCA ATATGGATGAAGGAATTCAGATGATAAAGAGGAGTCTCAGCTCTAA AAGGGTTCTTGTTGTTTTTGATGACGTGGATGATTTGATGCAAATAG AAAACTTGGCAGAAGAGCATATTTGGTTTGGTCCAAGAAGTAGGAT CATCACAACAACTAGACACAAACATTTTCTAACCCAATATGGAGTC ATAGAATCATATGAAGTTCCGAAACTACATGATGCAGAAGCTATTG AGCTGTTTAGTTGGTGGGCTTTCAAACAAAATCTTCCTAATGAAATT TATAAAAATCTCTCCTACCAGGTAGTAAATTATGCTAAAGGCCTCCC CCTCGCNCTCAATCACTAAGTGAATTCGCG	G G V G K T T I T K A V Y N D I S C Q F D G S S F L N N V R E R S K D N A L Q L Q Q E L L H G S L K G K S L K V S N M D E G I Q M I K R S L S S K R V L V V F D D V D D L M Q I E N L A E E H I W F G P R S R I I T T T R H K H F L T Q Y G V I E S Y E V P K L H D A E A I E L F S W W A F K Q N L P N E I Y K N L S Y R * * I M L K A S P S ? S I T K * I R

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVcin152	CCGCGGGAAATTCCCCTTTTGGGGGGGTGGGGAAGACGACTCCTGG GCCAAACTNAGTATATAATGATGGGAGAGTAGTTTGCTCATTTTGA GAAAAGAATGTGGGTTTGTGTTTCANANGAGTTTGATGTCAAAAGG TTAATAAAAGAAATCATTACTTCTGCCACTCATGGCAAATGTGATG ATTTACCCATGGATGAGTTGGCACGTCTTCTTATAAATGTATTAGAT GATAAAAAATTCTTACTTATCTTAGATGACGTGTGGAGTAAGAATC GAGATAAATGGCTTGAGTTAAAAGCCTTGCTAGATGGCGGTGCTAA GGGAAGTAAAATAATTGTCNCCACACGTGATAAATTGGTAGCCTCC GNCATGGGTACTTGTCCCATGTATGAATTAAAGGGTCTTTCTGATGA GGAGTGTTTGTCTCTATTTATTACATGTGCATTCNAGGATGATCGAG ATAAACANTATCCAAGACTTGTGGGGANTGGNAAGGATATTGTCNA AAANTGCNNAAGGCCTCCCCCTCGCCCTCAATCACTAGTGAATNCG CGGCCGC	G G W G R R L L G Q T ? Y I M M G E * F A H F E K R M W V C V S ? E F D V K R L I K E I I T S A T H G K C D D L P M D E L A R L L I N V L D D K K F L L I L D D V W S K N R D K W L E L K A L L D G G A K G S K I I V ? T R D K L V A S ? M G T C P M Y E L K G L S D E E C L S L F I T C A F ? D D R D K ? Y P R L V G ? ? K D I V ? ? C ? R P P P R P Q S L V N ? R P
rgVcin165	GAATTCGATTGGGGGGGTGGGGAAGACGACTATCGCCAAGGCTATT TATAATGAGATCTCAAATCAATATGATGGTAGGAGCTTTCTTAGAA ATATAAGAGAGAGATCCAAAGGTGATATACTTCAGTTACAGCAAGA ACTTCTTCATGGTATCCTGAGGGGAAAATTTTTTTAAATAAACAATG TTGATGAAGGAATTAGTATGATAAAGAGGTGTCTAACCTCCAATAG AGTTCTTGTTATTTTTTATGATGTGGATGAGTTGAAACAACTAGAAT ATTTGGTTGAAGAGAAAGATTGGTTTCATGCAAAAAGTACAATCAT CATTACAACTAGAGACAAACACGTGCTTGCTCAATATGGAGCGGAT ATACCATATGAGGTTTCAAAATTAAACAAGGAAGAAGCTACTGAGC TCTTTAGTTTGTGGGCCCTTAAACAAAATCATCCCCAAGAAGTTTAT AAAAACCTCTCATACAATATCATAGATTATGCTGATGGCCTCCCCCT CGCCCTCAATCACTAGTGAATTCG	G G V G K T T I A K A I Y N E I N Q Y D G R S F L R N I R E R S K G D I L Q L Q Q E L L H G I L R G K F F * I N N V D E G I S M I K R C L T S N R V L V I F Y D V D E L K Q L E Y L V E E K D W F H A K S T I I I T T R D K H V L A Q Y G A D I P Y E V S K L N K E E A T E L F S L W A L K Q N H P Q E V Y K N L S Y N I I D Y A D G L P L A L

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVrup103	GAATTCGATTGGGGGGGTGGGGAAGACGACTATCGCCAAGGCTATT TATAATGAGATCTCACATCAATATGATGGTAGTAGCTTTCTTATAAA TATCAGAGAGAGATCCAAAGGTGATATACTTCAGTTACAACAAGAA CTTCTTCATGGTATCCTGAGGGGAAAAAATTTTAAAATAAACAATG TTGATGAAGGAATTAGTATGATAAAGAGGTGTCTAACCTCCAATAG AGTTCTTGTTATTTTTTATGATATGGATGAGTTGAAACAACTAGAAT ATTTGGCTGAAGAAAAAGATTGGTTTCATGCAAAAAGTACAATCAT CATTACAACTAGAGACAAACACGTGCTTGCTCAATATGGAGCGGAT ATACCATATGAGGTTTCAACATTAAACAAGGAAGAAGCTACTGAGC TCTTTAGTTTGTGGGCCTTTAAACAAAATCATCCCCAAGAAGTTTAT AAAAACCTCTCATACAATATCATAGATTACGCTGATGGCCTCCCCCT CGCCCTCAATCACTAGTGAATTCGC	G G V G K T T I A K A I Y N E I S H Q Y D G S S F L I N I R E R S K G D I L Q L Q Q E L L H G I L R G K N F K I N N V D E G I S M I K R C L T S N R V L V I F Y D M D E L K Q L E Y L A E E K D W F H A K S T I I I T T R D K H V L A Q Y G A D I P Y E V S T L N K E E A T E L F S L W A F K Q N H P Q E V Y K N L S Y N I I D Y A D G L P L A L
rgVrup119	GGAATTCGATTGGGGGGGTGGGGAAGACGACCATGGTGAAACAGG TGGGTGCAAATGCTCACAGAGATGGGCTGTTTCAGCGTGTTGCAAT GGCTGTGATATCCCAGAATCCGGATTTGAGGAAAATTCAAGCCCAA ATTGCAGATATGTTGAACTTGAAGCTAGAAGAGGAGAGTGAAGCTG GGAGGGCAGCAAGGTTGAGGGAGAGGATAATGAGAGGCAAGAGTG TTCTCATAATCTTAGATGACATATGGAGAAGAATTGACTTATCTGAG ATAGGAATTCCCAGTACTGGCAGTGACCTTGATGCCTGCAAATCCA AAATCTTGCTGACCACAAGGCTTGAAAATGTGTGTCATGTCATGGA AAGCCAAGCAAAAGTCCCTCTGAATATTCTGTCTGAACAGGATTCTT GGACCTTGTTTGGGAGGAAAGCAGGGAGAGTCGTGGATTCTCCTGA TTTTCATAATGTAGCACAGAAGATTGTCAAAGAATGTGGAGGCCTC CCCCTCGCCCTCAATCACTAGTGAATTCGCGGCC	G G V G K T T M V K Q V G A N A HR D G L F Q R V A M A V I S Q N P D L RK I Q A Q I A D M L N L K L E E E S E A G R A A R L R E R I M R G K S V L I I L D D I W R R I D L S E I G I P S T G S D L D A C K S K I L L T T R L E N V C H VM E S Q A K V P L N I L S E Q D S W T L F G R K A G R V V D S P D F H N V A Q KI V K E C G G L P L A L

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVrup124	GAATTCCATTGGGGGGGTGGGGAAGACGACTTTGGCTCAAGTGGTC TTCAATGACAGGGAAATGGAGGCTCGTTTTGAGAGGAGGATGTGGG TGTCTGTCACTGGGACACCTAACGAAAAACGGATTTTGAGAAGCAT GTTGAGGAACTTGGGTGATATGAATGTGGGAGATGATTGTGGTGAG TTGTTGAGGAAAATAAATCAATACCTCTTGGGCAAGAGGTTTTTGCT TGTCATGGATGATGTGGGGGAAAACACAAATACTTGGTGGCGCAAA ATCAGTGATGGATTGCCTAAAGGAAATGGGAGTAGTATTATTATCA CTACAAGAACTAAGGAAGTCGCAACAATGATGGGAGTGGAGGAAG AGAGAACACATCGGCCCAAAGTCCTCAGTAAGGATGATAGTTGGTT GCTCTTCCGCAATGTAGCATTTGCTGCCAATGGAGGCATTTGTACTT CCTCTGAACTGGAGAACATTGGGAGGGAGATTGTACATAAATGTGG GGGCCTCCCCCTCGCCCTCAATCACTAGTGAAT	G G V G K T T L A Q V V F N D R E M E A R F E R R M W V S V T G T P N E KR I L R S M L R N L G D M N V G D D C G E L L R K I N Q Y L L G K R F L L V M DD V G E N T N T W W R K I S D G L P K G N G S S I I I T T R T K E V A T M M G VE E E R T H R P K V L S K D D S W L L FR N V A F A A N G G I C T S S E L E N IG R E I V H K C G G L P L A L
rgVrup126	GAATTCGATTGGGGGGGTGGGGAAGACGACCCTCTTGAAGAGAATC AACAACGAGTTCCTCCAAACATGCTATGAAGTCGATGTAGTCATTT GGGTTGTTGTGTCCCAACAAGGGAANGTGAAAAAGGTCCAGGAAA CCATTCTCAATAAGTTGGAGATTGCTGAATACAAATGGAAAGATAG GAGCGTGCATGAAAGGGCTGAAGAAATAATCAGTGTCTTGCAAACA AAGAAATTTGTGCTCTTGTTAGATGATATATGGAAGCAGCTTGATCT TTTGGAAGTGGGGATTCCTCCTTTGAATGATCAAAATAAGTCCAAA GTAATATTTACAACACGGTTTTCAACTGTGTGCCACGACATGGGAG CTAAAGCATTGAAGTTGAGTGCTTGGCATGGGAGGAAGCTTTTTCTT TGTTTCGGACCAAGGTAGGAGAAGACACCTTAGATTCTCATCCAGA TATACAAAAGGTTGCGGAGATTTTTGTCAAAGAGTGCANAGGCCTC CCCCTCGCCCTCAATCACTAGTGAATTCGCGGC	G G V G K T T L L K R I N N E F L Q T C Y E V D V V I W V V V S Q Q G ? VK K V Q E T I L N K L E I A E Y K W K DR S V H E R A E E I I S V L Q T K K F V L L L D D I W K Q L D L L E V G I P P L N D Q N K S K V I F T T R F S T V C H DM G A K A L K L S A W H G R K L F L C FG P R * E K T P * I L I Q I Y K R L R RF L S K S A ? A S P S P S I T S E F A

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVhyb101	GCGGGAATTCGATTGGAGGGGTGGGGAAGACAACTATCGCCAAGG CTGTTTATAATGATATCTCATATCAATTTGATGGTAGTAGTTTTCTTA ACAATGTTAGAGAAAGATCCAAAGACAATGCACTTCAATTACAACA AGAACTACTTCANGGTATCTTAAAGGGAAAAAGTCTAAAAGTAAGC AATATGGATGAAGGAATTCANATGATAAAGAGGAGTCTTTGCTCTA AAAGGGTTCTTGTTGTTTTTGATGACGTGGATGATTTGATGCAACTA NAAAACTTGGCANAAGAGCATAGTTGGTTTGGTCCAAGAAGTAGGA TCATCATAACAACTAGACATAAACGTTTTCTANCCCAATATGGAGTC AAAGAATCATATGAAGTTCAAAAACTGCATGATGAANAANCTATTG AGCTGTTTAGTTTGNGGGCTTTCCCACAAAATCTTCCTAGCGAAATT TATAGAAATCNCNCCTATCGGGTAGTANATTATGCTAANGGTCTTCC CCTTGCCCTTAATCACTAG	G G V G K T T I A K A V Y N D I S Y Q F D G S S F L N N V R E R S K D N A L Q L Q Q E L L ? G I L K G K S L K V S N M D E G I ? M I K R S L C S K R V L V V F D D V D D L M Q L ? N L A ? E H S W F G P R S R I I I T T R H K R F L ? Q Y G V K E S Y E V Q K L H D E ? ? I E L S L ? A F P Q N L P S E I Y R N ? ? Y R V V ? Y A ? G L P L A L
rgVrup102	CCGCGGGAATTCGATTGGAGGGGTAGGGAAGACGACAATTTCCCCA TGAATGGCACAAATGCAATTTGCCCTATAAAGCAGGGAATGTCAAA ACCTAAAGAGATCACATTTATTGCATCAGAATAAGAATATAAATAA CATTTAATTCAATAAAAGGCCACTGACAAACTATAAAGGCAGCAAA TCTCAAAAAATCTGTAAAAACAGCAAACAGATCACCATGGATATAG TCATTGTCTACCAGGCTGACCAAGAAATAACTGCTCTGTACCTGAA AAATATCAGTGTTATTTAGTTGCTGTCACTAAATTGAAAATATCTGT ATGTGAATATACGTATATATCAATTATAAACAACACCTTTAACACCA ACCTCCTCCAGCAAAATTCTTGATGGGTCTCCCCCTCGCTCTCAATC ACT	G G V G K T T I S P * M A Q M Q F A L * S R E C Q N L K R S H L L H Q N K N I N N I * F N K R P L T N Y K G S K S Q K I C K N S K Q I T M D I V I V Y Q A D Q E I T A L Y L K N I S V I * L L S L N * K Y L Y V N I R I Y Q L * T T P L T P T S S S K I L D G S P P R S Q S L V N S R P P

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVhyb110	TGCGATCCAGCTCCGGCCGCCATGGCGGCCGCGGGAATTCGATTGG GGGGGTGGGCAAAACAACCCTCTTGAACAGGGTCAACAATGAGTTC CTCAAATCCAGGGTTGAGTTTGACGCAGTGATTTGGGTGATTGTGTC CAGACCAGCAAATGTGGAGAAGGTTCAGCAAGTTCTTTTCAATAAA TTGGAGATTCCTAGTAACAACTGGGAAGGTAGAAGTGAGGATGAAA GGAAAGAAGCAATATTCAATGTCTTGAAGATGAAAAAAATTGTCGT CTTATTAGATGATATATGGGAGCTTCTTGATCTCTTTGCAGTGGGGA TTCCTCCTATAAATGATGAAAATAAGTCCAAGGTGGTATTTACAACC CGATTTTCAACTGTGTGCCGAGATATGGGAGCTAAGGGCATTGAAG TGAANTGCTTANCATGAGAGGAAGCATTTGCTCTATTTCAGGCATAT GTAGGAAAAGACACTA	G G V G K T T L L N R V N N E F L K S R V E F D A V I W V I V S R P A N V E K V Q Q V L F NK L E I P S N N W E G R S E D E R K E A I F N V L K M K K I V V L L D D I W E L L D L F A V G I P P I N D E N K S K V V F T T R F S T V C R D M G A K G I E V ?C L ? * E E A F A L F Q A Y V G K D T
rgVrup121	GGCGGGAAATTCGATTGAGGGCAAGTGGGAGGCCGTCACACTCTTT GGCAACCATCTCCGCCAGCTTTGGTATATCTGGATGAGAACTTATGG TGTCTGCTCCTACCTTGGTCTGAAACAGAGCAAAAGCTTCCTCCCAT GGGAGGCAATTCACTTCAATGCTCTTGGTAGATTCCATCTTTTGGCA CACTTGTTTAGATCGCGTTGTAAATACCATCTTCAACTTATCTTGAT GATTCAGAGGAGGAATACCAACTCTGGATAGATCCAGCCGCTCCCA TATGTCATCTAACAAAAGCACAAATTTCTTTGTCTTCAGGACATTGA ATATTTCTTCTGCCCTTTCATCCTCACTCCTATCTTCCCATTTATCTTT CCCAATCTCCAATTTATTGAAAAGAACTTGCTGAACCTTTTCCACAT TGGCTGGTCTGGACACAGTCACCCAAATCACTGCATCAAATTCAAC CCTGGTTTTGAGGAGCTCATTGTTGATCCTGGTCAAGAGGGTGGTTT TCCCGACGCCCCCAATCACTAGTG	G G K F D * G Q V G G R H T L W Q P S P P A L V Y L D E N L W C L L L P W S E T E Q K L P P M G G N S L Q C S W * I P SF G T L V * I A L * I P S S T Y L D D S E E E Y Q L W I D P A A P I C H L T K A Q I S L S S G H * I F L L P F H P H S Y L P I Y L S Q S P I Y * K E L A E P F P H W L V W T Q S P K S L H Q I Q P W F * G A H C * S W S R G W F S R R P Q S L V

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVhyb127	CGGGAATTCGATTGGGGGGGTGGGAAAAACAACCATCGCCAAGGTT TGTTTACAATGATATGTTAGATCAATTTAAACGCCATAGCTTTCTTG AAAATGTGAGAGAGAAATCTAAAGATGATCGTGGTGTGCTTCAATT ACAAGAAAAACTTCTTTGTGATATTCTAATGGAGAAAAATTTGAAG TTAAGTAATATTGATGAAGGAATCAAAANGATAATTAAGAGTAAGC ATCACTTGGAAAAGGTTCTTATTGTTCTTGATGATGTAGATTGTCCA AAACAATTAAAATTTTTTGCTCCTAATCCTGAATGGTTTCATCCAGG AAGCATAATCATTGTGACCATGAGAAATAAACGTTGCCTAGATGTA CATGAGTCATATTCATCATATGAGGCTAAGGGATTAGCACACAAGC AAGCTACGGAACTCTTTTGTTGGAATGCCTTTCAACAACACCATCCA AAAGAGAATTATGTGGACCTCTCTAATCGTATATTGGATTATGCTAA AGGCCTCCCCCTAGCTCTCAATCACTAGT	G G V G K T T I A K V C L Q * Y V R S I * T P * L S * K C E R E I * R * S W C A S I T R K T S L * Y S N G E K FE V K * Y * * R N Q ? D N * E * A S L GK G S Y C S * * C R L S K T I K I F C S* S * M V S S R K H N H C D H E K * T L P R C T * V I F I I * G * G I S T Q A S Y G T L L L E C L S T T P S K R E L C G P L * S Y I G L C * R P P P S S Q S L
rgVhyb139	TGGCNATCGCAGCTCCGGCCGCCATGGCGGCCGCGGGAATTCGATT GGGGGCGTGGGGAAAACGACTGGCATAACTCGTATNCACTCTCGGG CTCATTGTCAATGATAGACTACACATTTCGGCCATGTCTTGTTGGGT GACTGTCTCAGAAGACGTCCTTGCATGTCAGTATTGATGCATCGAA GCTATTGCCGCAAAGATGACTTCCTATTTCACCAAACAGGAGGATG AAACCATTTTAGCTGCTTTATTATCAAAACCATTGCCGGAAAGACA GAATTTTGTTCTAAAATTAGATGATGTCTGGGAATTTTATGCCCCAC CGCCAGGTTGGAATTCCCATTGGAGTTGACGGAGCAACCCTGATCA CAACCACCAGATCAAAGCTACGTGTGCCTAAGAATGGTATGCTGAG AAATCATCAAATGTGGCCCCACTTTCTGAGATAGAAGCATGGGAGC TTTTCAACCCAGCGCTTGAGTCGGTACACTGCCACTGATTCCAACAC AGAATACGAAA	G G V G KT T G I T R ? H S R A H C Q * * T T H FG H V L L G D C L R R R P C M S V L M H R S Y C R K D D F L F H Q T G G * N H F S C F I I K T I A G K T E F C S K I R *C L G I L C P T A R L E F P L E L T E QP * S Q P P D Q S Y V C L R M V C * E I I K C G P T F * D R S M G A F Q P S A *V G T L P L

Appendix Table 1A. Nucleotide and amino acid sequences of each representative RGA cloned from P-loop/GLPLAL-1 primer (continued).

Representative RGAs cloned	Nucleotide sequence	Amino acid sequence
rgVhyb149	GCGGGAAATTCGATTGGAGGGGTGGGAAAGACGACATTCCTTTAGG AAGATGGTNGGAAAGGTTTTCGGTTAATAAGGGTTTGATCAAATTA CAACAAAAATTTCTTTCTCAACCTTTGGAGGAAGAGAATCTAAATA TGAAAGGACTCACATCTATAAAGGCTAGACTCCGTTCAAAAAAGGT TCTTATTATTCTCGACAATGTGAATGATTCAAGAATATTGGAATGCT TAAATTGGAAATCGGGATTGGTTTGGTCGAGGAAGTAGAATTATTA TAACGACTAGAGATAAATGTTTGTTATTTTCACATGGAGTTAATTAT TATGAGGTTGAGAAATTCTATCGTGACGAAGCCTATGATTCCATTAT ACATCATTCGTTAACACATGAACTTCCTACAGCTTGATTTCTTGGAG CTTTCAAAATAATTGATATTTTTATCCTCAAGGTCTGCCGCTTGCCCT CATTCATTATTGCA	G G V G K T T F L * E D G ? K G F R L I R V * S N Y N K N F F L N L R K R I * I * K D S H L * R L D S V Q K R F L L F S T M * M I Q E Y W N A * I G N R D W F G R G S R I I I T T R D K C L L F S H G V N Y Y E V E K F Y R D E A Y D S I I H H S L T H E L P T A * F L G A F K I I D I F I L K V C R L P S F I I A

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents.

