ANTISERUM PRODUCTION FOR XANTHOMONAS AXONOPODIS PV. CITRI DETECTION AND IDENTIFICATION OF CANKER RESISTANCE GENE ANALOGS IN THAI HYBRID LIME M33

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การผลิตแอนติเซรุ่มเพื่อตรวจสอบเชื้อ *Xanthomonas axonopodis* pv. *citri* และ การหายีนด้านทานโรคแคงเกอร์ในมะนาวไทยลูกผสม M33

นางสาวผ่องพรรณ ทรงวัฒนา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาเทคโนโลยีชีวภาพ มหาวิทยาลัยเทคโนโลยีสุรนารี ปีการศึกษา **2553** ผ่องพรรณ ทรงวัฒนา: การผลิตแอนติเซรุ่มเพื่อตรวจสอบเชื้อ Xanthomonas axonopodis
 pv. citri และ การหายืนด้านทานโรคแคงเกอร์ในมะนาวไทยลูกผสม M33 (ANTISERUM
 PRODUCTION FOR XANTHOMONAS AXONOPODIS PV. CITRI DETECTION AND
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 HYBRID LIME M33 อาจารย์ที่ปรึกษา: ผู้ช่วยศาสตราจารย์ คร.มารินา เกตุทัต-คาร์นส์,
 82 หน้า.

โรคแคงเกอร์เป็นโรคที่สำคัญของมะนาวในประเทศไทย การทดลองนี้มีวัตถุประสงค์เพื่อ ทดสอบความด้านทานโรคแคงเกอร์ในมะนาว 3 พันธุ์ ได้แก่มะนาวลูกผสม M33 มะนาวแป้น และ มะนาวน้ำหอมพบว่า มะนาวพันธุ์น้ำหอม และM33 มีความด้านทานมากกว่าพันธุ์แป้นเชื้อไอโซ เลตที่ก่อโรคทั้งหมดสามารถตรวจสอบได้ด้วยคู่ไพเมอร์จำเพาะXAC01 และ XAC02 ด้วยวิธี PCR ผลการวิเคราะห์ลำดับเบลของ 16S rDNA ของเชื้อก่อโรครุนแรง (ไอโซเลต BP104 และ BP210) พบว่ามีความใกล้เคียงกับลำดับเบลของเชื้อไอโซเลต BP210 ไปผลิตแอนติเซรุ่มในกระต่าย จาก การทดสอบประสิทธิภาพและความจำเพาะเจาะจงของแอนติเซรุ่มที่เจือจาง 1:4,000 หรือน้อยกว่า พบว่ามีความจำเพาะเจาะจงต่อการตรวจสอบเชื้อไอโซเลต BP210 ไปผลิตแอนติเซรุ่มในกระต่าย จาก การทดสอบประสิทธิภาพและความจำเพาะเจาะจงของแอนติเซรุ่มที่เจือจาง 1:4,000 หรือน้อยกว่า พบว่ามีความจำเพาะเจาะจงต่อการตรวจสอบเชื้อXAC (BP210) ที่มีชีวิตในระดับความเข้มข้น 10⁶ CFU/ml และเชื้อตายที่ความเข้มข้น10⁵ CFU/ml อย่างไรก็ตาม แอนติเซรุ่มที่ความเข้มข้น 1:2,000 เหมาะสมต่อการตรวจสอบเชื้อ XAC มากที่สุด โดยเกิด cross-reaction กับเชื้อ *X. campestris* pv. *Vesicatoria* เพียงชนิดเดียว จากการตรวจสอบเชื้อ XAC ที่ปลูกเชื้อบนใบมะนาวด้วยแอนติเซรุ่ม ความเข้มข้น 1:2,000 พบว่าสามารถตรวจสอบเชื้อ XAC ที่ปลูกเชื้อบนใบมะนาวด้วยแอนติเซรุ่ม เกามะสมต่อการตรวจสอบเชื้อ XAC มากที่สุด โดยเกิด cross-reaction กับเชื้อ *X. campestris* pv. *Vesicatoria* เพียงชนิดเดียว จากการตรวจสอบเชื้อ XAC ที่ปลูกเชื้อบนใบมะนาวด้วยแอนติเซรุ่ม ความเข้มข้น 1:2,000 พบว่าสามารถตรวจสอบเชื้อ XAC ที่ปลูกเชื้อบนใบมะนาวด้วยแอนติเซรุ่ม เหมาะสมต่อการตรวจสอบเชื้อ XAC บาใบก่อนกรรเสดงอุกเชื้อแล้ว ดังนั้นแอนณิชรุ่มที่ผลิตจึงไม่ เหมาะสมต่อการตรวจสอบเชื้อ XAC บนใบที่ลูกปลูกเชื้อแล้ว ดังนั้นแอนณิชรุ่มที่ผลิตจึงไม่