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
1	361012	63.20	55.47	93.44	119.26	40.57	57.14	**70.70**	**72.33**	**71.51**	5
2	361013		15.26	3.83	6.99	3.88	4.47	**9.55**	**5.12**	**6.89**	1
3	361014		39.19	51.71	36.46	39.63	42.19	**45.45**	**39.42**	**41.83**	5
4	361019	6.37	5.72	4.39	11.59	8.21	15.10	**5.49**	**11.64**	**8.56**	1
5	361022	76.12		92.87	50.18	41.25		**84.50**	**45.71**	**65.10**	5
6	361023	12.67	8.90	9.33	44.11	20.06	10.15	**10.30**	**24.78**	**17.54**	3
7	361024	9.45	6.14	9.50	8.25	4.17	3.50	**8.36**	**5.31**	**6.84**	1
8	361025	48.81	56.52	24.31	10.72	10.62	24.52	**43.21**	**15.28**	**29.25**	4
9	361026	15.59	9.74	3.01	3.46	9.26	3.32	**9.45**	**5.35**	**7.40**	1
10	361029	12.13	20.30	6.81	3.48	5.63	4.92	**13.08**	**4.68**	**8.88**	1
11	361030	17.66	19.15	7.50	14.59	31.35	19.07	**14.77**	**21.67**	**18.22**	3
12	361031	12.63	8.27	8.41	20.37	7.74	7.67	**9.77**	**11.92**	**10.85**	2
13	361036		65.04		62.66		22.06	**65.04**	**42.36**	**49.92**	5
14	361041	8.80	6.36	14.47	6.54	6.19	7.11	**9.88**	**6.61**	**8.24**	1
15	361042	3.18	1.23	1.70	5.74	3.83	0.58	**2.04**	**3.38**	**2.71**	0
16	361051	39.78	28.73	18.06	68.17	38.59	32.49	**28.86**	**46.42**	**37.64**	4
17	361057	2.85	2.41	1.82	8.99	24.73	3.01	**2.36**	**12.24**	**7.30**	1
18	361058	5.63	3.83	5.44	9.69	14.73		**4.97**	**12.21**	**7.86**	1
19	361059	6.93	12.98	6.30	5.10	10.25	6.10	**8.74**	**7.15**	**7.94**	1
20	361061	8.88	13.14	4.32	15.08	7.74	9.14	**8.78**	**10.65**	**9.72**	1
21	361062	6.37	4.48	8.29	29.25	9.62	19.02	**6.38**	**19.30**	**12.84**	2

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
22	361065	27.73	57.41		25.52	54.70	18.49	**42.57**	**32.90**	**36.77**	4
23	361066	4.98	8.48	5.94	7.91	4.02	3.63	**6.47**	**5.19**	**5.83**	1
24	361067	5.79		3.14			12.00	**4.46**	**12.00**	**6.97**	1
25	361069	10.39	8.51	6.42	12.37	11.00	10.47	**8.44**	**11.28**	**9.86**	1
26	361071	3.49	0.56	2.67	1.80	4.02	0.46	**2.24**	**2.09**	**2.17**	0
27	361072	12.95	13.39	3.68	20.53	9.98	17.33	**10.01**	**15.95**	**12.98**	2
28	361075	8.68	6.97	3.28	5.29	4.15	5.04	**6.31**	**4.83**	**5.57**	1
29	361076	9.60	4.79	2.19	12.95	28.64	10.55	**5.53**	**17.38**	**11.45**	2
30	361077	14.77	13.32	9.37	5.97	6.91	11.09	**12.49**	**7.99**	**10.24**	2
31	361079	4.22	4.28	3.50		6.30	3.70	**4.00**	**5.00**	**4.40**	0
32	361081	55.90	20.72	21.05	45.18	33.06		**32.56**	**39.12**	**35.18**	4
33	361083	15.40	16.35	4.99	10.44	11.67		**12.25**	**11.06**	**11.77**	2
34	361086		32.23	24.51	13.62	9.24	15.31	**28.37**	**12.72**	**18.98**	3
35	361089	33.35	64.68	23.41	71.84	21.03	17.06	**40.48**	**36.64**	**38.56**	4
36	361090	36.85	10.91	3.28	11.67	8.89	21.38	**17.01**	**13.98**	**15.50**	3
37	361092	9.85	10.22	10.74	25.03	9.06	12.84	**10.27**	**15.64**	**12.96**	2
38	361093	3.51	2.34	4.25	3.39	4.71	15.72	**3.37**	**7.94**	**5.65**	1
39	361094	11.93	8.04	6.85	5.19		3.66	**8.94**	**4.43**	**7.13**	1
40	361095	7.16	9.21	10.24	4.00	5.85	8.73	**8.87**	**6.19**	**7.53**	1
41	361096	4.19	6.24	1.86	9.63	7.87	4.24	**4.10**	**7.25**	**5.67**	1
42	365004	5.07	5.05	7.62	16.97	8.76	10.52	**5.91**	**12.08**	**9.00**	1

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
43	365005	8.07	7.47	7.26	15.34	9.23	5.64	**7.60**	**10.07**	**8.84**	1
44	365006	10.84	14.70	16.00	8.14	6.30	4.22	**13.85**	**6.22**	**10.03**	2
45	365007	13.55	6.82	10.85	5.98	8.46	13.94	**10.41**	**9.46**	**9.93**	1
46	365010	6.62	2.00		10.57	4.62	10.53	**4.31**	**8.57**	**6.87**	1
47	365012	20.22	26.32	12.69	5.80	20.80	20.76	**19.75**	**15.78**	**17.76**	3
48	365013	54.86	31.14	46.08	25.63	52.67	15.43	**44.02**	**31.24**	**37.63**	4
49	365015	15.11	7.67	6.11	13.02	29.42	14.85	**9.63**	**19.10**	**14.36**	2
50	365017	11.49	6.99	15.58	20.47	9.99	18.32	**11.35**	**16.26**	**13.81**	2
51	365018		6.89	6.14	9.63	10.85	18.73	**6.51**	**13.07**	**10.45**	2
52	365019	8.96	10.96	7.05	4.98	4.66	6.77	**8.99**	**5.47**	**7.23**	1
53	365020	14.48	11.85	10.40	26.83	10.54	38.67	**12.25**	**25.35**	**18.80**	3
54	365021	14.31	12.16	9.00	21.27	12.73	10.71	**11.82**	**14.90**	**13.36**	2
55	365023	6.20	9.39		21.24		15.07	**7.79**	**18.15**	**12.97**	2
56	365024	13.58	6.24	20.60	25.95	15.63	23.26	**13.47**	**21.61**	**17.54**	3
57	365025	4.37	4.91	5.46	4.09	2.92	4.79	**4.92**	**3.93**	**4.42**	0
58	365027	63.75	53.74	50.94	81.01	19.15	33.84	**56.14**	**44.67**	**50.41**	5
59	365028	7.22	8.80	13.47	2.63	2.18	6.76	**9.83**	**3.86**	**6.84**	1
60	365029	17.40	13.40	13.77	9.69	7.38	6.85	**14.85**	**7.97**	**11.41**	2
61	365030	10.09	5.57	23.29	10.92		19.83	**12.98**	**15.38**	**13.94**	2
62	365032	6.66	24.38	32.40	20.81	21.81	19.84	**21.15**	**20.82**	**20.98**	3
63	365035	16.10		8.13	61.17	37.78	20.46	**12.12**	**39.80**	**28.73**	4

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
64	365036	18.56	23.49	14.13	37.59	34.72	42.88	**18.73**	**38.40**	**28.56**	4
65	365037		2.77	11.20	8.29	9.70	24.51	**6.99**	**14.16**	**11.29**	2
66	365038	12.06	8.46	19.87	38.89		18.22	**13.46**	**28.56**	**19.50**	3
67	365039	20.32	4.93	16.77	24.49	9.25	5.35	**14.00**	**13.03**	**13.52**	2
68	365044	18.32	6.24	3.67	11.03	21.19		**9.41**	**16.11**	**12.09**	2
69	365049	7.02	7.13	8.27	6.90	24.02	13.21	**7.47**	**14.71**	**11.09**	2
70	365051	72.65	39.86	35.25			54.44	**49.25**	**54.44**	**50.55**	5
71	365052	5.03	6.67	5.41	26.71	13.77	16.92	**5.71**	**19.14**	**12.42**	2
72	365053	4.73	11.92	2.94				**6.53**		**6.53**	1
73	365055	9.43	14.71	8.79	20.96	20.22	12.51	**10.98**	**17.90**	**14.44**	2
74	365056	8.35	6.05	4.92	22.38	8.58	19.47	**6.44**	**16.81**	**11.63**	2
75	365057	8.50	7.35	9.48	15.54	10.38	13.71	**8.44**	**13.21**	**10.83**	2
76	365059	10.99		9.52	6.69	3.70	14.88	**10.25**	**8.42**	**9.15**	1
77	365061		7.43	2.84	10.72	4.98	9.86	**5.14**	**8.52**	**7.17**	1
78	365062	16.14	12.71	12.32	7.11	5.42	5.21	**13.72**	**5.91**	**9.82**	1
79	365063	41.35	40.65	25.58	53.09		18.00	**35.86**	**35.54**	**35.73**	4
80	365065	23.66	35.66	18.27	70.75	37.57	35.83	**25.86**	**48.05**	**36.96**	4
81	365066	15.27	30.69	19.89	17.85	34.08	22.63	**21.95**	**24.85**	**23.40**	3
82	365067	12.15		10.40	4.49	2.53	4.06	**11.28**	**3.69**	**6.73**	1
83	365074	3.85	18.21	35.08	16.03	14.74	17.10	**19.05**	**15.96**	**17.50**	3
84	365075	6.14	1.44	13.78		3.08	11.33	**7.12**	**7.21**	**7.16**	1

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
85	365076	65.91		28.38	127.75			**47.15**	**127.75**	**74.02**	5
86	365077	48.82	61.97	55.95	14.04	19.03	38.98	**55.58**	**24.02**	**39.80**	4
87	365078	44.59	37.70	36.32		30.60	40.79	**39.54**	**35.70**	**38.00**	4
88	365084			1.73	4.83	4.28	6.97	**1.73**	**5.36**	**4.45**	0
89	365085	3.56		7.45	1.11	6.02	8.36	**5.51**	**5.16**	**5.30**	1
90	365086	3.73	5.86		9.26	13.04	27.52	**4.80**	**16.60**	**11.88**	2
91	365087	44.54	48.62	44.85	15.82	3.82	10.00	**46.01**	**9.88**	**27.94**	4
92	366008	6.36		2.01	2.34			**4.19**	**2.34**	**3.57**	0
93	366010	2.78	18.39	4.19	7.21	2.05	1.41	**8.45**	**3.56**	**6.00**	1
94	366013	21.22	9.23	6.87	32.95	22.88	12.37	**12.44**	**22.73**	**17.59**	3
95	366015	12.08	12.75	10.92	13.20	7.84	36.37	**11.92**	**19.14**	**15.53**	3
96	366017	41.42	57.64	44.85	69.94			**47.97**	**69.94**	**53.47**	5
97	366018	22.19	26.20	17.44	17.70	22.40	12.79	**21.94**	**17.63**	**19.79**	3
98	366020	14.83	15.33	12.72	13.75	9.09	27.87	**14.30**	**16.90**	**15.60**	3
99	366022	4.73	7.81	3.35	4.73	1.11	5.42	**5.30**	**3.75**	**4.52**	0
100	366023		11.39	3.98	9.07	3.38	12.74	**7.69**	**8.40**	**8.11**	1
101	366026	12.06	11.20	16.18	13.56	18.24	14.08	**13.15**	**15.29**	**14.22**	2
102	366027	13.09	9.85	17.04		0.00	1.62	**13.32**	**0.81**	**8.32**	1
103	366030	3.67	3.38	18.73		1.10	11.61	**8.60**	**6.35**	**7.70**	1
104	366032	9.37	12.90	8.80		9.32	9.49	**10.36**	**9.40**	**9.98**	1
105	366034	2.22	8.47	4.93	26.41	4.57	1.70	**5.21**	**10.89**	**8.05**	1

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
106	366035	19.14	18.49	8.73	16.96			**15.45**	**16.96**	**15.83**	3
107	366037	11.14	18.11	8.19	17.66		1.22	**12.48**	**9.44**	**11.26**	2
108	366038	11.55	8.68	19.80	10.53	16.39	7.97	**13.34**	**11.63**	**12.49**	2
109	366039	3.99	4.33	10.33			17.47	**6.22**	**17.47**	**9.03**	1
110	366040	41.45	25.16	27.57		28.61	20.12	**31.39**	**24.37**	**28.58**	4
111	366041	5.08	5.34		7.76	6.92	31.93	**5.21**	**15.54**	**11.40**	2
112	366042	4.92	13.00	5.38	21.65	2.04	20.41	**7.77**	**14.70**	**11.23**	2
113	366043	18.26	12.72	14.38	27.28	13.64	20.20	**15.12**	**20.37**	**17.75**	3
114	366045	4.12	5.29	8.98	4.54	3.86		**6.13**	**4.20**	**5.36**	1
115	366046	4.50	14.43	16.96	5.30	16.09	25.56	**11.96**	**15.65**	**13.81**	2
116	366047	6.36	9.75	4.33				**6.81**		**6.81**	1
117	366048	0.57	10.17	5.40	3.78	16.79	5.85	**5.38**	**8.81**	**7.09**	1
118	366049	19.24	32.16	21.42	18.54	21.61		**24.27**	**20.08**	**22.59**	3
119	366050	79.03	56.80	59.19		85.89	46.42	**65.01**	**66.15**	**65.46**	5
120	366055	8.48	8.23	7.61	10.05		4.87	**8.11**	**7.46**	**7.85**	1
121	366056	55.08	44.58	31.39	65.28		77.70	**43.68**	**71.49**	**54.81**	5
122	366057		7.74	19.69	21.00	21.09	16.73	**13.72**	**19.61**	**17.25**	3
123	366058	15.76	14.40	15.64	3.44	6.48	11.55	**15.26**	**7.16**	**11.21**	2
124	366059	9.29	4.68	9.94	4.21	3.27		**7.97**	**3.74**	**6.28**	1
125	366060		9.73	13.20	15.69			**11.47**	**15.69**	**12.87**	2
126	366062		174.03	106.36	152.73	117.89	59.54	**140.20**	**110.06**	**122.11**	5

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
127	366063	7.94	10.39	13.12	12.94		26.50	**10.48**	**19.72**	**14.18**	2
128	366064	17.39	7.04	7.05	10.74	14.30		**10.49**	**12.52**	**11.31**	2
129	366066	16.97	7.69	10.73	5.78	7.90	18.72	**11.80**	**10.80**	**11.30**	2
130	366070	19.92	12.97	28.45	15.76	23.44	20.69	**20.45**	**19.96**	**20.20**	3
131	366072	6.10	23.10	3.61		3.01	12.32	**10.94**	**7.67**	**9.63**	1
132	366074	20.18	16.57	12.71		29.56	20.09	**16.48**	**24.83**	**19.82**	3
133	366075	14.50	18.01	15.68	29.04	23.96	17.63	**16.06**	**23.54**	**19.80**	3
134	366076	10.73	5.77	8.05	4.00	6.67	12.56	**8.18**	**7.74**	**7.96**	1
135	366079	8.40	8.90	12.41	7.84	5.18	5.34	**9.90**	**6.12**	**8.01**	1
136	366081	20.41	10.25	9.14	13.27			**13.27**	**13.27**	**13.27**	2
137	366085	37.58	21.70	12.65	14.22	8.22	9.91	**23.98**	**10.78**	**17.38**	3
138	366086	22.85	7.20	7.98	35.33	7.83		**12.68**	**21.58**	**16.24**	3
139	366087	13.01	17.65	20.03	0.57	11.63	17.38	**16.90**	**9.86**	**13.38**	2
140	366091	13.11	12.38	17.55	16.33	18.86		**14.35**	**17.60**	**15.65**	3
141	366092	3.94	10.72	19.25	18.47	17.45	10.75	**11.30**	**15.56**	**13.43**	2
142	366093	8.90	11.52	18.75	11.34	9.43	12.61	**13.06**	**11.13**	**12.09**	2
143	366094	14.04	18.83	8.04	13.53	20.78	3.76	**13.64**	**12.69**	**13.16**	2
144	366095	16.34	40.85		52.89	43.49	16.93	**28.60**	**37.77**	**34.10**	4
145	366097	30.98	20.25	10.31	12.63	34.36	17.68	**20.51**	**21.56**	**21.03**	3
146	366102	10.16	12.00	8.66	11.06	14.14	10.33	**10.27**	**11.84**	**11.06**	2
147	366103	87.38	64.35	80.85	119.74			**77.53**	**119.74**	**88.08**	5

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
148	366104	9.35	8.00	13.55				**10.30**		**10.30**	2
149	366105	15.15		13.61	20.51		21.10	**14.38**	**20.81**	**17.59**	3
150	366106	8.89	9.76	3.92	1.02			**7.52**	**1.02**	**5.90**	1
151	366107	7.65	4.55	1.70		4.33	0.91	**4.63**	**2.62**	**3.83**	0
152	367001	9.54	15.41	32.19	40.90	33.85	12.03	**19.05**	**28.93**	**23.99**	3
153	367002	20.28	16.48	14.01	37.15	22.06	31.69	**16.92**	**30.30**	**23.61**	3
154	367003	97.75	94.64	68.95	69.32	65.92	43.78	**87.11**	**59.67**	**73.39**	5
155	367004	15.43	19.73	31.23	4.72	32.32	10.75	**22.13**	**15.93**	**19.03**	3
156	367005	16.41	10.57	7.29	6.83	6.75	12.92	**11.42**	**8.83**	**10.13**	2
157	367006	64.50	33.98	21.56	42.88	60.02	38.39	**40.01**	**47.09**	**43.55**	5
158	367007	16.35	13.73	21.18	28.72	15.36	26.34	**17.09**	**23.47**	**20.28**	3
159	367008	82.97	28.96	81.89		97.45		**64.61**	**97.45**	**72.82**	5
160	367009		4.54	4.81	7.51	14.22	16.96	**4.68**	**12.90**	**9.61**	1
161	367011	9.09	10.79	4.68	19.51	13.34	14.73	**8.19**	**15.86**	**12.03**	2
162	367014		10.63		9.68	4.68	17.88	**10.63**	**10.75**	**10.72**	2
163	367015	14.82		5.66	11.88	45.99	34.66	**10.24**	**30.84**	**22.60**	3
164	367018	23.58	18.16	15.54	20.77	16.31		**19.09**	**18.54**	**18.87**	3
165	367019	32.46	27.34	16.74	36.42	20.04		**25.51**	**28.23**	**26.60**	4
166	367020	9.71	10.62	11.32	37.16	17.97	23.69	**10.55**	**26.27**	**18.41**	3
167	367021	15.49	14.06	9.06	8.56	16.72		**12.87**	**12.64**	**12.78**	2
168	367022	6.28	9.02	11.62	21.49		40.55	**8.97**	**31.02**	**17.79**	3

Appendix Table 2A. Average number of spores/25 cm^2 leaf area and downy mildew resistance score of 179 seedlings from cross 'Horizon x Ill. 547-1' and their parents (continued).

		Number of spores/ 25 cm^2 leaf area						The 1st Exp.	The 2nd Exp.	Avr.	Downy mildew score
		The 1st inoculation			The 2nd inoculation						
	Genotype	Node 5	Node 6	Node 7	Node 5	Node 6	Node 7				
169	367024	11.78	9.78	24.59	38.43	21.91		**15.38**	**30.17**	**21.30**	3
170	367026	5.79		1.53	9.16		3.04	**3.66**	**6.10**	**4.88**	0
171	367031	12.76	6.19	15.41		10.25	5.39	**11.45**	**7.82**	**10.00**	1
172	367032	11.36	28.62	24.42				**21.47**		**21.47**	3
173	367034	11.87	11.28	9.99	12.22	8.13	8.34	**11.05**	**9.56**	**10.31**	2
174	367036	6.24	14.72	19.58	14.66	9.77	1.72	**13.51**	**8.72**	**11.12**	2
175	367037	21.74	15.40	20.98	14.90	3.95	24.89	**19.37**	**14.58**	**16.98**	3
176	367038	47.46	34.02	33.53	27.64	31.99	22.63	**38.34**	**27.42**	**32.88**	4
177	367039	16.08	25.55	21.71	24.05	29.17	34.09	**21.11**	**29.10**	**25.11**	4
178	367040	46.20	26.52	10.05	31.72	26.49	12.20	**27.59**	**23.47**	**25.53**	4
179	367041	8.76	17.69	14.52	9.82	10.58	12.12	**13.66**	**10.84**	**12.25**	2
180	Horizon	11.42	16.51	15.68	22.65	13.79	10.61	**14.54**	**15.68**	**15.11**	3
181	Ill. 547-1	1.82	3.35	2.32	3.63	0.85	1.14	**2.49**	**1.87**	**2.18**	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population.

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
361012	0	0	1	1	1	1	1		0
361013	1	1	1	1	1	1	1	0	0
361014	0	1	1	1	1	1	1	0	1
361019	0	1	1	1	1	0	1	0	0
361022	1	0	1	1	0	0	1	0	1
361023	1	1	1	1	1	1	1	0	1
361024	1	1	1	1	1	0	1	1	0
361025	1	1	1	1	1	1	1		1
361026	1	1	1	1	1	1	1	1	1
361029	1	0	0	1	1	1	1	0	0
361030	0	1	1	1	1	1	0	1	1
361031	1	1	0	1	1	1	1	1	1
361036	0	1	1	1	1	1	1	1	0
361041	1	1	0	0	1	1	1	0	0
361042	1	1	1	1	0	0	1	1	1
361051	1	1	1	1	0	0	1		0
361057	0	1	1	1	1	1	1	1	0
361058	1	1	1	1	0	0	1	1	1
361059	1	1	1	0	1	0	1	1	1
361061	1	1	0	1	1	0	1	0	1
361062	1	1	0	0	1	1	1	0	1
361065	1	1	1	1	1	0	1	0	1
361066	0	1	1	1	1	0	1	0	1
361067	1	0	1	1	0	1	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
361069	1	1	1	0	1	0	1	1	0
361071	1	1	1	1	1	1	1	1	0
361072	1	1	1	0	1	1	1	1	1
361075	1	1	1	1	1	1	0	1	0
361076	0	1	1	0	1	0	1	1	1
361077	1	0	1	0	0	1	1	1	1
361079	0	1	1	0	0	1	1	1	0
361081	1	1	1	0	1	0	1	1	0
361083	1	1	1	0	1	1	1	1	0
361086		1	1	0	0	1	1	1	1
361089	0	1	1	0	1	0	1	1	1
361090	0	1	1	0	0	0	1	0	0
361092	1	1	0	0	0	0	1	1	1
361093	0	0	0	0	0	0	1	1	0
361094	0	0	0	0	0	1	1	1	1
361095	0	0	0	0	0	1	1	0	0
361096	0	0	1	1	0	1	1	1	1
365004	1	1	1	0	0	1	1	1	1
365005	0	1	1	0	0	0	0	1	1
365006	1	1	1	1	1	0	1	1	1
365007	1	1	1	0	1	1	0	1	1
365010	1	1	1	1	1	0	0	1	1
365012	1	0	1	1	0	1	0	1	1
365013	1	1	1	1	0	1	1	0	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
365015	1	1	1	1	0	1	1	1	1
365017	1	0	0	1	0	1	1	1	1
365018	1	0	1	0	0	0	0	1	1
365019	1	0	0	0	0	1	1	1	1
365020	1	0	1	1	1	0		1	1
365021	0	1	0	1	0	1		1	1
365023	1	1	1	1	0	1	0	1	1
365024	1	1	0	1	1	0	1	1	1
365025	1	1	1	1	0	1	1		0
365027	1	1	0	0	0	1	1	1	0
365028	1	1	0	1	1	1	1		0
365029	1	1	1	1	1	1	1	1	1
365030	1	1	0	1	0	1		1	1
365032	1	1	1	1	1	1	0	0	1
365035	1	1	1	1	0	1	1	1	0
365036	1	0	1	1	1	1	1	1	1
365037	1	0	1	1	1	0		1	0
365038	1	0	1	1	0	1	1	1	0
365039	1	1	0	0	0	0	1	1	1
365044	0	0	1	1	0	0	1	1	1
365049	1	1	0	1	0	0	0	0	1
365051	0	1	1	1	0		1	0	1
365052	1	1	0	1	0	1	1	1	0
365053	0	1	1	1	1	0	0	1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
365055	0	0	1	1	1	0	1	1	1
365056	0	1	0		1	1	1	0	0
365057	0	0	1	1	1	0	1	1	1
365059	1	0	0	1	0	0	1	1	1
365061	1	1	0	0	0	0	0	0	1
365062	0	1	0	0	1	1	1	1	1
365063	1	0	0	0	0	0	1	1	0
365065	0	1	1	0	1	0	0	1	0
365066	1	1	1	1	0	1	1	1	0
365067	1	0	1	0	1	0	1	1	1
365074	1	1	0	0	0	1	1	1	1
365075	1	1	0	0	1	0	1	1	1
365076	0	1	1	0	1	1	1	1	1
365077	1	0	1	0	1	0	1	1	1
365078	0	0	0	1	1	0	0	1	0
365084	0	1	1	1	0	0	0	1	1
365085	0	0	1	1	1	0	1	1	1
365086	1	1	0	1	0	0	1	1	1
365087	1	1	0	1	0	0	1		0
366008	1	1	0	0	0	1	1	1	1
366010	1	0	0	1	0	1	1	1	1
366013	1	0	0	1	0	1	0	1	1
366015	1	1	1	1	0	1	1	0	1
366017	0	1	1	0	0	0	0	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
366018	1	0	1	1	0	0	1	0	1
366020	1	1	1	0	0	1	1	0	0
366022	1	1	0	0	0	1	0	0	0
366023	1	1	1	1	0	1	1	1	1
366026	0	1	1	1	0	1	1	0	1
366027	0	0	1	1	0	1	1	1	1
366030	1	1	1	1	0	1	1	1	1
366032	1	1	1	1	0	0	1	1	1
366034	1	0	0	0	0	0	1	1	1
366035	1	1	0	1	0	0	1	0	1
366037	1	1	0	1	1		0	0	0
366038	1	1	0	0	1	1	1	1	0
366039	0	1	0	1	0	0	1	1	1
366040	0	1	0	1	0	1	1	1	1
366041	0	1	1	1	0	1	1	1	1
366042	1	1	1	1	0	1	1	1	1
366043	1	1	1	1	1	0	1	1	1
366045	1	1	1	1	0	0	1	1	0
366046	1	1	1	1	0	0	1	0	1
366047	1	1	1	1	1	0	1	1	1
366048	0	1	1	1	0	0	1	0	1
366049	1	1	1	1	0	1	1	1	1
366050	1	0	1	0	1	0	1	1	1
366055	1	0	1	1	0	0	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
366056	1	0	1	1	0	1	1	1	1
366057	1	1	1	0	0	0	1	1	1
366058	1	0	1	1	0	1	1	1	1
366059	1	0	1	1	0	1	1	1	1
366060	1	1	1	0	0	1	1	0	1
366062	1	0	1	1	1	0	1	0	1
366063	0	0	1	1	0	0	1	0	0
366064	1	1	1	1	0	1	1	0	1
366066	1	1	1	1	1	1	1	0	1
366070	1	1	1	0	0	0	1	1	1
366072	1	0	0	1	1	1	1	1	1
366074	1	0	1	1	0	0		0	1
366075	1	1	1	1	0	0	1	1	1
366076	1	0	0	1	1	0	1	1	1
366079	1	0	1	1	0	0		0	0
366081	1	1	0	0	0		1	1	1
366085	1	1	1	1	0	1	1	1	1
366086	1	0	0	1	1	1	1	1	0
366087	1	1	1	1	0	0	1	1	1
366091	1	1	1	1	1	0	1	1	1
366092	1	1	1	0	0	0	1	1	1
366093	1	1	1	0	1	1	1	1	0
366094	1	1	1	1	0	1	1	1	1
366095	1	1	0	0	0	1	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
366097	1	1	0	0	0			0	0
366102	1	0	1	1	1	1	1	1	0
366103	0	0	1	1	0	0	1	1	0
366104	1	1	1	1	1	0	0	0	0
366105	1	1	1	1	0	0	0	0	0
366106	1	1	1	1	1		1	1	0
366107	1	0	1	1	1	1	1	1	0
367001	1	1	1	1	0	1	1	1	0
367002	1	1	1	1	0	0	0	0	0
367003	1	0	1	1	0	0	1	1	0
367004	0	0	0	1	0	1		1	0
367005	1	0	1		0	1	1	1	0
367006	0	1	1	1	0	1	1	1	0
367007	1	1	1	1	0		1	1	0
367008	1	0	1		0	0	1	1	0
367009	1	1	1		1	1	1	1	0
367011	1	1	1		0	0		0	0
367014	0	1	0		0	0	1	0	0
367015	1	1	1	1	0	0	1	1	0
367018	0	0	0		1	0	1	1	0
367019	0	1	1	1	1	0	1	1	1
367020	0	1	1	0	0	0	1	1	1
367021	1	1	1	1	0	1	1	0	1
367022	1	0	0	0	0	1	1	0	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	CAP rgVrip064	CAP rgVamu085	CAP stkVa011	SSCP rgVrip145	SSCP GLPL6-1	SSCP rgVrip158	SSCP rgVamu100	SSCP rgVamu092	SSCP rgVamu111
367024	1	1	1	0	0	1	1	0	0
367026	1	1	1	1	1	1	1	0	0
367031	1	0	0	0	0	0	1	1	0
367032	1	1	1	0	0	1	1		0
367034	0	1	0	1	1	1	1	1	0
367036	0	1	1	1	0	0	1	1	0
367037	0	1	1	0	1	1	1	1	0
367038	1	1	1	0	0	0	1	1	1
367039	1	0	0	0	1	1	1		1
367040	0	0	0	0	0	1		1	
367041	1	1	0	1	1	1	1	1	1
Horizon	1	0	0	1	0	1	1	1	0
Ill. 547-1	1	1	0	1	1	1	0	1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
361012	1	1	0	0	0	0	1	0
361013	0	1	0	0	0	0	0	0
361014	1	1	1	0	1	1	1	0
361019	0	0	0	1	0	1	0	1
361022	1	1	0	1	1	0	1	0
361023	1	0	1	1	1	1	1	1
361024	0	0	1	0	0	1	0	0
361025	1	1	1	0	0	1	1	0
361026	1	0	1	1	0	1	1	1
361029	1	1	1	0	0	0	0	0
361030	0	1	0	1	0	1	1	0
361031	1	0	1	1	0	1	1	0
361036	1	1	1	1	0	1	1	0
361041	1	1	1	0	0	1	1	0
361042	1	1	1	0	0	0	0	1
361051	0	1	1	0	0	0	0	0
361057	1	1	1	0	0	1	1	0
361058	1	1	1	0	1	1	1	1
361059	1	1	0	1	0	0	1	0
361061	0	1	0	1	1	1	0	0
361062	0	1	0		1	0	1	1
361065	0	1	0	1	0	1	0	0
361066	1	1	1	0	0	1	1	0
361067	1	1	1	1	1	0	1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
361069	0	1	1	1	0	0	0	0
361071	1	1	0	1	1	1	1	0
361072	1	1	0	1	1	1	1	0
361075	1	1	0	1	0	0	0	0
361076	1	0	0	0	0		0	1
361077	1	1	0	1	1	0	0	1
361079	1	1	0	0	1	0	0	1
361081	1	1	1	0	0	0	1	0
361083	1	1	1	1	1	0	0	1
361086	1	0	1	1	0	0	0	1
361089	0	1	0	0	0	1	1	0
361090	0	1	0		0	0	1	0
361092	1	1	1	1	0	0	1	0
361093	0	1	0		0	0	0	0
361094	1	0	1	1	0	0	1	0
361095	1	1	1	1	0	1	1	1
361096	1	1	1	1	0	0		0
365004	0	1	0	0	0	1	0	0
365005	1	1	0	0	0	0	0	0
365006	1		0	0	0	0	0	1
365007	1	1	1	1	0	0	0	1
365010	0	1	1	1	0	1	1	0
365012	1	1	0	1	0	0	1	0
365013	0	1	0	0	1	0	0	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
365015	1	1	1	1	0	0	1	0
365017	0	1	0	1	0	1	0	0
365018	1	1	0	0	1	1	0	0
365019	1	0	1	1	0	0	0	0
365020	0	1	1	1	1	1	0	0
365021	1	1	1	0	0	1	0	0
365023	1	0	0	1	1	0	0	0
365024	1	1	1	0	0	1	1	0
365025	1	0	1	0	1	0	0	0
365027	1	0	1	1	1	0	1	0
365028	1	0	1	1	1	0	0	0
365029	1	0	1	1	1	0	0	0
365030	1	0	1	1		0	1	0
365032	1	1	1	0	1	1	1	0
365035	0	1	1	1	1	1	1	0
365036	1	0	1	1	1	0	1	0
365037	1	1	1	1	0	1	0	0
365038	1	1	1	1	1	0	1	0
365039	1	0	1	0	1	1	1	0
365044	1	0	1	1	1	1	1	0
365049	1	1	1	1	0	0	1	0
365051	1	0	1	0	1	1	0	0
365052	0	1	1		0	0	0	0
365053	1	0	1	1	0	0	0	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
365055	0	1	1	0	1	1	1	0
365056	0	1	1	1	0	0	0	0
365057	0	1	1	0	1	0	0	0
365059	1	1	1	1	1	0	0	0
365061	1	0	1	0	1	0	0	0
365062	1	1	1	1	1	0	0	0
365063	0	0	1	1	1	0	1	0
365065	1	1	1	0	0	0	0	0
365066	1	1	1	0	1	1	1	0
365067	1	1	1	1	1	1	1	0
365074	0	0	1	1	1	1	0	0
365075	1	1	1	1	1	1	1	0
365076	1	1	1	0	1	1	1	0
365077	1	0	1	0	1	1	0	0
365078	0	1	0	1	1	1	0	0
365084	1	0	1	0	1	1	1	0
365085	1	0	1	1	1	0	1	0
365086	1	1	0	0	0	1	0	0
365087	0	1	0		0	1	0	0
366008	1	1	1	0	1	1	1	0
366010	1	1	0	0	0	0	1	0
366013	1	0	1	1	0	0	0	0
366015	1	1	0	1	1	0	0	1
366017	1	1	1	0	0	1	1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
366018	1	1	1	1	1	0	0	0
366020	1	1	1	1	0	1	0	0
366022	1	1	0	0	0	0	0	1
366023	1	1	0	1	0	1	0	1
366026	0	0	1		0	1	0	0
366027	1	1	1	1	0	1	0	0
366030	1	1	1	0	0	1	1	0
366032	1	1	1	0	0	0	0	0
366034	1	1	1	1	0	1	1	1
366035	1	1	1	0	0	1	0	1
366037	1	1	0	1	0		0	1
366038	0	0	1	1	0		0	0
366039	0	1	1	0	0	0	0	0
366040	1	1	0	0	0	1	1	0
366041	1	0	1	1	1	1	1	1
366042	1	1	1	1	0	1	0	0
366043	1	0	1	0	1	1	1	0
366045	1	0	1	1	1	0	0	0
366046	1	1	1		1	1	1	0
366047	1	1	1	0	1	1	1	0
366048	1	1	1	1	1	1	1	0
366049	1	1	1	1	1	1	1	0
366050	0	0	1	1	0	1	0	0
366055	1	1	1	1	1	0	0	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
366056	1	1	1	1	0	0	0	1
366057	1	1	0	1	1	1	0	0
366058	1	0	1	1	1	1	0	0
366059	1	0	1	1	1	1	0	0
366060	1	1	1	0	0	1	1	0
366062	1	0	1	1	1	0	1	0
366063	0	0	1	1	0	0	1	0
366064	1	1	1	1	0	1	1	0
366066	1	1	1	1	1	1	1	0
366070	1	1	1	0	0	0	1	1
366072	1	0	0	1	1	1	1	1
366074	1	0	1	1	0	0		0
366075	1	1	1	1	0	0	1	1
366076	1	0	0	1	1	0	1	1
366079	1	0	1	1	0	0		0
366081	1	1	0	0	0		1	1
366085	1	1	1	1	0	1	1	1
366086	1	0	0	1	1	1	1	1
366087	1	1	1	1	0	0	1	1
366091	1	1	1	1	1	0	1	1
366092	1	1	1	0	0	0	1	1
366093	1	1	1	0	1	1	1	1
366094	1	1	1	1	0	1	1	1
366095	1	1	0	0	0	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
366097	1	1	0	0	0			0
366102	1	0	1	1	1	1	1	1
366103	0	0	1	1	0	0	1	1
366104	1	1	1	1	1	0	0	0
366105	1	1	1	1	0	0	0	0
366106	1	1	1	1	1		1	1
366107	1	0	1	1	1	1	1	1
367001	1	1	1	1	0	1	1	1
367002	1	1	1	1	0	0	0	0
367003	1	0	1	1	0	0	1	1
367004	0	0	0	1	0	1		1
367005	1	1	0		1		0	1
367006	0	1	1	1	1		1	0
367007	1	1	0	0	0		0	0
367008	1	1	0	0	0		1	0
367009	1	1	0	0	0		1	1
367011	1	0	0		0		0	0
367014	0	1	0	0	0		0	0
367015	1	1	0	0	0		0	0
367018	1	0	0	1	1		0	0
367019	1	1	1	0	1		0	0
367020	1	1	0	0	0		0	0
367021	0	1	1	0	0		0	0
367022	1	0	1	1	0		1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVcin109	SSCP rgVcin111	SSCP rgVcin123	SSCP rgVcin125	SSCP rgVcin127	SSCP rgVcin139	SSCP rgVcin165	SSCP rgVrup103
367024	0	1	1		1		0	0
367026	0	0	1	1	0		0	1
367031	1	1	1		0		1	0
367032	0	1	1		0		0	0
367034	1	1	1	1	1		1	1
367036	0	1	1	1	1		0	0
367037	1	1	1	0	1		1	0
367038	1	1	1	1	1		1	1
367039	1	1	1	0	1		1	0
367040		1	1	1	1		0	1
367041	0	0	1	1	0		0	0
Horizon	1	1	1	0	0		0	0
Ill. 547-1	1	1	1	1	1		0	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
361012	0	0	0	1	0	0	0	0	0
361013	0	0	0	1	1	0	1	1	0
361014	0	1	0	0	1	0	1	1	1
361019	0	0	1	0	0	0	0	0	0
361022	0	0	0	1	0	1	1	0	1
361023	0	0	0	0	0	1	1	1	1
361024	0	0	0	1	0	0	1	0	0
361025	1	1	0	1	0	0	0	1	0
361026	1	1	1	0	1	1	1	0	1
361029	0	0	0	0	1	0	1	1	1
361030	1	0	0	1	0	1	0	0	0
361031	0	1	0	1	0	0	1	0	1
361036	1	1	1	1	1	0	1	0	1
361041	0	1	0	1	0	0	1	0	1
361042	1	0	0	0	0	0	1	1	1
361051	1	0	0	0	1	0	1	0	0
361057	0	0	0	0	1	0	1	1	1
361058	0	0	1	0	0	0	0	1	1
361059	1	0	0	0	1	1	1	1	1
361061	1	0	0	0	0	1	0	1	0
361062	1	0	0	0	0	0	1	1	0
361065	0	0	0	1	0	1	0	0	0
361066	0	0	1	1	0	0	0	0	0
361067	1	1	0	0	1	1	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
361069	1	0	0	1	1	0	0	1	1
361071	0	0	0	0	1	0	0	1	1
361072	1	0	0	0	1	0	1	1	1
361075	1	0	0	1	1	1	1	0	1
361076	1	0	0	0	0	0	0	1	0
361077	1	0	0	0	0	1	0	1	0
361079	0	0	0	1	0	0	0	0	0
361081	0	1	1	1	0	0	1	0	1
361083	1	0	0	0	0	0	0	0	1
361086	0	0	0	1	0	0	1	1	1
361089	0	0	0	1	0	0	1	0	0
361090	0	0	0	1	0	1	0		0
361092	0	0	0	1	0	1	1	0	0
361093	1	0	0	1	0	0	0		1
361094	0	0	0	1	0	1	1	1	1
361095	0	1	0	1	0	1	1	0	0
361096	0	0	0	1	1	1	1	0	0
365004	0	0	0	0	1	1	0	0	0
365005	0	0	0	1	1	0	1	0	1
365006	0	0	0	0	1		1	1	1
365007	0	0	1	1	0	1	1	0	1
365010	0	0	1	1	0	1	0	1	0
365012	0	0		0	0	1	0	1	1
365013	0	1	0	1	1	0	0	0	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
365015	0	1	1	0	0	1	1	1	0
365017	0	1	0	1	0	1	0	1	0
365018	0	1	1	1	1	0	0	0	0
365019	0	0	0	0	0	1	0	1	0
365020	0	1	1	1	0	1	0	0	0
365021	0	1	0	1	1	0	0	0	0
365023	0	1	0	1	1	1	1	0	0
365024	0	1	0	0	0	1	1	1	0
365025	1	0	1	0	1	0	1	1	0
365027	0	0	0	0	1	1	1	1	0
365028	0	0	1	0	1	1	0	1	0
365029	1	0	0	0	0	1	1	1	0
365030	0	1	0	0	1	1	1	1	0
365032	1	0	0	1	1	0	0	0	1
365035	0	0	0	1	0	0	1	0	0
365036	1	0	0	0	0	1	0	1	0
365037	0	0	0	1	1	1	1	0	0
365038	0	0	0	0	0	1	1	1	0
365039	0	0	0	1	1	0	1	0	0
365044	0	0	0	1	1	1	1	0	0
365049	0	0	0	0		1	1	1	0
365051	0	0	0	1		0	1	0	1
365052	0	0	0	0	0	0	0	0	0
365053	0	1	1	0	1	1	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
365055	0	1	0	1	1	0	1	0	0
365056	0	0	0	0	0	0	0	0	0
365057	0	0	0	1	0	0	1	0	0
365059	0	0	1	1	0	1	0	1	0
365061		0	0	1	0	0	0	0	0
365062	0	1	0	0	0	0	1	1	0
365063	0	1	1	0	0	1	1	1	
365065	0	0	0	0	0	0	1	1	0
365066	0	0	0	1	0	0	1	0	0
365067	0	0	1	1	1	1	1	1	0
365074	0	0	0	1	1	1	1	0	0
365075	0	0	1	1	0	0	1	0	0
365076	0	1	0	1	1	0	1	0	0
365077	0	1	0	1	0	0	0	0	0
365078	0	0	0	0	1	0	0	0	0
365084	0	0	0	1	0	0	1	1	0
365085	0	1	0	0	0	1	0	1	0
365086	0	0	0	1	0	0	0	0	0
365087	0	0	1	0	0	0	0	0	0
366008	1	0	0	1	0	0	1	0	0
366010	0	0	0	0	0	0	1	1	0
366013	0	0	0	1	0		0	0	1
366015	0	0	0	0	1	1	0	1	0
366017	0	0	0	1	1	0	1	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
366018	1	0	0	0	0	0		0	1
366020	1	0	1	1	1	1	1	1	1
366022	1	0	0	0	1	0	0	1	1
366023	1	0	0	0	1	1	1	1	1
366026	1	0	1	1	0	1	0	1	1
366027	1	0	1	0	0	1	1	1	1
366030	1	0	1	1	1	0	1	1	1
366032	1	0	1	0	0	0	1	1	1
366034	1	0	0	1	1	1	1	1	1
366035	1	0	1	1	0	0	0	1	0
366037	1	0	1	0	0	0		0	0
366038	1	0	1	0	0	0	0	1	
366039	1	0	1	1	0	0	0	1	1
366040	1	0	1	1	1	0	1	1	1
366041	1	0	0	1	1	0	0	1	1
366042	1	0	0	0	1	0	1	1	1
366043	1	0	0	1	0	0	0	1	1
366045	1	0	0	0	0	0	0	1	1
366046	1	0	0	0	1	0	1	1	1
366047	1	0	0	0	0	1	1	1	1
366048	1	0	1	0	0	1	1	1	1
366049	1	0	0	0	0	0	0	1	1
366050	1	0	1	0	0	1	1	1	1
366055	1	0	1	0	1	1	0	1	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
366056	1	0	0	0	0	1	0	1	1
366057	1	0	1	1	0	1	1	1	1
366058	1	0	0	1	0	0	0	1	1
366059	1	0	0	1	1	1	1	1	1
366060	1	0	0	0	0	0	1	1	1
366062	0	0	1	1	0	0	0	1	0
366063	1	0	1	0	1	0	1	1	1
366064	1	0	0	1	0	1	0	1	1
366066	1	0	1	1	1	1		1	1
366070	1	0	1	1	0	1	1	1	1
366072	1	0	1	1	0	0	1	1	1
366074	1	0	1	1	0	1	1	1	1
366075	1	0	1	0	0	1	1	1	1
366076	0	0	0	0	0	0	0	1	1
366079	1	0	0	1	0	0	1	1	1
366081	1	0	0	0	1	1	1	1	1
366085	0	0	1	0	1	1	1	1	1
366086	1	1	0	1	0	0	1	1	1
366087	1	0	1	1	0	1	0	1	1
366091	1	0	0	1	0	0	1	1	1
366092	0	0	0	0	0	0	0	1	1
366093	1	0	0	1	1	0	0	0	1
366094	1	0	1	1	0	1	0	0	0
366095	0	0	1	1	0	0	0	1	0

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
366097	0	0	0	0		0	0	0	
366102	0	0	0	0	0	0	1	1	0
366103	0	0	1	1	1	1	1	1	1
366104	0	0	1	0	0	0	0	1	1
366105	0	0	0	0	0	0	0		0
366106	0	0	0	0		0	0	0	0
366107	0	1	1	1	1	1	1	0	1
367001	0	0	0	1	0	0	0	1	0
367002	0	0		0	0	0			0
367003	0	0	0	1	0	0	0	0	0
367004	0	0		0	1		0		0
367005	1	0	1	0	1	1	1	1	0
367006	1	0	1	1	0	1	0	0	1
367007	1	0	0	1	0	0	0	0	0
367008	1	1	1	1	1	1	1	0	1
367009	1	1	0	1	0	0	1	0	0
367011	1	0	0	0	0	0	0	0	0
367014	1	0	1	1	0	0	0	0	0
367015	1	0	0	1	0	0	1	1	0
367018	1	0	1	1	0	1	0	1	0
367019	1	0	0	1	0	0	1	0	0
367020	1	1	0	1	0	0	1	1	0
367021	1	0	1	0	1	0	0	1	0
367022	1	1	1	1	1	1	0	0	1

Appendix Table 3A. Segregation of 26 markers in 'Horizon x Ill. 547-1' population (continued).