การพัฒนาเครื่องหมายโมเลกุลที่บ่งชี้ ลักษณะความด้านทานโรคในพืชนั้นมีความสำคัญต่อ การพัฒนาสายพันธุ์พืชให้มีความด้านทานในอนาคตได้ ในการทดลองนี้ จึงได้ทำการศึกษายืนที่ เกี่ยวข้องต่อความด้านทานโรคแคงเกอร์ในมะนาวลูกผสม M33 เปรียบเทียบกับมะนาวพันธุ์พ่อและ แม่ โดยเริ่มศึกษาในกลุ่มยืน Nucleotide binding site (NBS) Leucine-rich repeat (LRR) ด้วยเทคนิค PCR ร่วมกับการตัดด้วยเอมไซม์ตัดำเพาะ จากการประเมินระดับความด้านทานของM33 เทียบกับ มะนาวแป้น และมะนาวน้ำหอม โดยการปลูกเชื้อXAC บนใบ พบว่า M33 และมะนาวน้ำหอม มี ลักษณะการต้านทานต่อโรคโดยเกิด hypersensitive response ซึ่งให้ผลสอดคล้องกับผลของยีน *Pt9/Alu*1, *Pt14/Bfa*1 และ *16R1-19 Tru*1I ว่ามีความเกี่ยวข้องกับลักษณะการต้านทานโรคแคงเกอร์ ในมะนาว

สาขาวิชาเทคโนโลยีชีวภาพ ปีการศึกษา 2553 ถายมือชื่อนักศึกษา_____ ถายมือชื่ออาจารย์ที่ปรึกษา_____ PONGPAN SONGWATTANA : ANTISERUM PRODUCTION FOR XANTHOMONAS AXONOPODIS PV. CITRI DETECTION AND IDENTIFICATION OF CANKER RESISTANCE GENE ANALOGS IN THAI HYBRID LIME M33. THESIS ADVISOR : ASST. PROF. MARIENA KETUDAT-CAIRNS, Ph.D., 82 PP.

CANKER RESISTNCE/M33/HYBRID LIME/CITRUS CANKER/RESISTANCE GENE ANALOGS

Citrus canker is a serious disease of lime in Thailand. This study aims to evaluate the resistance characteristic of hybrid lime M33, Pan lime and Nam Hom lime. The results indicated that, Nam Hom and M33 showed higher resistance level than that of Pan lime. PCR amplification using *Xanthomonas axonopodis* pv. *citri* (XAC) specific primers (XAC01 and XAC02) could detect all pathogenic bacterial isolates. The 16S rDNA sequencing confirmed that the virulent bacteria (BP104 and BP210) were 99% identical with XAC (AE008923.1). Bacterial isolate BP210 was used for antiserum production by rabbit injection. The efficiency and sensitivity of antiserum from this study showed that, antiserum at the dilution of 1:4,000 or lower was able to detect XAC (BP210) bacteria at 10⁶ CFU/ml for live cells and 10⁵ CFU/ml for dead cells. However, the suitable dilution of this antiserum was 1:2,000 which could cross-react with only *X. campestris* pv. *vesicatoria* but not other *Xanthomonas* tested. The pathogen on infected leaves (10⁵, 10⁴ and 10³ CFU/ml) was able to detect the pathogen on infected leaves 4 days post-inoculation when symptom had already

appeared. Thus, this antiserum has low detection efficiency and cannot be used to detect low pathogen concentration or before canker lesion can be observed.

The development of molecular markers can help identify genes that linked to resistance characteristic on resistance plant. This methodology can be used to improve commercial favorable crop species to be disease resistant in the future. In this study, the citrus canker resistance (R) marker genes within M33 and its parents were screened using the Nucleotide binding site (NBS) Leucine-rich repeat (LRR) genes by PCR amplification in combination with restriction enzymes digestion. The resistant evaluation of hybrid lime and its parents was performed by inoculation with XAC on young leaves. The hypersensitive response phenotype on M33 and Nam Hom (resistant lime) confirmed that the marker Pt9/Alu1, Pt14/Bfa1 and 16R1-19/Tru1I were closely linked with the citrus canker resistance genes.