DNA sample	SSCP rgVrup119	SSCP rgVrup124	SSCP rgVrup126	SSCP rgVhyb101	SSCP rgVhyb102	SSCP rgVhyb110	SSCP rgVhy121	SSCP rgVhyb127	SSCP rgVhyb149
367024	1	0	1	1	1	1	1	0	0
367026	1	0	0	0	0		1	0	
367031	1	1	1	0	1	1	1	1	1
367032	1	0	0	0	0	0	0	1	0
367034	1	0	1	1	0	1	1	1	0
367036	1	0	0	1	1	0	1	0	0
367037	1	0	0	0	1	0	1	0	1
367038	1	1	0	0	1	1	0	1	1
367039	1	1	0	0	0	0	1	1	0
367040		1	1	1	1	1	1	0	1
367041	1	0	1	1	1	1	1	0	1
Horizon	1	0	0	1	0	0	1	1	0
Ill. 547-1	0	0	1	0	1	1	0	0	0

Appendix Table 4A. Chi-square analysis for marker segregation at 1:1 and 3:1 ratios.

Marker	Band = 0	Band = 1	Missing data	Expected 0	Expected 1	Chi-square	
Segregation1:1							
rgVamu085	56	125	0	90.5	90.5	26.30387	
GLPL6-1	106	75	0	90.5	90.5	5.309392	**
rgVamu100	23	148	10	85.5	85.5	91.37427	
rgVcin125	71	88	22	79.5	79.5	1.81761	*
rgVcin127	103	76	2	89.5	89.5	4.072626	*
rgVcin139	63	72	46	67.5	67.5	0.6	*
rgVrup119	75	102	4	88.5	88.5	4.118644	*
rgVrup126	120	58	3	89	89	21.59551	
rgVhyb101	85	96	0	90.5	90.5	0.668508	*
rgVhyb102	108	68	5	88	88	9.090909	
rgVhyb110	102	75	4	88.5	88.5	4.118644	**
rgVhyb121	76	100	5	88	88	3.272727	*
rgVhyb127	73	103	5	88	88	5.113636	**
Segregation3:1							
rgVrip064	48	132	1	45	135	0.266667	*
stkVa011	55	126	0	45.25	135.75	2.801105	*
rgVrip145	58	116	7	43.5	130.5	6.444444	**
rgVrip158	82	93	6	43.75	131.25	44.58857	
rgVamu092	46	127	8	43.25	129.75	0.233141	*
rgVamu111	69	111	1	45	135	17.06667	
rgVcin109	50	130	1	45	135	0.740741	*

* significant ($P < 0.05$)

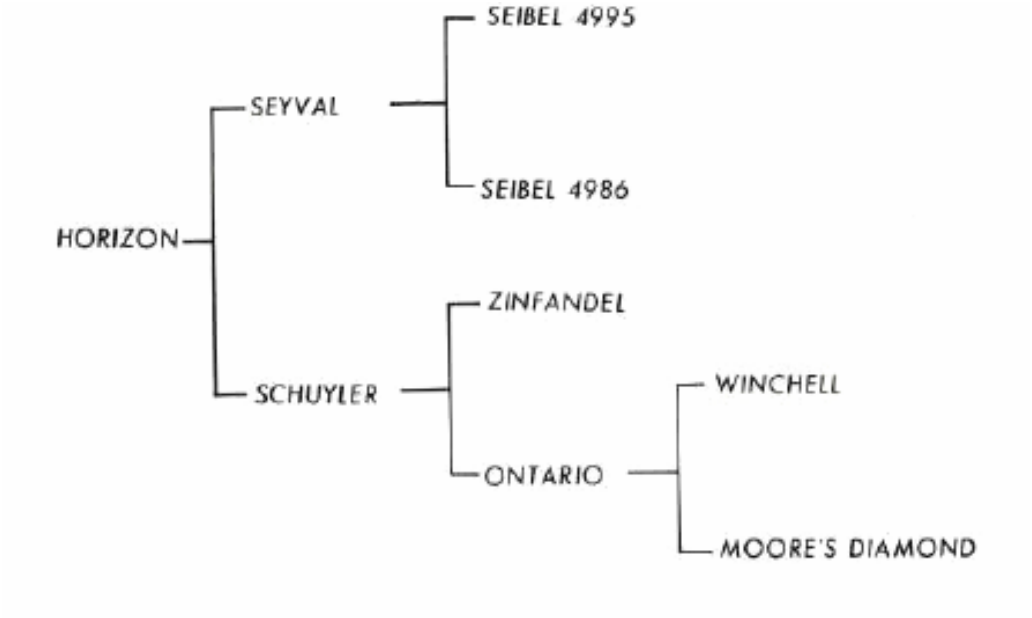
** highly significant ($P < 0.01$)

Appendix Table 4A. Chi-square analysis for marker segregation at 1:1 and 3:1 ratios (continued).

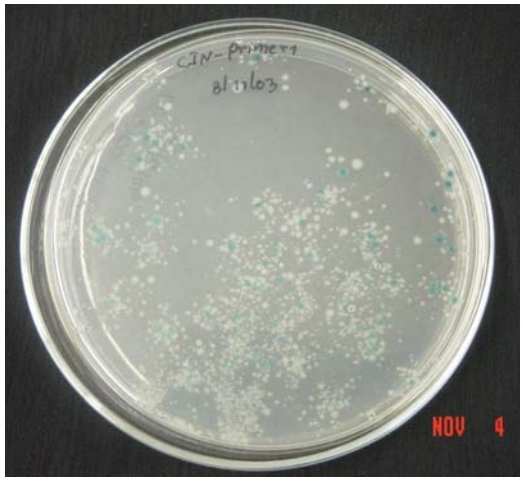
Marker	Band = 0	Band = 1	Missing data	Expected 0	Expected 1	Chi-square	
rgVcin111	56	124	1	45	135	3.585185	*
rgVcin123	56	125	0	45.25	135.75	3.405157	*
rgVcin165	103	75	3	133.5	44.5	27.87266	
rgVrup103	145	34	2	134.25	44.75	3.443203	*
rgVrup124	146	35	0	135.75	45.25	3.095764	*
rgVhyb149	92	85	4	132.75	44.25	50.03578	

* significant ($P < 0.05$)

** highly significant ($P < 0.01$)



Appendix Figure 1A. Pedigree of *Vitis* hybrid 'Horizon' from Cornell grape breeding program:



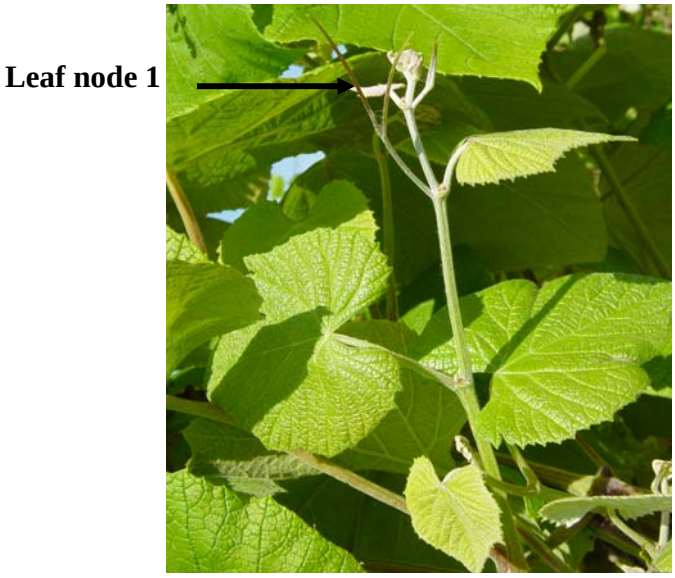
Appendix Figure 2A. White and blue colonies of *E. coli* carrying inserted and wild type vectors, respectively on selective plate.

Appendix Figure 3A. Mapping population is cultivated at NYSAES, Cornell University, Geneva, New York:

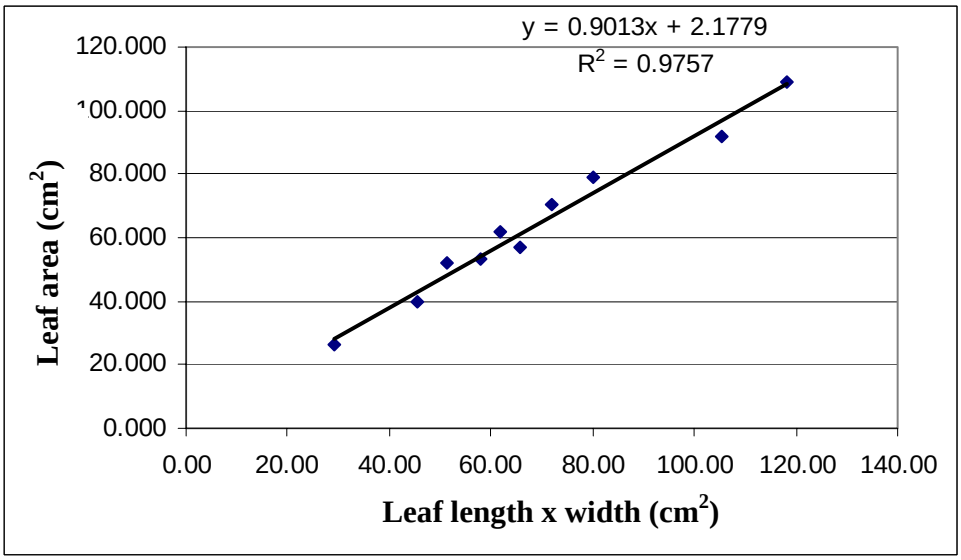
A) Female parent *V. hybrid* 'Horizon'

B) Segregating population

C) Male parent Ill. 547 – 1.



Appendix Figure 4A. Leaf node 1 is the first expanded leaf from the top.



Appendix Figure 5A. Correlation between leaf length x width and leaf area that was measured from leaf area meter.

Appendix Table 1B. Downy mildew evaluation of female parents.

Genotypes	Number of spores/25cm^2 leaf area				Rating score				Avr.	Phenotype
	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7		
Black Queen										
1	61.4	60.3		27.1	5	5		4	4.7	highly susceptible
2		33.4	22.5	51.5		4	3	5	4.0	susceptible
3	8.8	24.8	73.6		1	3	5		3.0	moderate susceptible
4	17.3	45.0	19.4		3	5	3		3.7	susceptible
5	62.4	87.2	178.5		5	5	5		5.0	highly susceptible
6	106.3	28.7	>48.1	29.7	5	4	5	4	4.5	highly susceptible
Avr.									**4.15**	**highly susceptible**
Carolina Black Rose										
1		11.8	17.7	21		2	3	3	2.7	moderate susceptible
2	25.5	21.0	72.0		4	3	5		4.0	susceptible
3	51.6		43.8	39.1	5		5	4	4.7	highly susceptible
4	13.6	14.0	25.6	86.3	2	2	4	5	3.3	moderate susceptible
5	21.5	39.8	25.1	16.9	3	4	4	3	3.5	susceptible
Avr.									**3.64**	**susceptible**
Italia										
1	10.9	10.5	36.3	53.4	2	2	4	5	3.3	moderate susceptible
2	15.6	18.1	23.8	55.8	3	3	3	5	3.5	susceptible
3	12.4	13.8		22.4	2	2		3	2.3	moderate
4	19.5	44.8	28.1	65.5	3	5	4	5	4.3	susceptible
5	62.5	90.0	34.2	64.4	5	5	4	5	4.8	highly susceptible
6	43.3	67.5	73.9	75.3	5	5	5	5	5.0	highly susceptible
Avr.									**3.87**	**susceptible**

Appendix Table 2B. Downy mildew evaluation of male parents.

Genotypes	Number of spores/25cm^2 leaf area				Rating score				Avr.	Phenotype
	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7		
NY 88.0517.01										
1	0.0	5.1	7.9	1.7	0	1	1	0	0.5	highly resistant
2	19.7	8.0	9.9	4.0	3	1	1	0	1.3	resistant
3	0.0	5.2	2.4		0	1	0		0.3	highly resistant
4	5.7	3.3	2.7	0.9	1	0	0	0	0.3	highly resistant
5	0.5	1.0	3.0	0.0	0	0	0	0	0.0	highly resistant
Avr.									**0.48**	**highly resistant**
NY 65.0550.04										
1	5.9	8.7		13.7	1	1		2	1.3	resistant
2	9.4	10.4		6.1	1	2		1	1.3	resistant
3	6.4	5.1		4.1	1	1		0	0.7	resistant
4	0.0	7.6	14.5	5.2	0	1	2	1	1.0	resistant
5	4.4	2.4	3.0	12.5	0	0	0	2	0.5	highly resistant
Avr.									**0.96**	**resistant**
NY 65.0551.05										
1	1.3	1.0	2.9		0	0	0		0.0	highly resistant
2	3.7	5.7	4.9	6.1	0	1	0	1	0.5	highly resistant
3	5.9	10	10	2.3	1	1	1	0	0.8	resistant
4	7.0	5.2	5.4	5.5	1	1	1	1	1.0	resistant
5	0.0	2.6	0.5	2.5	0	0	0	0	0.0	highly resistant
Avr.									**0.46**	**highly resistant**

Appendix Table 3B. Downy mildew evaluation of progenies of nine hybridization crosses.

	Number of spores/25cm^2 leaf area				Rating score					
Seedling #	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7	Avr.	Phenotype
Black Queen x NY 88.0517.01										
6	25.2	252.1	45.7		4	5	5		4.7	highly susceptible
7		101.1	155.1	54.5		5	5	5	5.0	highly susceptible
8	31.1		16.4	29.7	4		3	4	3.7	susceptible
9	33.0	66.5	75.9	117.3	4	5	5	5	4.8	highly susceptible
13	9.0	21.9	7.4	6.7	1	3	1	1	1.5	moderate
15	9.0		13.0	18.1	1		2	3	2.0	moderate
19	18.1	22.8	15.0	23.6	3	3	2	3	2.8	moderate susceptible
20	3.3	7.1	6.7		0	1	1		0.7	resistant
24	0.0	0.0	1.6		0	0	0		0.0	highly resistant
27	1.7	13.3	12.8	18.6	0	2	2	3	1.8	moderate
29		6.9	21.4	24.1		1	3	3	2.3	moderate
30	87.7	88.8	25.6		5	5	4		4.7	highly susceptible
32	6.1	7.3	7.7	6.4	1	1	1	1	1.0	resistant
33	0.0		3.6	2.9	0		0	0	0.0	highly susceptible
Black Queen x NY 65.0550.04										
1	43.3	194.3		33.3	5	5		4	4.7	highly susceptible
6	29.6	24.8	32.6		4	3	4		3.7	susceptible
8	6.2	12.2	9.4	12.6	1	2	1	2	1.5	moderate
14	38.5	11.2	17.8		4	2	3		3.0	moderate susceptible
30	26.8	19.0	36.2	47.8	4	3	4	5	4.0	susceptible
54	2.9	6.3	8.4	8.5	0	1	1	1	0.8	resistant

Appendix Table 3B. Downy mildew evaluation of progenies of nine hybridization crosses (continued).

	Number of spores/25cm^2 leaf area				Rating score					
Seedling #	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7	Avr.	Phenotype
Black Queen x NY 65.0551.05										
2	30.8	36.5	32.7		4	4	4		4.0	susceptible
3	51.9	235.8	43.0		5	5	5		5.0	highly susceptible
5	235.2	111.1	222.8	159.2	5	5	5	5	5.0	highly susceptible
6	247.0	>154.2	>133.9	102.5	5	5	5	5	5.0	highly susceptible
7		124.6	84.3	172.8		5	5	5	5.0	highly susceptible
8	54.4	53.7	51.3	66.3	5	5	5	5	5.0	highly susceptible
9		74.0	53.3	99.6		5	5	5	5.0	highly susceptible
10	42.7	47.5	51.9	59.9	5	5	5	5	5.0	highly susceptible
16	241.3	430.4	90.4	45.4	5	5	5	5	5.0	highly susceptible
19	317.0	118.9	131.2	81.4	5	5	5	5	5.0	highly susceptible
20	3.4	7.2	10.2	7.3	0	1	2	1	1.0	resistant
21	268.4	147.7	97.8	289.6	5	5	5	5	5.0	highly susceptible
24	28.5	80.3	40.7	21.6	4	5	5	3	4.3	susceptible
25	14.1	21.2		19.4	2	3		3	2.7	moderate susceptible
27	23.3	33.4	39.2	55.7	3	4	4	5	4.0	susceptible
28	53.1	28.8	79.2	52.2	5	4	5	5	4.8	highly susceptible
31	12.5		12.0	10.2	2		2	2	2.0	moderate
32		18.6	22.9	10.0		3	3	1	2.3	moderate
47	185.4	50.9	100.1	41.8	5	5	5	5	5.0	highly susceptible

Appendix Table 3B. Downy mildew evaluation of progenies of nine hybridization crosses (continued).

	Number of spores/25cm^2 leaf area				Rating score					
Seedling #	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7	Avr.	Phenotype
Carolina Black Rose x NY 88.0517.01										
2	53.1		40.1	161.2	5		5	5	5.0	highly susceptible
3	111.9	35.5	29.5	201.5	5	4	4	5	4.5	highly susceptible
8	1.3	1.7	1.0	5.1	0	0	0	1	0.3	highly resistant
11	2.1	1.2	4.2	0.0	0	0	0	0	0.0	highly resistant
12	0.0	2.0	1.7	11.5	0	0	0	2	0.5	highly resistant
14	169.4	50.7	58.5		5	5	5		5.0	highly susceptible
15		60.9	44.2	48.0		5	5	5	5.0	highly susceptible
20	34.2	78.4		32.6	4	5		4	4.3	susceptible
36		1.8	2.9	3.1		0	0	0	0.0	highly resistant
40	4.7	1.8	3.2		0	0	0		0.0	highly resistant
Carolina Black Rose x NY 65.0550.04										
2		9.5	5.3	10.0		1	1	1	1.0	resistant
3	8.9	0.0	6.2		1	0	1		0.7	resistant
5		226.7	31.6	102.8		5	4	5	4.7	highly susceptible
13	5.6	3.2	9.2	5.0	1	0	1	0	0.5	highly resistant
14	9.4	9.0	7.1	11.1	1	1	1	2	1.3	resistant
15	11.4	11.1		10.9	2	2		2	2.0	moderate
17	1.4	6.2		1.7	0	1		0	0.3	highly resistant
19	5.0	5.8	3.4		0	1	0		0.3	highly resistant

Appendix Table 3B. Downy mildew evaluation of progenies of nine hybridization crosses (continued).

	Number of spores/25cm^2 leaf area				Rating score					
Seedling #	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7	Avr.	Phenotype
Carolina Black Rose x NY 65.0551.05										
1	63.3	38.2	65.1	70.9	5	4	5	5	4.8	highly susceptible
2	73.8	54.9	114.1	79.3	5	5	5	5	5.0	highly susceptible
4	27.6		39.7	46.3	4		4	5	4.3	susceptible
5	148.0	244.8	85.7	217.8	5	5	5	5	5.0	highly susceptible
7		25.5	20.4	13.6		4	3	2	3.0	moderate susceptible
8	142.3	75.2	171.8	128.5	5	5	5	5	5.0	highly susceptible
9	32.1	40.8		548.4	4	5		5	4.7	highly susceptible
18	47.6	25.3	81.5	37.1	5	4	5	4	4.5	highly susceptible
20	26.4	43.1	24.8	83.7	4	5	3	5	4.3	susceptible
21	36.6	47.4	99.4	165.2	4	5	5	5	4.8	highly susceptible
22	6.8	2.1	0.6	2.8	1	0	0	0	0.3	highly resistant
27	43.2	68.2	37.5		5	5	4		4.7	highly susceptible
29	312.5	76.8	216.4	173.4	5	5	5	5	5.0	highly susceptible

Appendix Table 3B. Downy mildew evaluation of progenies of nine hybridization crosses (continued).

	Number of spores/25cm^2 leaf area				Rating score					
Seedling #	Node 4	Node 5	Node 6	Node 7	Node 4	Node 5	Node 6	Node 7	Avr.	Phenotype
Italia x NY 88.0517.01										
3		0.0	0.0	14.9		0	0	2	0.7	resistant
6	12.7	12.5		20.8	2	2		3	2.3	moderate
Italia x NY 65.0550.04										
1	396.4	152.8		231.0	5	5		5	5.0	highly susceptible
2		352.5	224.2	121.3		5	5	5	5.0	highly susceptible
6	58.7	191.3	26.6	101.9	5	5	4	5	4.8	highly susceptible
10	214.1	105.6	41.8		5	5	5		5.0	highly susceptible
12	6.9	4.4	5.8	7.3	1	0	1	1	0.8	resistant
15	10.5	5.4	5.5	7.2	2	1	1	1	1.3	resistant
18	15.3		10.9	15.6	3		2	3	2.7	moderate susceptible
Italia x NY 65.0551.05										
3		4.3	2.3	12.7		0	0	2	0.7	resistant
4	94.5	33.7	34.4	294.6	5	4	4	5	4.5	highly susceptible
6	39.4	26.1	50.7	30.1	4	4	5	4	4.3	susceptible
21	10.5	10.5	13.2	17.3	2	2	2	3	2.3	moderate

Appendix Figure 1B. Three cultivars of *V. vinifera* used as female parents for gca and sca analysis:

A) Black Queen: black blue fruit, large cluster, long oval shape and large berry size, thin skin, vigorous and excellent productive vines, very susceptible to downy mildew

B) Carolina Black Rose: blue fruit, medium cluster, large berry size, good texture firmness, thin skin, highly vigorous vines, susceptible to downy mildew

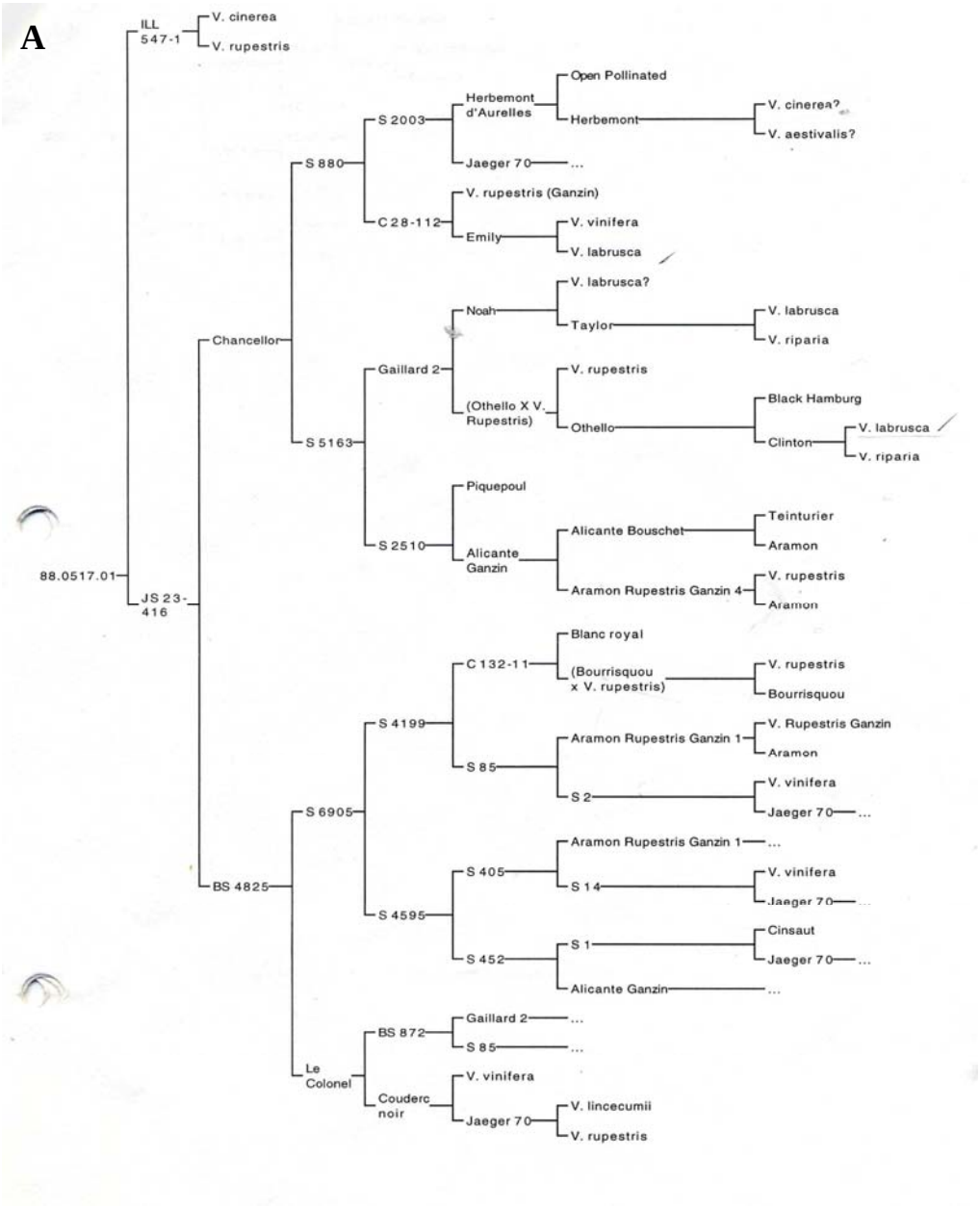
C) Italia: dull yellow skin fruit, medium cluster, large cluster berry size, firmness texture, tough skin, vigorous and productive vines, susceptible to downy mildew.

Appendix Figure 2B. Three downy mildew resistant lines developed in the Cornell grape breeding program used as male parents for gca and sca analysis:

A) NY 88.0517.07: blue fruit, large cluster somewhat loose, small berry size, late maturity with high acidity and wild flavor in New York, vigorous and productive vines, very high downy mildew and powdery mildew resistance

B) NY 65.0550.04: blue fruit, large cluster, medium berry size, relatively neutral flavor, moderately vigorous and quite productive vine, excellent downy mildew and powdery mildew resistance

C) NY 65.0551.05: White fruit, large cluster, medium-large berry size, neutral flavor often doesn't accumulate much sugar, moderately vigorous vine and production, black rot disease on fruit in some years, can have heavy powdery mildew on leaves, but generally little on fruit.

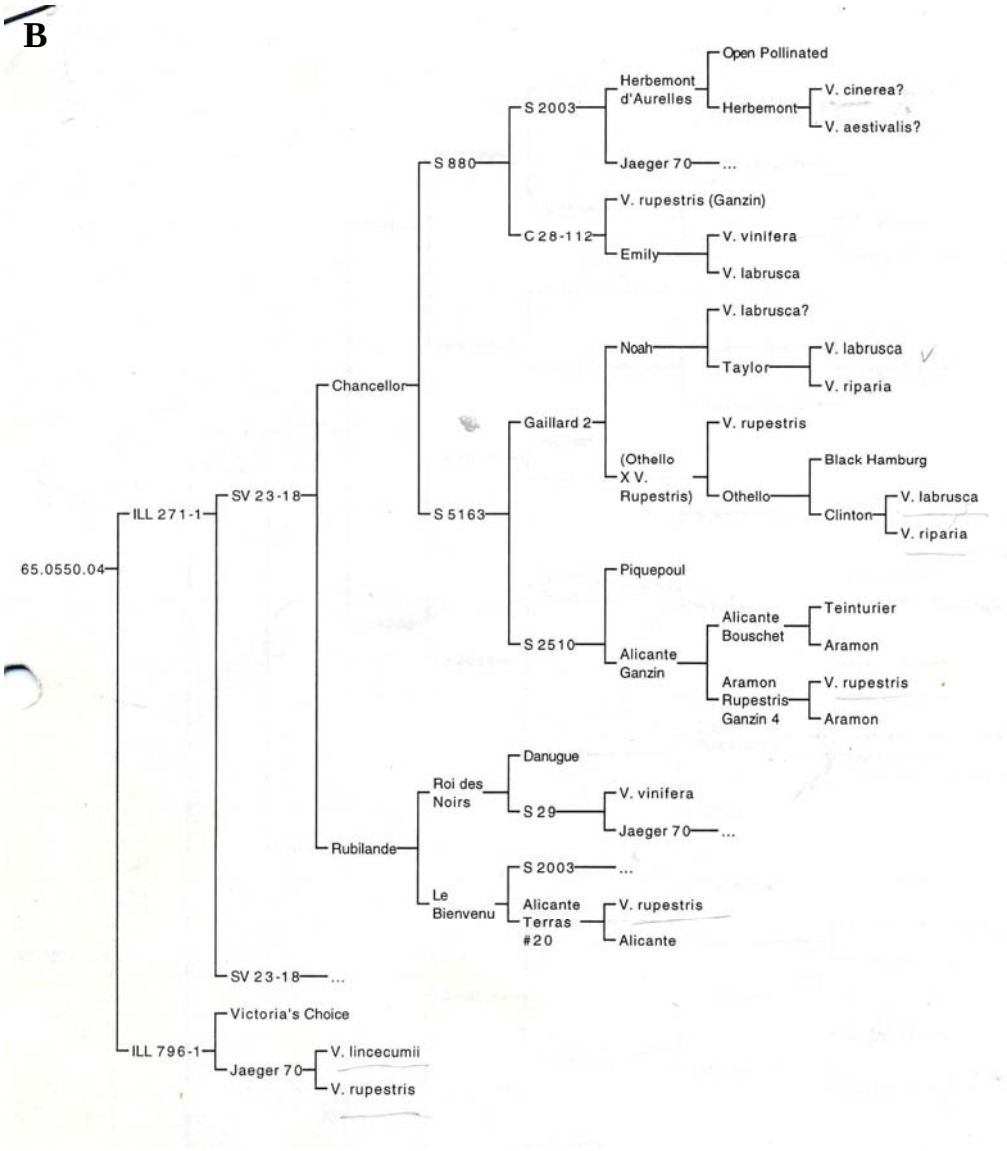


Appendix Figure 3B. Pedigree of downy mildew resistant lines from Cornell grape breeding program:

A) NY 88.0517.01

B) NY 65.0550.04

C) NY 65.0551.05.

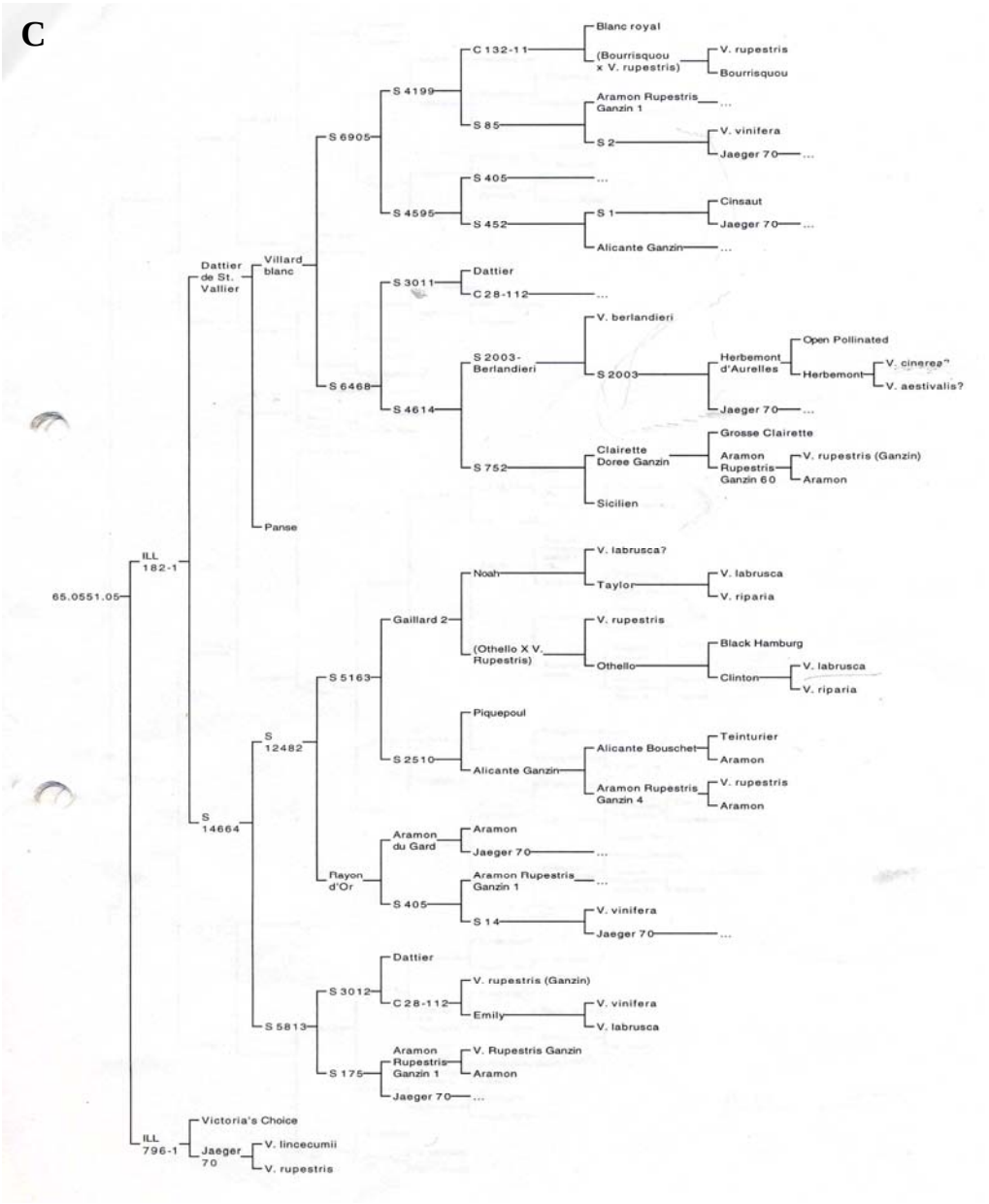


Appendix Figure 3B. Pedigree of downy mildew resistant lines from Cornell grape breeding program (continued):

A) NY 88.0517.01

B) NY 65.0550.04

C) NY 65.0551.05.

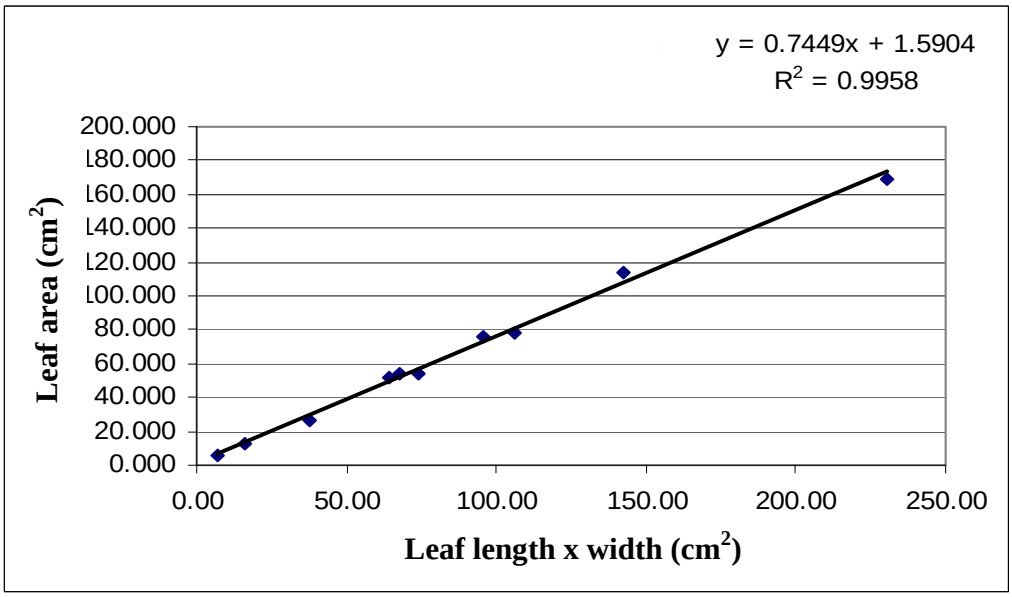


Appendix Figure 3B. Pedigree of downy mildew resistant lines from Cornell grape breeding program (continued):

A) NY 88.0517.01

B) NY 65.0550.04

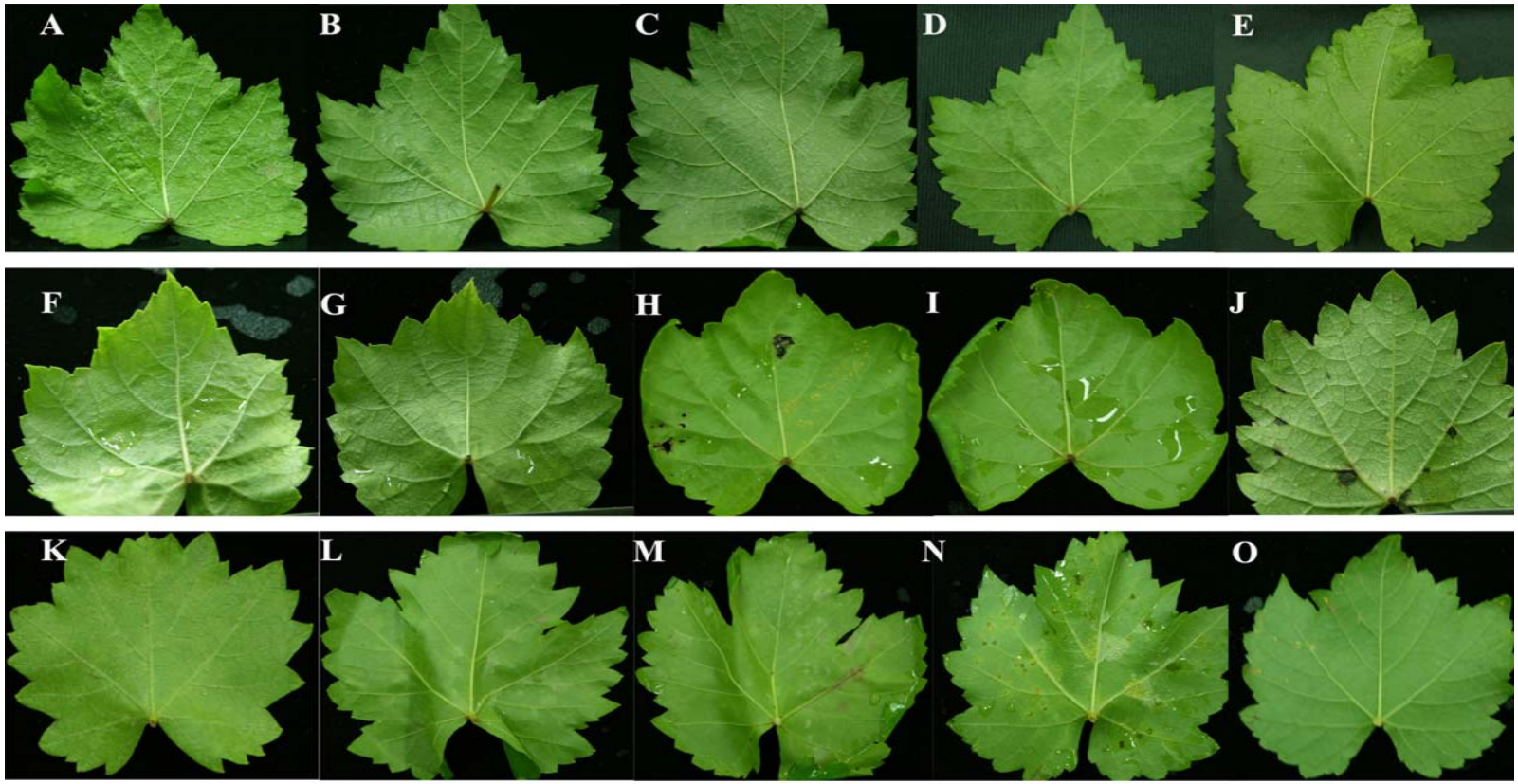
C) NY 65.0551.05.



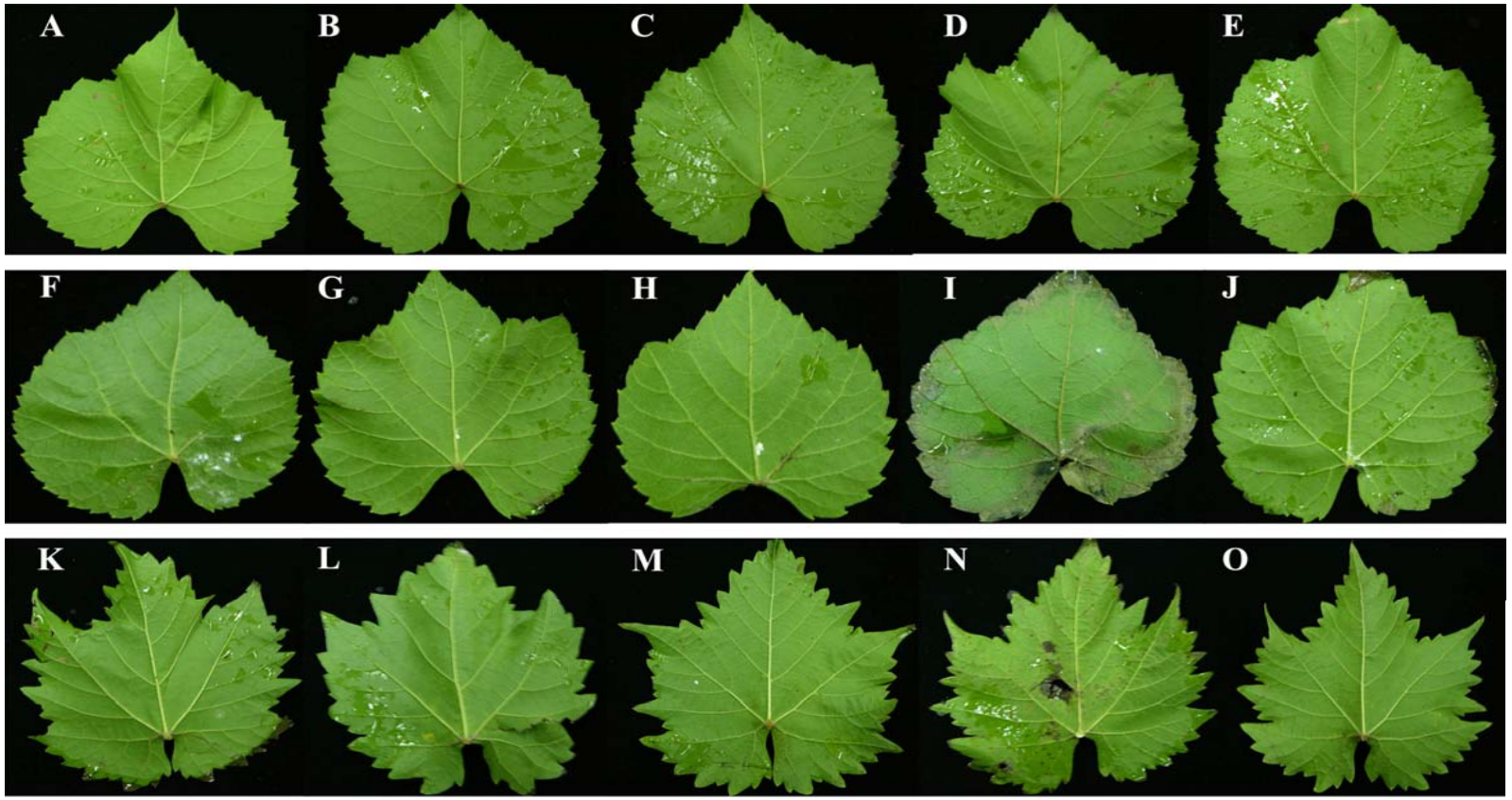
Appendix Figure 4B. Correlation between leaf length x width and leaf areas that measured from a leaf area meter.

Appendix Figure 5B. Harvested, fully ripened pollinated clusters from A) Carolina Black Rose x NY 88.0517.01 B) Carolina Black Rose x 65.0550.04; C) Carolina Black Rose x NY 65.0551.05.

Appendix Figure 6B. Seedlings of progenies from nine hybridizations crosses in nursery.