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LIST OF ABBREVIATIONS

ALP	=	alkaline phosphatase
Avr	=	avirulence
BP	=	Banpaew, Samut Sakhon
bp	=	base pair
°C	=	degree celcius
CFU	=	colony forming unit
DNA	=	deoxynucleotide
dNTP	=	deoxynucleotide 5' triphosphate
ELISA	=	enzyme-linked immunosorbent assay
ETI	=	effector-triggered immunity
et al.	=	Et alia (and other)
Fig.	=	figure
g	=	gram
h	=	hour
HR	=	hypersensitive response
К	=	Kokkruad, Nakhon Ratchasima
L	=	liter
LRR	=	leucine-rich repeat
LPS	=	lipopolysaccharides
mAbs	=	monoclonal antibodies
mg	=	milligram

LIST OF ABBREVIATIONS (Continued)

mg	=	milligram
min	=	minute
ml	=	milliliter
mM	=	millimolar
NBS	=	nucleotide-binding site
nm	=	nanometer
ng	=	nanogram
0.D.	=	optimal density
pAbs	=	polyclonal antibodies
PAMPs	=	plant-associated molecular patterns
PCR	=	Polymerase Chain Reaction
РЈ	=	Phichit
PR	=	pathogenesis-related genes
PTA-ELISA	=	plate-trapped antigen ELISA
PTI	=	PAMP-triggered immunity
RGAs	=	resistance gene analogs
rpm	=	revolution per minute
rRNA	=	ribosomal RNA
sec	=	second
SUT	=	Suranaree University of Technology, Nakhon Ratchasima
Та	=	annealing temperature
TIR	=	Toll and Interleukin-1 receptors

LIST OF ABBREVIATIONS (Continued)

U	=	unit
UV	=	ultraviolet
V	=	volume
v/v	=	volume per volume
w/v	=	weigth per volume
XAC	=	Xanthomonas axonopodis pv.citri
μg	=	microgram
μl	=	microlitre
μΜ	=	micromolar

CHAPTER I

INTRODUCTION

1.1 Background

Lime (*Citrus aurantifolia* Swingle) is a fruit tree in the family of Rutaceae, genus *Citrus*. It is an important economic crop in Thailand because it is often used to enhance the flavor of foods and beverages. Recently, lime has been planted in every regions of Thailand. The planted areas of lime in Thailand are about 116,000 rai dominating in the central plain (69,000 rai). The total yield of lime was around 163,000 tons with the value of 6,000 million baht (Office of agricultural economics, 2008).

Citrus canker is a serious disease in citrus plants and also in Thai lime. It is caused by *Xanthomonas axonopodis* pv.*citri* bacteria which is globally distributed. Moreover, this disease causes losses in yield and quality annually. It can infect leaves, twigs and fruit through stomata and wounds and induce callus-like lesion with water-soaked margins and often surrounded by a chlorotic halo. This bacterium can be dispersed by rain splash and wind to other area (Graham et al., 2004). Most commercial limes are susceptible host for citrus canker, including Pan lime.

Recently, the prevention and control of citrus canker are usually the use of copper-based spray programs or sometime complementary with antibiotics, especially in the young stages (Medina-Urrutia and Stapleton, 1986). However, copper

bactericides and antibiotics are disadvantages after long-term uses. Moreover, the accumulation of copper metal in soils causes phyto-toxic and environmental effects (McManus and Stockwell, 2001). This pathogenic bacterium can survive from one crop season to the next as latent infection in propagation organs or epiphytic populations on plant surfaces. These events often contain very low number of bacterial populations but they represent the primary source of inoculum for introduction of disease into subsequent crops. Therefore, the method for their detection is necessary.

Currently, many methods have been developed and are available in the laboratory. Polymerase chain reaction (PCR) method is a highly sensitive detection method (Cubero and graham, 2002; Cubero et al., 2001) but, laboratory equipment and specialized training are required. In contrast, immunodiagnostic is an easy method to use at the site where disease is suspected. They are not required neither special equipments nor training to perform the detection. The immunodiagnostic technique is based on the ability of an antibody to recognize and bind to a specific antigen, a substance associated with a plant pathogen. This method is known as enzyme-liked immunosorbent assay (ELISA). Therefore, this methodology can be used as diagnostic tool for pathogen detection in the field (Ward et al., 2004).

Resistance plant development is an alternative way that can be used to improve commercial favorable crop species to be resistant to disease. Resistant lime with commercially favor fruits has been successfully developed from Phichit research center (Thailand) by conventional breeding. This new resistant lime, named Phichit lime or M33 was crossed between canker susceptible Pan lime (female) and canker resistant Namhom lime (male) (Dangpium, 2003).