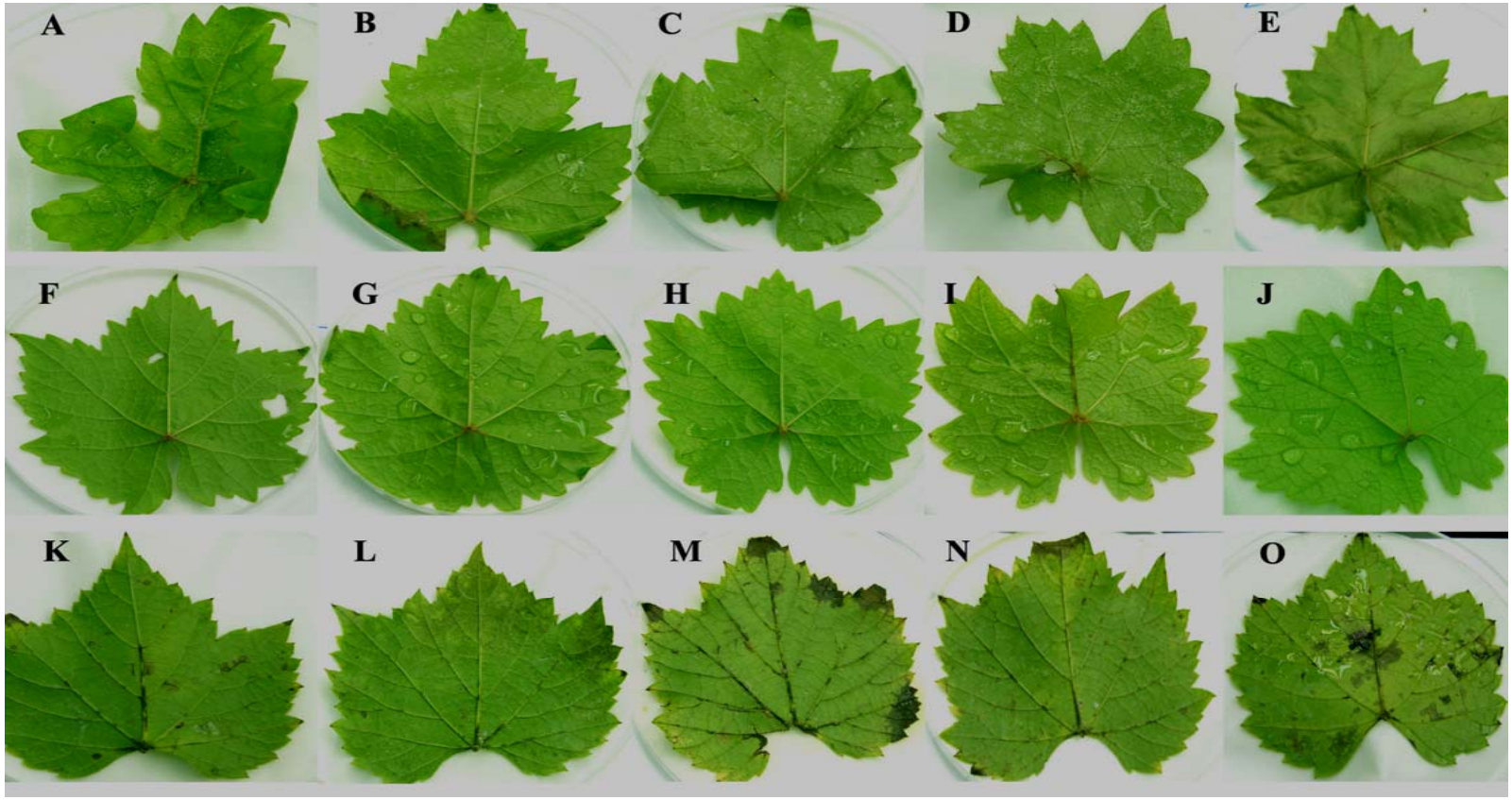


Appendix Figure 7B. Downy mildew evaluation of female parents which were highly susceptible to downy mildew in Black Queen and susceptible in Carolina Black Rose and Italia. A – E) From left to right Black Queen nodes 4; 5, 6, 7 and control (uninoculated); F – J) From left to right Carolina Black Rose nodes 4, 5, 6, 7 and control; K – O) From left to right Italia nodes 4, 5, 6, 7 and control**.**

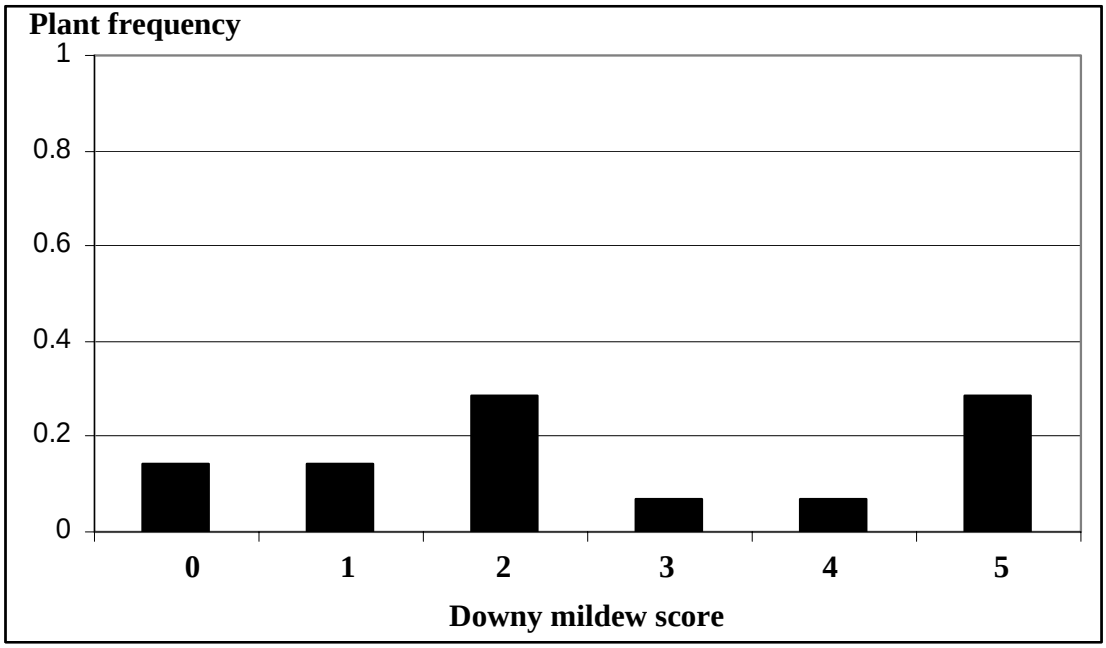


Appendix Figure 8B. Downy mildew evaluation of female parents which showed resistance to downy mildew in all lines

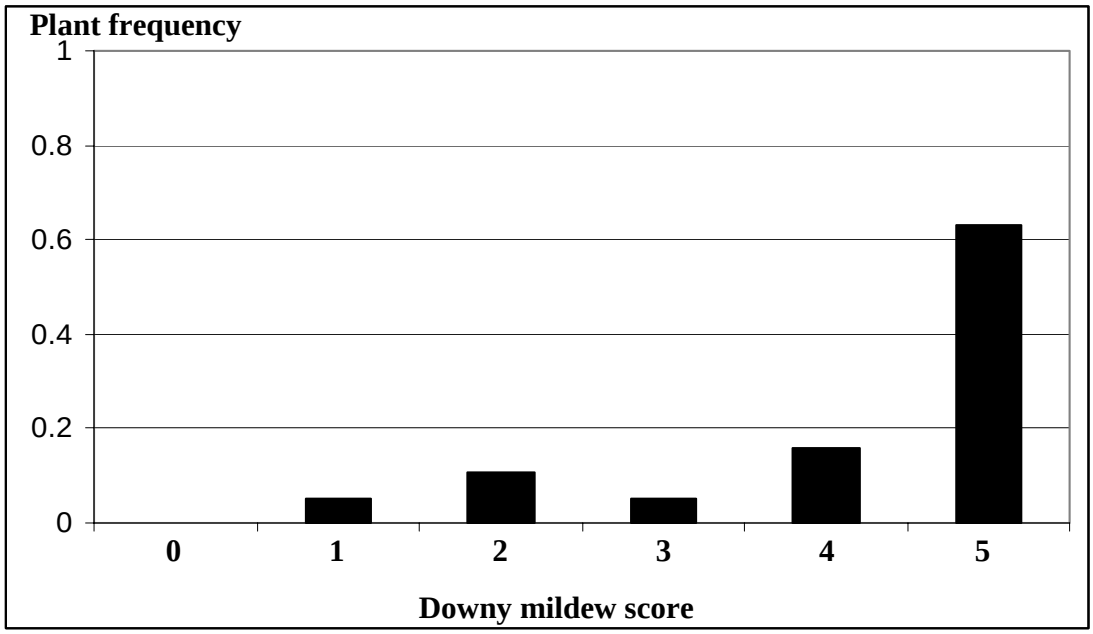
A – E) From left to right NY 88.0517.01 nodes 4, 5, 6, 7 and control (uninoculated); F – J) From left to right NY 65.0550.04 nodes 4, 5, 6, 7 and control; K – O) From left to right NY 65.0551.05 nodes 4, 5, 6, 7 and control.



Appendix Figure 9B. Downy mildew evaluation of F_1 hybrids A – E) From left to right Black Queen x NY 88.0517.01 (# 19) nodes 4, 5, 6, 7 and control (uninoculated) with high susceptibility, F – J) From left to right Carolina Black Rose x NY 88.0517.01 (# 36) nodes 4, 5, 6, 7 and control with high resistance, K – O) From left to right Italia x NY 88.0517.01 (# 6) nodes 4, 5, 6, 7 and control with intermediate resistance.



Appendix Figure 10B. Downy mildew segregation of progenies from cross 'Black Queen x NY 88.0517.01'.



Appendix Figure 11B. Downy mildew segregation of progenies from cross 'Black Queen x NY 65.0551.05'

APPENDIX C

MORPHOLOGICAL DIVERSITY OF HYBRID SEEDLINGS

6.1 Abstract

According to descriptor list of grape morphology published by Organization International of Vine and Wine (O.I.V.), twelve characteristics were selected for morphological study in the remaining 79 seedlings from nine crosses. Twelve characteristics consisted of eight shoot characteristics, including color at dorsal and ventral sides of node and internode, length of tendril, shoot diameter (cm), shoot height (cm), number of shoots per plant, and four leaf characteristics including color of young leaf, size of blade, shape of blade and number of lobes. *Vitis vinifera* L. cultivars Black Queen, Carolina Black Rose and Italia, used as female parents appeared completely green and green with red striped on shoot. On the other hand, completely green, green with red striped and completely red were color of shoot in three male parents (NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05). Similarly, each of six parents had specific leaf characteristics. The variation in shoot and leaf characteristics were also found in 79 seedlings. Moreover, some characteristics were different from their parents. These data indicate that hybrid seedlings have various combinations of parental allele(s) derived from sexual recombination of highly heterozygous parents. These hybrid seedlings are good genetic resource for breeding program due to their genetic diversity. The average shoot diameter, average shoot height and average number of shoots per plant of hybrid seedlings were 0.76 cm, 91.51 cm and three shoots, respectively. Even

though, 21 seedlings were characterized as resistant and highly resistant to downy mildew, three of these may be excluded from future grape breeding program due to their low vegetative growth. Other characteristics especially those associated with reproductive phase will be evaluated when seedlings are planted in the field. Consequently, seedlings with high disease resistance, good vegetative and reproductive growth will be selected. These will increase potential to develop new hybrids with disease resistance for current grape breeding program.

6.2 Introduction

The descriptor list for grapevine varieties and *Vitis* species were set for standardization of grape varieties described by Organization International of Vine and Wine (O.I.V.) in 1983 (Dettweiler, 1994). The list contains descriptions for 128 characteristics. Morphology descriptions comprise shoot, inflorescence, leaf, bunch and berry characteristics (I.P.G.R.I. et al., 1997). The morphology descriptions of 41,648 grape accessions collected from 30 countries were first published at "Repertoire mondial des collections de Vitis" in 1987 based on standard of O.I.V. (Dettweiler, 1994). In 1994, the Institute for Grapevine Breeding Geilweilerhof has carried out a second survey of grape collected from 40 countries with 30,311 accessions and was published in "World list of grapevine collections, 2nd edition" (Dettweiler, 1994). Nowadays, the differentiation and identification of European grape varieties reported at European *Vitis* database is available on http://www.genres.de/eccdb/vitis/. Moreover, databases on conservation of genetic resources and ability to exchange germplasm are also available on this website (Dettweiler, 2003).

The 83 seedlings from nine crosses (three resistant genotypes and three susceptible cultivars) were observed for variation of downy mildew resistant levels as

reported in Chapter IV. In this chapter, the morphological characterization of the remaining seedlings (79 seedlings) could provide information specific for individual hybrids as well as the diversity of hybrid population. In particular, specific information of each seedling will estimate the potential of 21 downy mildew resistant seedlings for future grape disease resistance breeding program.

6.3 Materials and methods

Eight characteristics of the shoot and four characteristics of the leaf were observed on 79 seedlings from crosses between three susceptible cultivars (*V. vinifera* cv. Black Queen, Carolina Black Rose and Italia) and three resistant genotypes (NY 88.0517.01, NY 65.0550.04 and NY 65.0551.05). These seedlings were approximately two years old and had been grown in six inch pots containing 1 part peat moss, 1 part soil, ½ part burnt rice-chaff, 1 part perlite, 1 part vermiculite, ¾ part sand. Characteristics of the shoot and leaf of the seedlings were recorded following standardization of O.I.V. as reported on "Descriptors for grapevine (*Vitis* spp.)" (I.P.G.R.I et al., 1997).

Eight characteristics selected for this study were color of dorsal side at internode, color of ventral side at internode, color of dorsal side at node, color of ventral side at node (Figure 1), length of tendril, shoot diameter (cm), shoot length (cm) and number of shoots. Color of dorsal side and ventral side at node and internode were recorded as 1 for completely green; 2 for green and red striped and 3 for completely red. Length of tendril was recorded as 1 for very short (<11 cm); 3 for short (14-16 cm); 5 for medium (19-21 cm); 7 for long (24-26 cm) and 9 for very long (>30 cm). Vernier calipter was used to measure diameter of shoot at the base. Shoot length was measured from the base to the first expanded leaf.

Leaf characteristics selected for this study are color of upper surface on young leaves (distal leaves; Figure 2) and recorded as 1 for green; 2 for green with bronze spots; 3 for yellow; 4 for yellow with bronze spots; 5 for copper yellow; 6 for copper; 7 for reddish and 99 for other. Mature leaf was studied on size of blade as 1 for very small (<5 cm width); 3 for small (6 – 10 cm width); 5 for medium (11 – 15 cm width); 7 for large (16 – 20 cm width) and 9 for very large (>21 cm), shape of blade (1 for cordate; 2 for wedge-shaped; 3 for pentagonal; 4 for circular; 5 for reniform and 99 for other; Figure 3) and number of lobes (1 for none; 2 for three; 3 for five; 4 for seven and 5 for more than seven; Figure 4).

Due to high number of hybrid seedlings, progenies from two crosses, 'Black Queen x NY 88.0517.01' and 'Black Queen x NY 65.0551.05', were chosen for diversity evaluation (13 and 19 seedlings, respectively). The unweighted pair group method with arithmetic averaging (UPGMA) cluster analysis from the NTSYS-pc version 1.8 software package was used to generate dendogram based on morphological similarity among these progenies (Sneath and Sokal, 1973; Rohf, 1993).

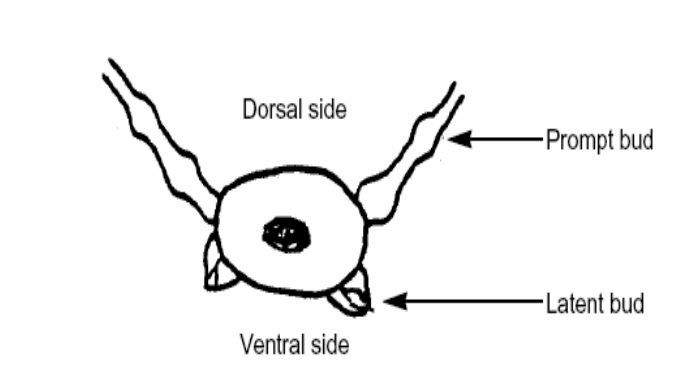


Figure 1. Shoot: dorsal and ventral sides.

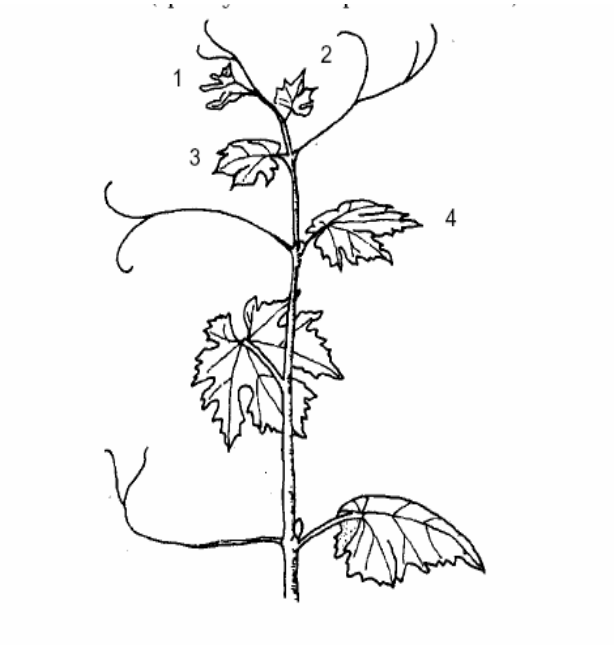


Figure 2. Young leaves: 4 distal leaves.

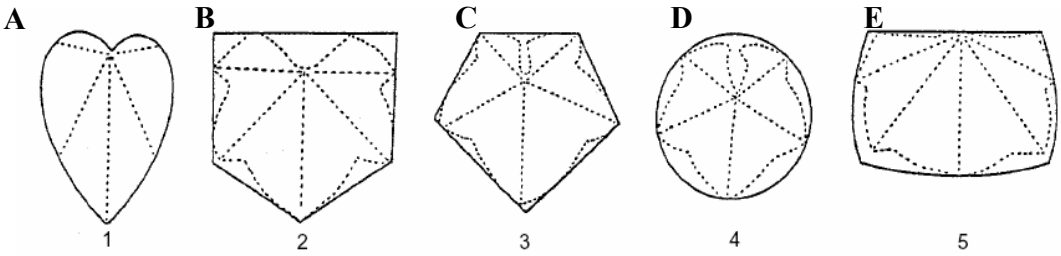


Figure 3. Mature leaf: shape of blade A) cordate; B) wedge-shaped; C) pentagonal; D) circular; E) reniform.

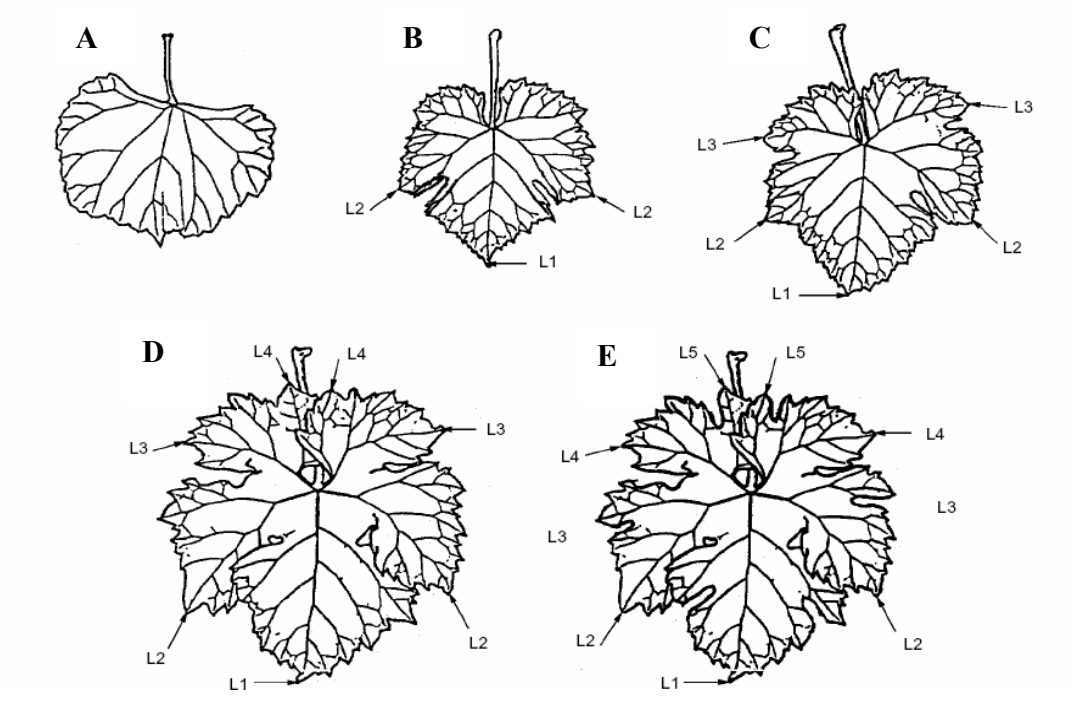


Figure 4. Mature leaf: number of lobes A) non; B) three; C) five; D) seven; E) more than seven.

6.4 Results

V. vinifera (cv. Italia) that was used as a female parent appeared completely green at dorsal and ventral sides of both internode and node of shoot (Figure 5; Table 1). While, other two female parents (*V. vinifera* cv. Black Queen and Carolina Black Rose), were completely green and green with red striped as shown on Figure 5 and Table 1. On the other hand, two male parents (NY 88.0517.01 and NY 65.0550.04), the downy mildew resistant genotypes with genetic composition of *V. vinifera* and some American species, showed all three colors (completely green, green with red striped and completely red) at their shoots as shown on Figure 6 and Table 1. In contrast, shoot color of NY 65.0551.05 was completely green similar to *V. vinifera* cv. Italia (Figure 5, 6; Table 1).

Because none of the parents grown in greenhouse was propagated from seeds as hybrid seedlings, some of shoot characteristics such as shoot diameter, shoot height and numbers of shoots per plant were not observed. Moreover, male and female parents were grown in different containers. The male parents were grown in large containers (approximately 20 inch diameter), while the female parents were grown in only six-inch pots. However, observation in the greenhouse and/or the field indicated that no significant difference in vegetative growth was found among male parents. In contrast, Italia had slightly lower vegetative growth when compared with the other two female parents.

Unfortunately, four out of 83 seedlings that were evaluated for downy mildew resistance as described in Chapter IV had died, therefore the morphological characterization of the shoot and leaf was performed on the remaining 79 seedlings. Variation in color of dorsal and ventral sides at internode and node of seedlings from all crosses was found (Figure 7; Table 2). These data indicated that the hybrids had various combinations of gene(s) from their parents. Some hybrids showed colors other than those found on the parents; for example green with red striped from parents that were completely green (cross between Italia with NY 65.0551.05; Table 2). This stem color could result from the combination of alleles from heterozygous parents especially the recessive ones. These recessive alleles would not be expressed when paired with dominant alleles in heterozygous parents.

The average shoot diameter of 79 seedlings from nine crosses was 0.76 cm. The seedling number 27 from a cross ‘Black Queen x NY 88.0517.01’ had the smallest shoot diameter (0.42 cm), while the largest shoot (2.81 cm) was found in seedling number 10 from a cross ‘Black Queen x NY 65.0551.01’ (Table 2). The variation on shoot height was also found among 79 seedlings (Table 2) with the average height of the 91.51 cm.

The shortest was the seedling number 3 from a cross 'Carolina Black Rose x NY 65.0550.04' (10 cm) and the highest seedling number 8 from a cross 'Carolina Black Rose x NY 65.0551.05' (378 cm; Table 2). Moreover, the average shoot number was approximately three shoots per plant. Some of the seedlings such as seedling number 2 from a cross 'Black Queen x NY 65.0551.05' had high shoot number of up to eight shoots per plant (Table 2). In general, seedlings that had Italia as female parent showed lower vegetative growth when compared with other female parents. However, there was no significant difference found on average shoot diameter, shoot height and number of shoots per plant among crosses as shown on Table 3-5.

Table 1. Shoot characteristics of three susceptible cultivars (female parents) and three resistant genotypes (male parents).

Genotypes	Color of internode[a]		Color of node[a]		Length of
	Dorsal	Ventral	Dorsal	Ventral	tendril[b]
Black Queen	1	2	1	2	1
Carolina Black Rose	2	1	2	1	1
Italia	1	1	1	1	1
NY 88.0517.01	2	3	2	2	3
NY 65.0550.04	1	3	1	3	1
NY 65.0551.05	1	1	1	1	3

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Four leaf characteristics of the parents, which include color of upper surface, size of blade, shape of blade and number of lobes, are shown on Figure 5, 6 and Table 6. Similarly, variation on leaf characteristics was also found among hybrid seedlings (Figure 8, 9, 10, 11; Table 7). Some seedlings had leaf characteristics such as shape of

blade and number of lobes different from their parents, which may be explained by differentiation on gene(s) combination and/or gene(s) expression as previously suggested on the variation of shoots.

Dendogram of the seedlings from a cross 'Black Queen x NY 88.0517.01' based on twelve characteristics of shoot and leaf indicated substantial phenotypic variation among progenies with similarity coefficients of 0.36 to 0.85 (Figure 12). Groups of seedlings that were closely related were clustered together such as seedling number 7 and number 15 or seedling number 6 and number 33 (Figure 12). On the other hand, seedling number 19 was very different from other seedlings derived from the same parent (Figure 12). Similarly, phenotypic variation was found in the progenies from a cross 'Black Queen x NY 65.0551.05' with similarity coefficients of 0.34 to 0.88 as shown on Figure 13.

6.5 Discussion

Characteristics of shoot and leaf such as color of shoot, color of young leaf, size of blade, shape of blade and number of lobes were very diverse among seedlings from nine crosses. Phenotypic variation and segregation of traits in this progeny population resulted from sexual reproduction of highly heterozygous parents. Thus, genetic variation in each individual plant is a novel combination of parental alleles. Moreover, highly heterozygous parents may lead to different characteristics of seedlings from their parents. Genetic distance among progenies from two cross combinations ('Black Queen x NY 88.0517.01'; Figure 12 and 'Black Queen x NY 65.0551.05'; Figure 13) ranged from 0.15 to 0.64 and 0.12 to 0.66, respectively. Overall, the genetic diversity of this population based on vegetative morphological variation suggested that it was a good genetic resource for grape breeding program. Moreover, variation of reproductive

morphology is also expected in this population and desirable characteristics such as better quality of berries and size of cluster, etc. from genetic recombination might also be possible. However, further field trials are needed in the future.

Miller et al. (1996) reported that increasing of shoot number resulted in increasing of total leaf area, shoot length and flower cluster length of whole plant. High number of shoots may allow plants to develop greater leaf areas enabling the production of more vegetative sinks of carbohydrate for themselves. Therefore, dry weight harvested from three and six shoots per plant are 22.0% and 20.0% higher than one shoot per plant, respectively (Miller, 1996). Even though, hybrid seedlings were grown in pots in greenhouse, they still showed ability to produce approximately three shoots per plant and should be able to grow vigorously in the field.

Due to the similar levels of vegetative growth of three male parents, there was no significant difference in the average shoot diameter, shoot height and number of shoots among hybrid seedlings of each female parent when crossed with different male parents. However, the largest shoots and highest number of shoots were found in seedlings derived from crosses that had Black Queen and Carolina Black Rose as female parents, respectively. These data were in agreement with higher vegetative and reproductive growth of Black Queen and Carolina Black Rose than Italia as mentioned earlier in Chapter IV, and demonstrated that these characteristics could be introgressed into their progenies. However, it should be noted that these quantitative characteristics were measured based on a single plant basis and should be considered preliminary.

Approximately 83 seedlings were evaluated for downy mildew resistance and 21 seedlings were resistant and highly resistant to the disease (Appendix Table 3B). Therefore, these 21 seedlings are valuable genetic resources for grape breeding program. When morphology of these seedlings were evaluated, the ranges of shoot diameter and

shoot height were from 0.43 to 2.30 cm, and 50.00 to 310.00 cm with means of and 1.06 and 120.06, respectively, indicating that the majority of them had good vegetative growth. However, seedling number 15 from a cross 'Italia x NY 65.0550.04' was already dead. While the other two seedlings, seedling number 30 from a cross 'Black Queen x NY 88.0517.01' and seedling number 3 from a cross 'Carolina Black Rose x NY 65.0550.04', had very low vegetative growth (Table 2). Therefore, these three seedlings will be excluded from future grape breeding program.

In the future, the reproductive characteristics especially those of inflorescence and berry as described on standardization of O.I.V. will also be evaluated when seedlings are planted in the field. These characteristics such as bud bursting, fruit maturity, cluster weight, berry weight, number of berries per cluster, number of clusters per shoot, number of seeds per berry and 100-seed weight will provide information on grape reproduction and quality of the grape berry (Eibach, 1989; Wagner, 1989), which could greatly affect the acceptance of farmers and consumers. For example, number of clusters per shoot directly relates to sugar content in the berry (Eibach, 1989). All of the results will provide information necessary for selecting seedlings with high potential on disease resistance, vegetative and reproductive growth for use as materials for developing new grape cultivars in the future.

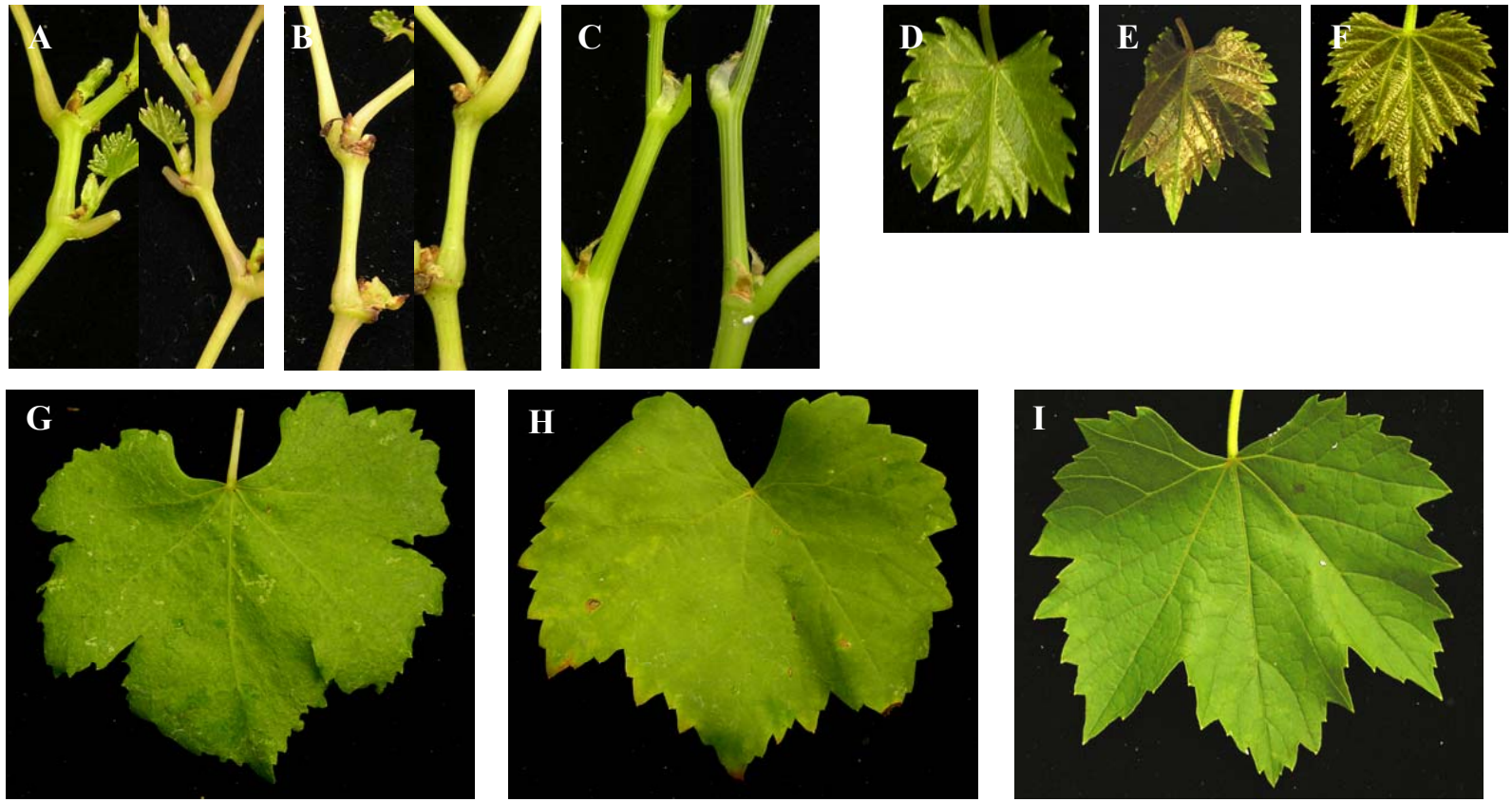


Figure 5. Shoot and leaf characteristics A) dorsal and ventral sides of Black Queen; B) dorsal and ventral sides of Carolina Black Rose; C) dorsal and ventral sides of Italia; D) young leaf of Black Queen; E) young leaf of Carolina Black Rose; F) young leaf of Italia; G) mature leaf of Black Queen; H) mature leaf of Carolina Black Rose; I) mature of Italia.

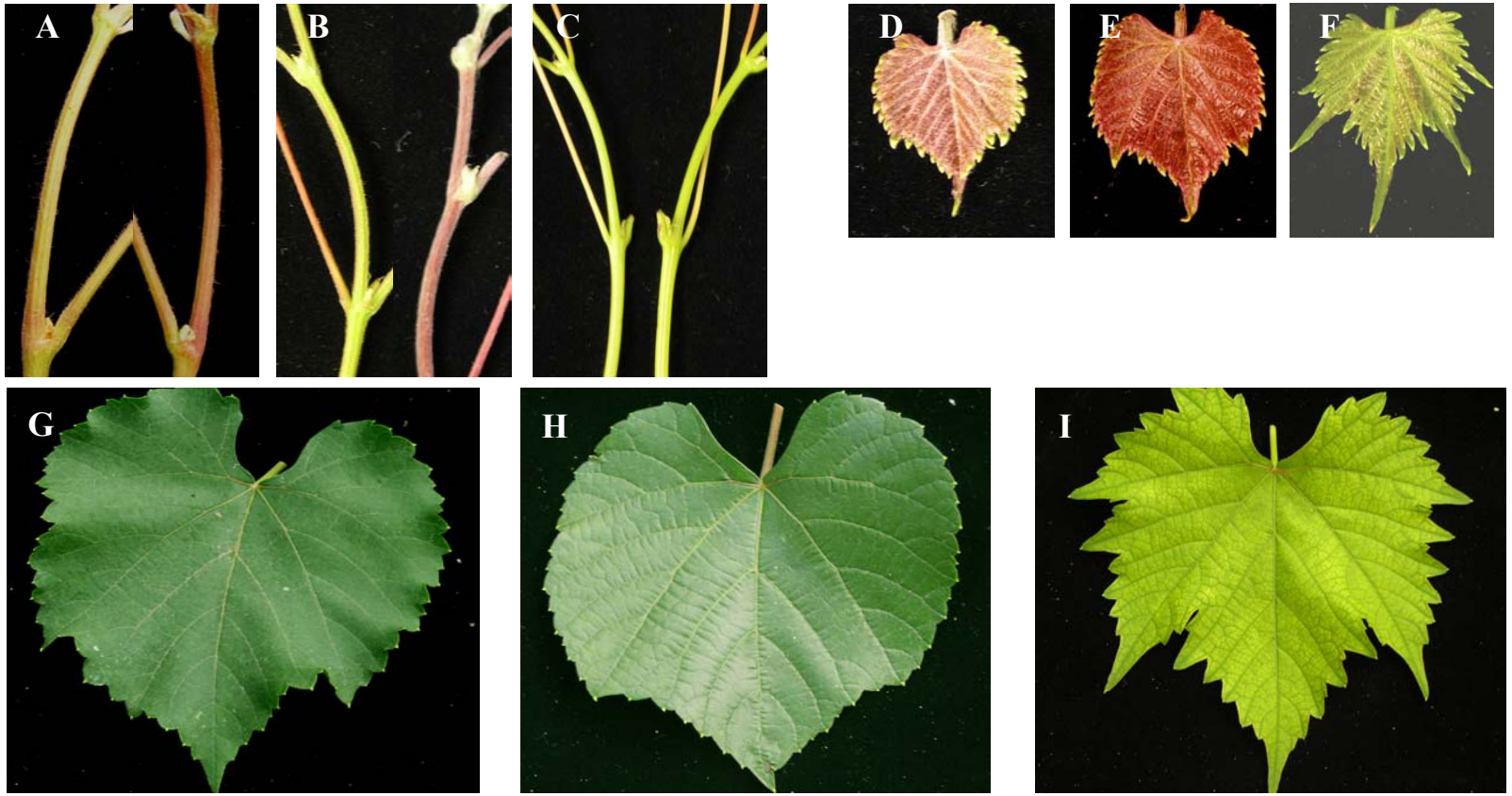


Figure 6. Shoot and leaf characteristics A) dorsal and ventral sides of NY 88.0517.01; B) dorsal and ventral sides of NY 65.0550.04; C) dorsal and ventral sides of NY 65.0551.05; D) young leaf of NY 88.0517.01; E) young leaf of NY 65.0550.04; F) young leaf of NY 65.0551.05; G) mature leaf of NY 88.0517.01; H) mature leaf of NY 65.0550.04; I) mature leaf of NY 65.0551.05.

Table 2. Shoot characteristics of seedlings from nine crosses.

Seedling #	Color of internode[a]		Color of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Black Queen x NY 88.0517.01								
6	1	1	1	1	0.48	33.5	2	-
7	1	2	1	2	1.82	94.0	3	1
8	2	2	1	2	0.95	80.0	4	-
13	2	3	2	3	0.63	55.0	6	1
15	1	2	1	2	1.17	130.0	2	-
19	2	3	1	3	1.87	130.0	3	3
20	2	2	2	2	0.83	60.0	5	1
24	2	2	1	1	1.10	58.0	6	1
27	2	2	2	2	0.42	43.0	4	-
29	2	2	2	2	1.32	60.0	3	1
30	2	3	1	1	0.92	13.0	2	-
32	2	2	2	2	2.39	156.0	6	1
33	1	1	1	1	0.93	68.0	3	-
Average					1.14	75.42	3.77	

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Color of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Black Queen x NY 65.0550.04								
1	1	2	1	2	1.88	100.0	5	1
6	1	3	1	2	1.23	67.0	5	1
8	2	2	1	1	0.74	160.0	3	1
14	1	2	1	1	0.64	53.0	2	3
30	2	3	2	3	0.64	120.0	3	1
54	3	3	1	2	1.43	160.0	1	1
Average					1.09	110.10	3.17	
Black Queen x NY 65.0551.05								
2	1	2	1	1	1.95	144.0	8	3
3	1	1	1	1	0.74	40.0	3	1
5	1	2	1	2	1.02	40.0	3	-
6	3	3	3	3	0.72	30.0	2	-
7	1	1	1	1	1.80	175.0	3	1
8	1	1	1	1	0.58	160.0	2	-
9	2	2	1	2	0.50	180.0	2	-

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Color of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Black Queen x NY 65.0551.05								
10	1	1	1	1	2.81	350.0	4	3
16	2	3	1	3	1.16	60.0	6	-
19	3	3	2	2	0.83	57.0	4	1
20	2	2	1	1	0.62	160.0	3	-
21	1	2	1	2	1.28	166.4	6	3
24	1	3	1	1	0.96	170.0	3	1
25	1	3	1	2	0.66	160.0	1	1
27	1	2	1	2	0.70	204.0	3	1
28	3	3	2	2	1.36	49.0	6	-
31	1	1	1	1	1.46	200.0	2	3
32	1	2	1	1	2.37	265.0	3	1
47	1	1	1	1	0.52	110.0	3	-
Average					1.16	143.18	3.53	

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Color of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Carolina Black Rose x NY 88.0517.01								
2	2	2	1	2	1.16	74.0	3	1
3	1	2	1	2	0.83	40.0	2	-
8	1	2	1	2	1.06	114.0	2	3
11	1	1	1	1	1.35	310.0	5	-
12	2	2	2	2	0.83	122.0	4	-
14	1	3	1	3	0.83	124.0	3	-
15	1	1	2	2	1.87	170.0	2	-
20	2	2	1	2	1.20	16.0	2	-
36	1	1	1	1	0.91	63.0	4	-
40	2	3	1	3	2.30	211.6	6	1
Average					1.23	124.46	3.30	
Carolina Black Rose x NY 65.0550.04								
2	2	2	1	2	1.15	205.0	3	1
3	1	2	1	2	1.04	10.0	1	-
5	1	2	1	2	1.19	246.0	2	3

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Color of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Carolina Black Rose x NY 65.0550.04								
13	1	2	1	2	0.62	84.0	1	-
14	2	3	1	3	1.14	102.0	2	1
15	1	2	1	2	1.19	74.0	2	1
17	2	3	1	1	0.69	58.0	3	1
19	2	3	2	3	0.43	50.0	2	1
Average					0.93	103.63	2.00	
Carolina Black Rose x NY 65.0551.05								
1	1	2	1	1	0.45	45.0	3	1
2	2	2	2	2	1.87	30.0	4	-
4	1	2	1	1	0.90	222.0	2	-
5	2	2	1	1	0.65	84.0	2	-
7	1	2	1	1	1.82	79.0	5	1
8	1	1	1	1	1.41	378.0	2	3
9	1	1	1	1	0.69	100.0	4	-
18	2	2	1	2	0.98	79.0	5	-

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Colour of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Carolina Black Rose x NY 65.0551.05								
20	1	2	1	1	0.93	85.0	3	3
21	1	2	1	1	0.65	53.0	1	-
22	1	2	1	2	0.70	154.0	1	1
27	1	1	1	1	0.84	350.0	5	1
29	1	3	1	2	0.55	80.0	3	3
Average					0.94	125.19	3.25	
Italia x NY 88.0517.01								
3	2	2	2	2	0.56	28.5	1	1
6	2	2	1	1	0.78	110.0	4	1
Average					0.67	69.25	2.50	

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 2. Shoot characteristics of seedlings from nine crosses (continued).

Seedling #	Color of internode[a]		Colour of node[a]		Diameter	Shoot	Number	Length of
	Dorsal	Ventral	Dorsal	Ventral	(cm)	length (cm)	of shoot	tendril[b]
Italia x NY 65.0550.04								
2	2	3	1	2	1.27	124.0	3	3
6	2	3	2	3	0.83	130.0	2	-
12	2	2	2	2	0.80	105.0	3	1
18	2	2	2	2	0.75	41.5	2	-
Average					0.91	100.13	2.50	
Italia x NY 65.0551.05								
3	1	1	1	1	0.95	130.0	4	3
4	2	2	1	1	0.75	90.0	1	1
6	2	2	2	2	1.45	45.4	3	-
21	1	1	1	1	1.39	116.0	5	1
Average					1.14	95.30	3.25	

[a]Color of shoot: 1 = completely green; 2 = green with red striped; 3 = completely red
[b]Length of tendril: 1= very short (<11 cm); 3 = short (14 – 16 cm)

Table 3. Average shoot diameter (cm) from progenies of each cross.

Male	Female			Average
	Black Queen	Carolina Black Rose	Italia	
NY88.0517.01	1.14	1.23	0.67	1.01
NY65.0550.04	1.09	0.93	0.91	0.98
NY65.0551.05	1.16	0.94	1.14	1.08
Average	1.13	1.03	0.91	

Table 4. Average shoot height (cm) from progenies of each cross.

Male	Female			Average
	Black Queen	Carolina Black Rose	Italia	
NY88.0517.01	75.42	124.46	69.25	89.71
NY65.0550.04	110.10	103.63	100.13	104.62
NY65.0551.05	143.18	125.19	95.30	121.22
Average	109.57	117.76	88.27	

Table 5. Average number of shoots per plant from progenies of each cross.

Male	Female			Average
	Black Queen	Carolina Black Rose	Italia	
NY88.0517.01	3.77	3.30	2.50	3.19
NY65.0550.04	3.17	2.00	2.50	2.56
NY65.0551.05	3.53	3.25	3.25	3.34
Average	3.49	2.85	2.75	

Table 6. Leaf characteristics of three susceptible cultivars (female parents) and three resistant genotypes (male parents).

Genotypes	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Black Queen	5	5	2	4
Carolina Black Rose	6	5	4	2
Italia	6	5	3	3
NY 88.0517.01	7	9	4	3
NY 65.0550.04	7	5	4	1
NY 65.0551.05	6	7	3	4

[a]Color of young leaf: 5 = copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven

Table 7. Leaf characteristics of seedlings from nine crosses.

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Black Queen x NY 88.0517.01				
6	3	1	2	3
7	5	7	2	5
8	6	3	2	3
13	3	3	3	2
15	1	7	2	3
19	7	9	2	5
20	7	1	2	2
24	7	5	2	2
27	5	3	2	2
29	3	5	3	3
30	5	1	3	1
32	5	5	2	3
33	1	5	2	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses (continued).

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Black Queen x NY 65.0550.04				
1	5	5	2	3
6	1	3	3	2
8	1	3	2	2
14	1	5	2	2
30	5	5	3	4
54	1	5	3	2
Black Queen x NY 65.0551.05				
2	3	7	2	3
3	3	7	2	5
5	1	7	3	4
6	3	3	3	2
7	1	5	3	3
8	3	5	2	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses.

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Black Queen x NY 65.0551.05				
9	5	5	3	4
10	5	9	3	5
16	5	3	3	2
19	1	5	3	3
20	3	5	3	3
21	7	7	3	4
24	5	5	2	3
25	6	5	3	4
27	3	7	3	3
28	3	3	3	2
31	5	9	3	3
32	5	9	2	5
47	5	5	3	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses (continued).

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Carolina Black Rose x NY 88.0517.01				
2	7	1	3	3
3	1	1	2	3
8	7	3	2	2
11	7	3	4	2
12	1	3	4	1
14	1	3	4	1
15	7	5	2	4
20	5	1	3	2
36	3	1	2	3
40	3	5	2	3
Carolina Black Rose x NY 65.0550.04				
2	5	7	2	4
3	5	5	2	4

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses (continued).

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Carolina Black Rose x NY 65.0550.04				
5	7	5	2	4
13	5	3	3	2
14	6	5	2	2
15	7	3	3	3
17	1	3	2	2
19	5	1	3	2
Carolina Black Rose x NY 65.0551.05				
1	5	5	2	4
2	5	3	3	2
4	3	9	3	5
5	5	3	2	2
7	6	3	3	3
8	7	7	3	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses (continued).

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Carolina Black Rose x NY 65.0551.05				
9	1	3	2	2
18	5	3	3	3
20	3	9	2	4
21	1	5	2	2
22	2	5	3	4
27	7	9	3	5
29	5	5	2	3
Italia x NY 88.0517.01				
3	1	5	3	3
6	5	5	2	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Table 7. Leaf characteristics of seedlings from nine crosses (continued).

Seedling #	Young leaf	Mature leaf		
	color of upper side[a]	Size of blade[b]	Shape of blade[c]	Number of lobes[d]
Italia x NY 65.0550.04				
2	7	5	3	5
6	1	1	3	2
12	5	3	2	4
18	7	3	3	3
Italia x NY 65.0550.04				
3	5	9	3	4
4	5	5	3	3
6	7	3	3	2
21	5	7	3	3

[a]Color of young leaf: 1 =green; 3 = yellow; 5= copper yellow; 6 = copper; 7 = reddish
[b]Size of blade: 1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
[c]Shape of blade: 2 = wedge-shaped; 3 = pentagonal; 4 = circular
[d]Number of lobes: 1 = none; 2 = three; 3 = five; 4 = seven; 5 = more than seven

Figure 7. Shoot characteristics in seedlings A) dorsal and ventral sides show completely green; B) dorsal and ventral sides show green with red striped; C) dorsal and ventral sides show completely red.

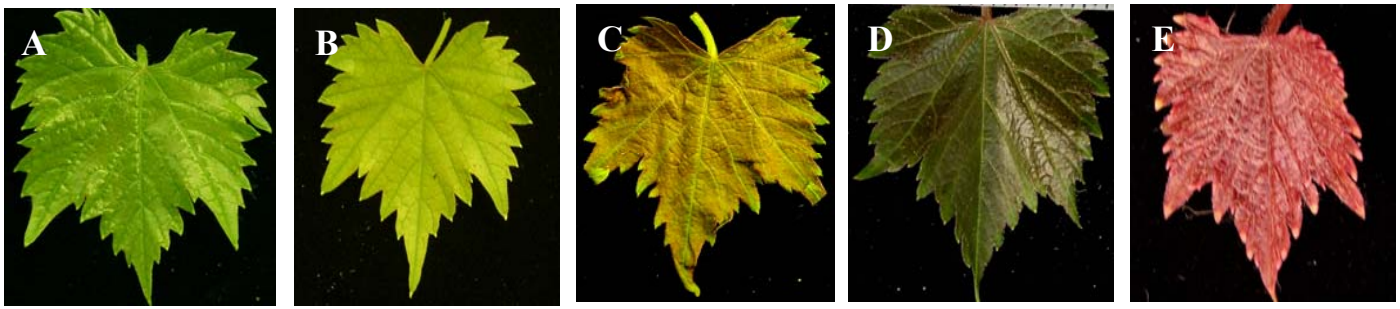


Figure 8. Young leaf: color of distal leaf in seedlings A) green; B) yellow; C) copper yellow; D) copper; E) reddish.

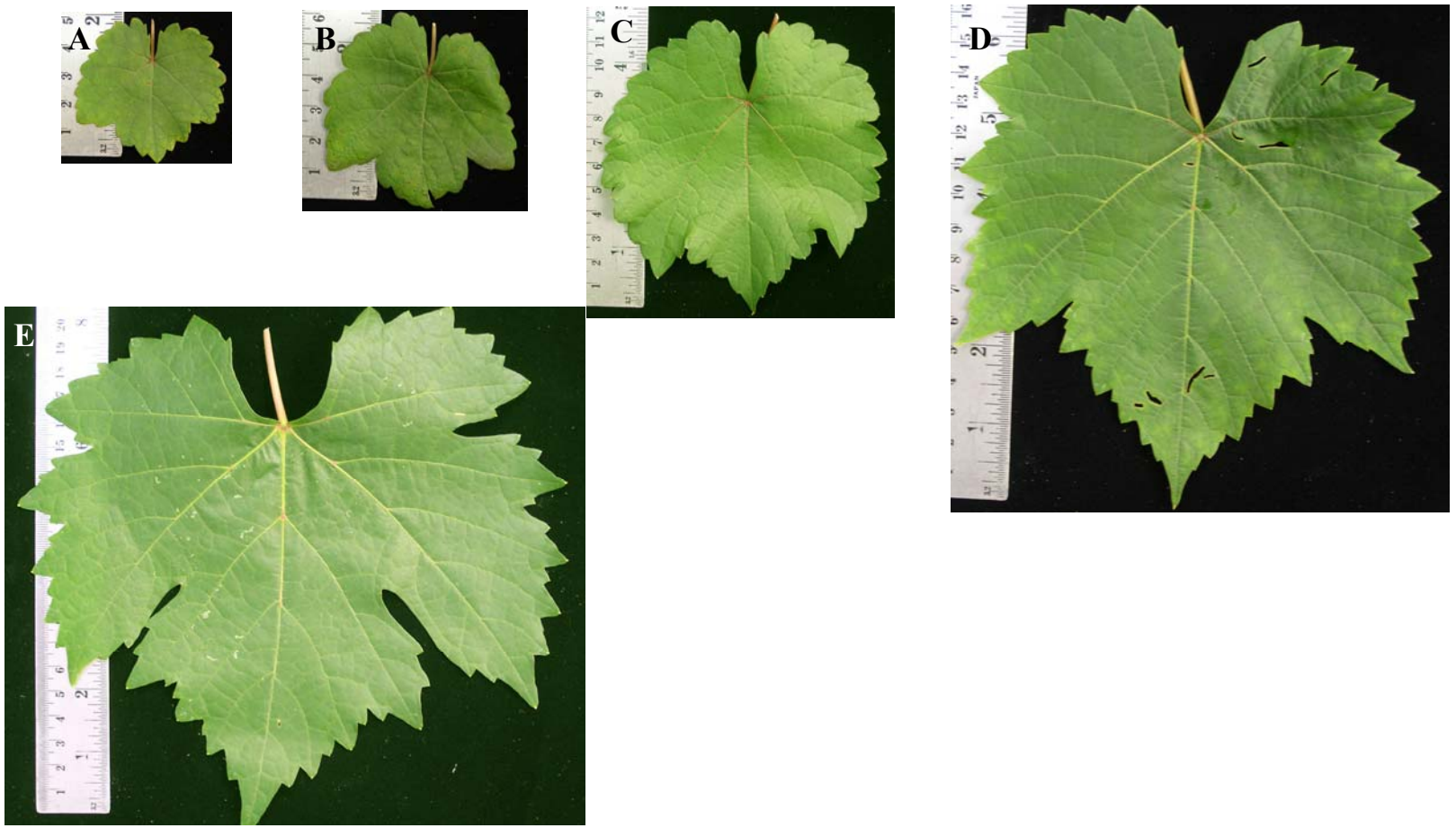


Figure 9. Mature leaf: size of blade in seedlings A) very small; B) small; C) medium; D) large; E) very large.

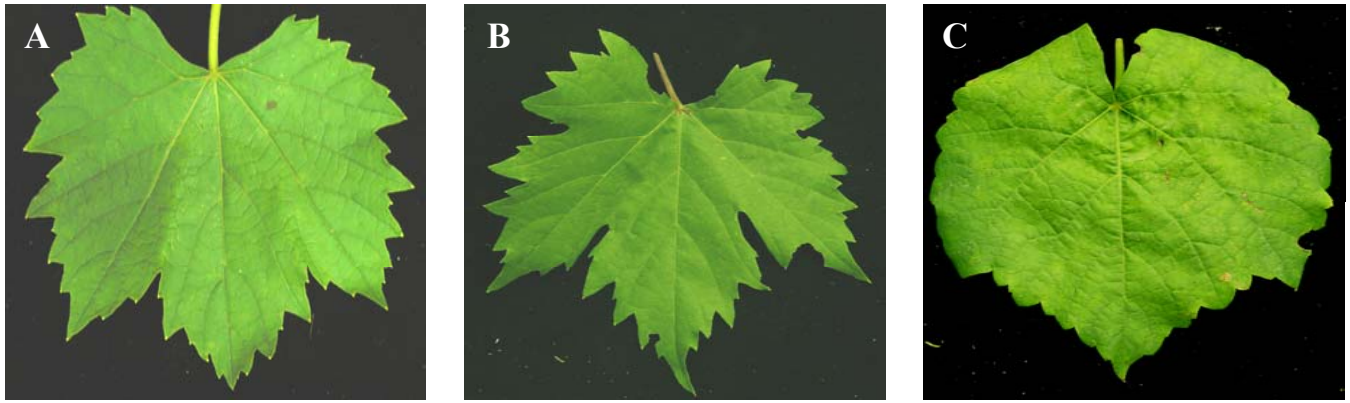


Figure 10. Mature leaf: shape of blade in seedlings A) wedge-shaped; B) pentagonal; C) circular.

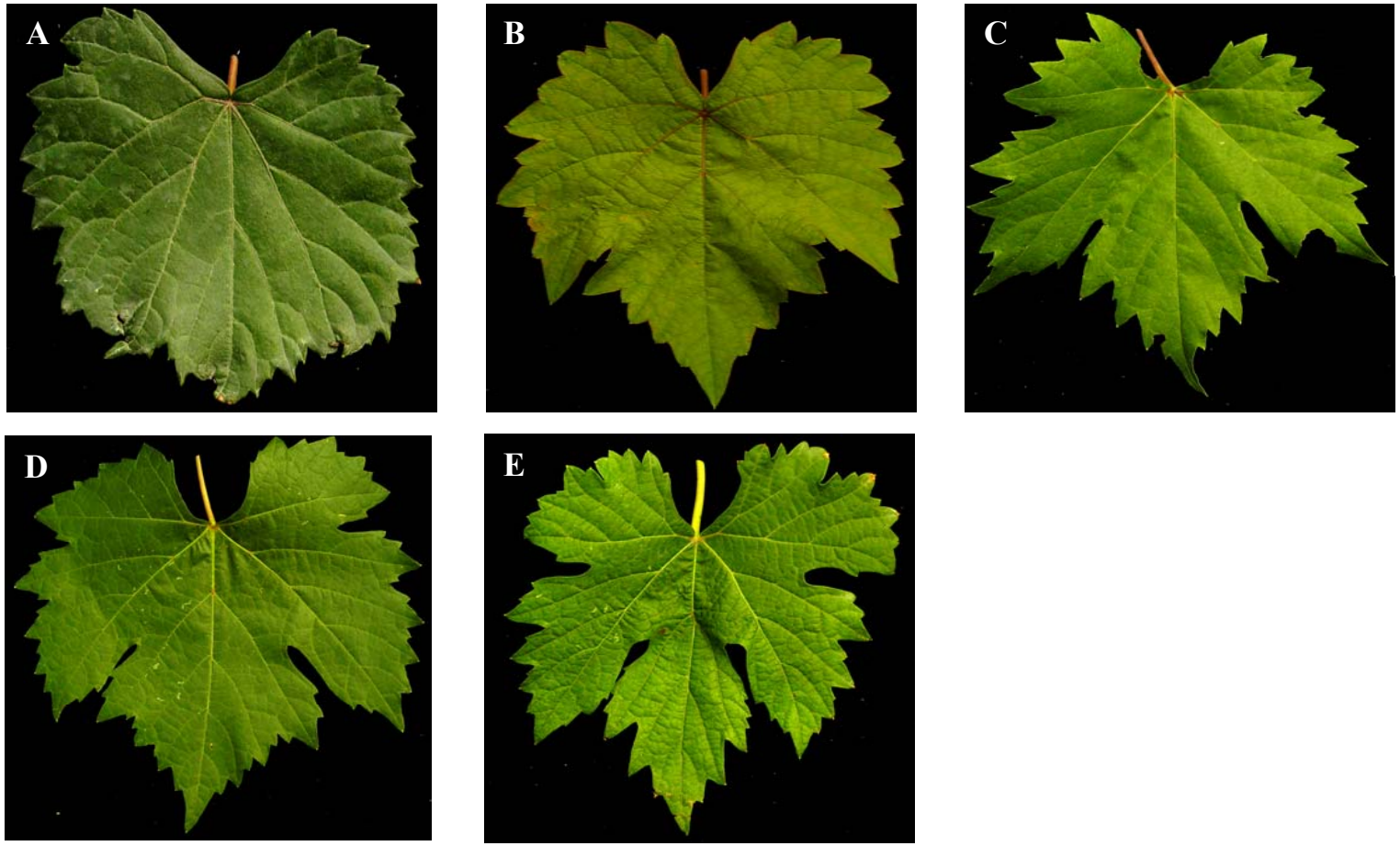


Figure 11. Mature leaf: number of lobes A) none; B) three; C) five; D) seven; E) more than seven.

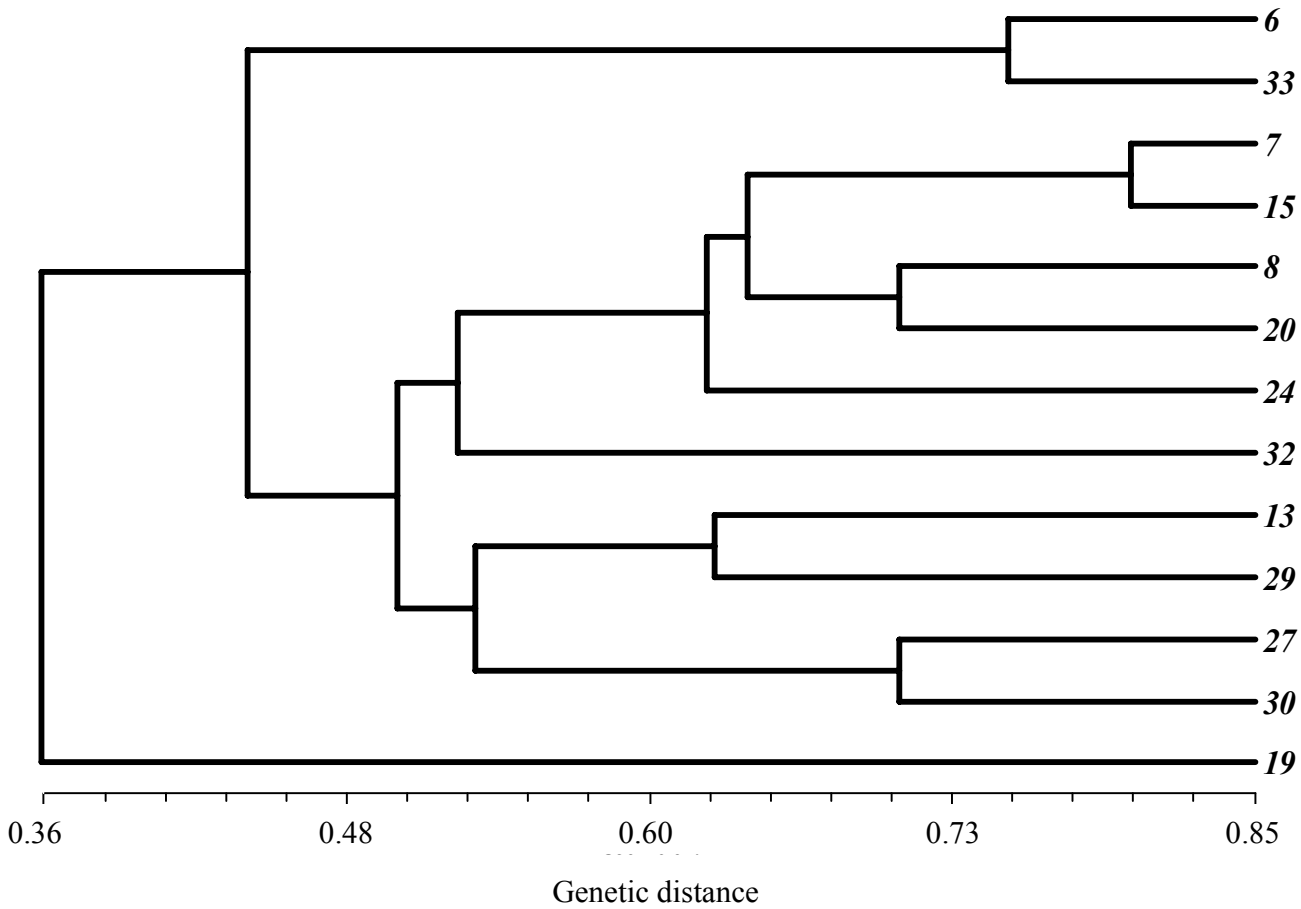


Figure 12. Dendogram of progenies from a cross ‘Black Queen x NY 88.0517.01’.

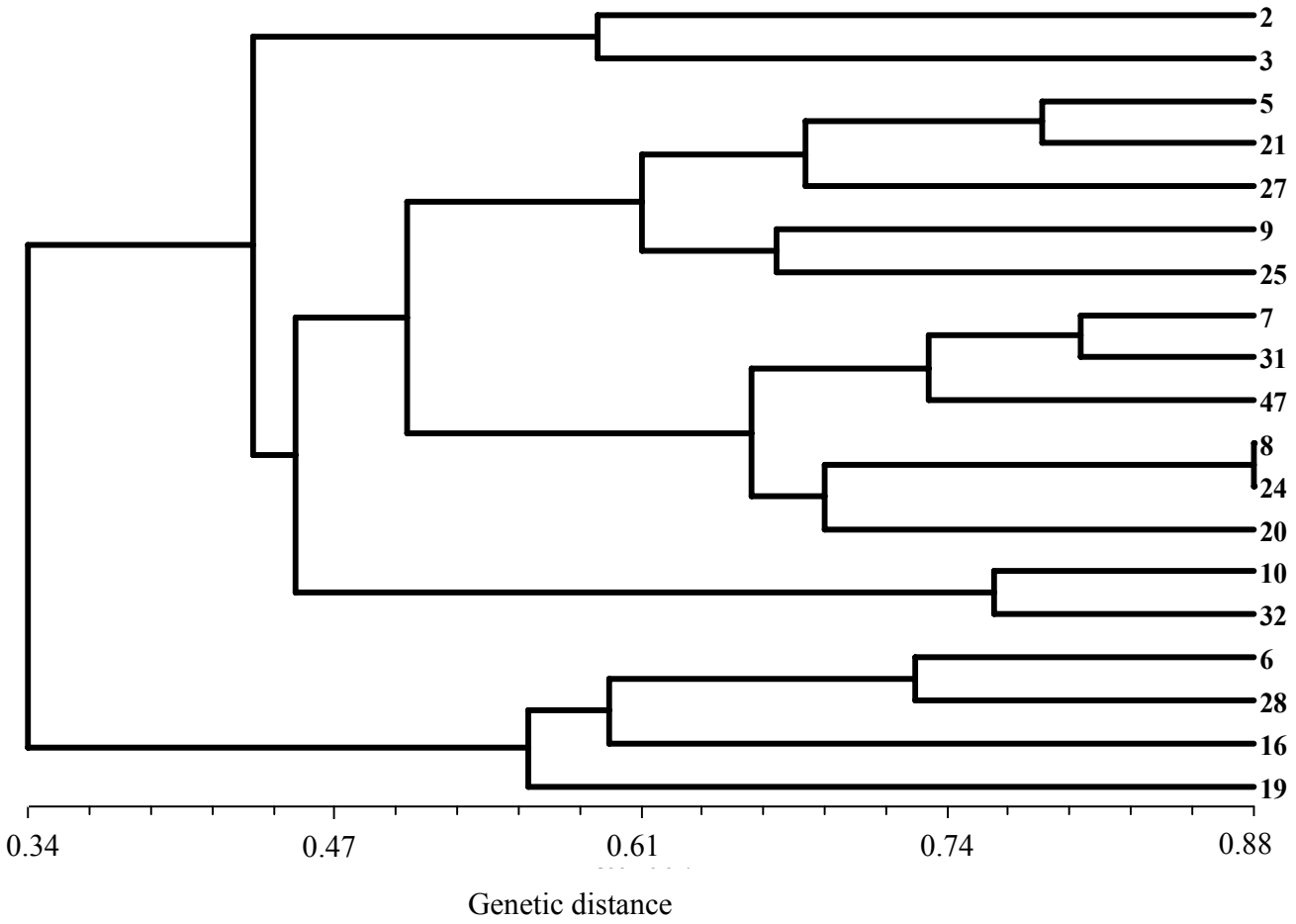


Figure 13. Dendogram of progenies from a cross ‘Black Queen x NY 65.0551.05’.

6.6 References

Dettweiler, E. (1994). Database for grape varieties and species. In **Proc. 6th Int. Symp. on Grape Breeding** (pp 21-23). Crimea, Ukraine.

Dettweiler, E. (2003). The European *Vitis* database: status quo. In **First Meeting of the ECP/GR Working Group on *Vitis*** (pp 2-3). Palic, Serbia and Montenegro.

Eibach, R. (1989). Investigations about the influence of some physiology and phenological characteristics on quality and their heredity. In **Proc 5th Int. Symp. on Grape Breeding** (pp 149-158). Pfalz, Germany.

I.P.G.R.I., U.P.O.V. and O.I.V. (1997). Descriptors for grapevine (*Vitis* spp.). International Union for the Protection of New Varieties of Plants, Geneva, Switzerland/Office International de la Vigne et du Vin, Paris, France/International Plant Genetic Resources Institute, Rome, Italy.

Miller, D.P., Howell, G.S. and Flore, J.A. (1996). Effect of shoot number on potted grapevines: I. canopy development and morphology. **Amer. J. Enol. Vitic.** 47: 244-250.

Rohf, F.G. (1993). NTSYS-pc. Numerical taxonomy and multivariate analysis: version 1.8. Applied Biostatistics. New York.

Sneath, P.H.A. and Sokal, R.R. (1973). **Numerical Taxonomy**. San Francisco: W.H. Freeman and Company.

Wagner, R. (1989). Study of phenotypic variation by analyzing data gathered together by O.I.V. on new varieties of table grape. In **Proc 5th Int. Sym. on Grape Breeding** (pp 178-186). Pfalz, Germany.

BIOGRAPHY

Siraprapa Mahanil was born on August 4, 1978 in Nan province. She received Bachelor degree of Science (Crop Production Technology) in 2000 from Crop Production Technology, Suranaree University of Technology. In 2001, she pursues Master degree and was granted the scholarship from the Center for Agricultural Biotechnology. Later in 2004, she was granted the scholarship from the Thailand Research Fund under the Royal Golden Jubilee Program to switch to Ph. D. degree in the School of Crop Production Technology, Suranaree University of Technology under the supervision of Prof. Dr. Paisan Laosuwan. At the RGJ-Ph. D. Congress VIII in 2007, she received Certification for outstanding research (oral) presentation. Her publications are:

1. Mahanil, S., Thipyapong, P., Reisch, B., Owens, C. and Laosuwan, P. (2007). Resistance Gene Analogs (RGAs) from *Vitis cinerea*, *V. rupestris* and *V.* hybrid 'Horizon'. **Amer. J. Enol. Vitic.** 58
2. Mahanil, S., Laosuwan, P. and Thipyapong, P. (submitted). Inheritance of downy mildew (*Plasmopara viticola*) resistance in table grape (*Vitis* spp.).
3. Mahanil, S., Attajarusit, J., Stout, M.J. and Thipyapong, P. (submitted). Overexpression of Tomato Polyphenol Oxidase Increases Resistance to Common Cutworm.