The development of molecular markers to identify the genes linked to the disease resistance characteristics would be helpful for further development of more resistant limes. This methodology can facilitate indirect selection of resistant plants and reduce the time required to identify resistant breeding line or segregating lines in the future. Generally, the activation of plant defense to pathogen attacks is often conferred by resistance (R) protein that controlled by *R* genes. These genes play important roles in improvement of crop species in the plant breeding programs. They also can lead to the understanding of the plant-pathogen interactions. However, the inheritance of citrus canker resistant traits are still unclear, the biochemical mechanism and the number of gene loci controlling this resistance have yet to be determined. An approach under consideration is cloning of *R* gene analogs (*RGAs*), from the resistant relative which includes cloning and screening diversity of *RGAs*, followed by functional tests of co-segregating analogs in transgenic plant and their expression (Hammond-Kosack and Kanyuka, 2007; Zimmerman et al., 2006; Manosalva et al., 2009).

Recently, many RGA sequences have been served as molecular makers for genetic mapping and molecular cloning of plant R genes for their tightly linkage or co-segregating with known disease resistant loci. R genes have been isolated and classified into several classes based on the structure of their predicted protein products. The largest R gene class in the plant genome is the nucleotide-binding site with leucine-rich repeats (NBS-LRR) class. Currently, a larger number of NBS-LRR genes have been cloned from different plant species such as citrus plant (Deng et al., 2000). They used PCR base method with degenerate primers designed from the conserved domains.

1.2 Objectives

This research aims to produce polyclonal antibody for citrus canker pathogen (*X. axonopodis* pv. *citri*) detection and also to identify molecular markers linked to the canker resistance gene(s) in Phichit lime (M33) and their parents using 12 specific primer pairs of citrus NBS-LRR class *RGAs* (Deng et al., 2000) in combination with restriction enzymes.

CHAPTER II

LITERATURE REVIEWS

2.1 The importance of canker disease

Lime is a small citrus fruit widely grown in the tropical and subtropical regions of the world especially, in Asia. Limes in Thailand have been planted in every regions dominating in the central plain. The main region for lime production in Thailand is Phetchaburi, Samut Sakhon, Ratchaburi and Phichit provinces (table 1.1).

 Table 2.1 Harvested areas and production statistics of lime in Thailand (Office of Agricultural Economics, 2008).

Regions	Harvested areas (Rai)	Production (Tons)
North	19,600	26,900
Northeast	870	260
Central	69,000	124,000
South	16,000	12,000
Total	106,000	163,000
Provinces	Harvested areas (Rai)	Production (Tons)
Prachinburi	31,000	71,000
Samut Sakhon	16,000	24,000
Ratchaburi	31,000	12,000
Phichit	7,000	11,000

The sour limes are a great source of vitamin C (29 mg/100 ml; USDA National nutrient database, n.d.) and citric acid contents (1.38 g/oz; Kristina et al., 2008). Thus, it can be used as a fresh fruit for garnish and for processing into a wide range of value-added product, such as beverage, sauces and some kind of traditional drug.

However, the large problem of lime planting area is citrus canker disease from *X. axonopodis* pv. *citri* bacteria. This disease has a long history in Florida. It was first discovered by P J. Wester around 1910 when the disease was introduce into North Florida and other Gulf states on *Poncitras trifoliate* citrus rootstock material from Japan. This disease was declared eradicated from Florida and the adjacent states in 1933 (Schubert and Sun, 2003, Dopson, 1964). Currently, citrus canker has been spread worldwide into warm, moist, citrus-growing coastal regions. This disease cause losses in yield and quality annually by defoliation, blemished fruit, premature fruit drop, die-back of twigs and general debilitation of the tree (Goto and Yaguchi, 1979).

2.2 The pathogen description

The genus *Xanthomonas* are gram-negative, rod-shaped and polarly-flagellated bacteria. The fagella allow the bacteria to move throughout an infected plant. *Xanthomonas* can infect a wide variety of species including pepper, tomato (*X. axonopodis* pv. *vesicatoria*), rice (*X. oryzae* pv. *oryzae*), citrus (*X. axonopodis* pv. *citri*), cotton (*X. axonopodis* pv. *malvacearum*), brassicas (*X. campestris* pv. *campestris*), bean (*X. axonopodis* pv. *phaseoli*) and soybeans (*X. axonopodis* pv. *glycines*). *Xanthomonas* infection is dependent on the type III protein secretion

system, which relies on transport protiens, secreting several hypersensitive reaction proteins and outer proteins, causing an interaction with the plant (Gürlebeck, et al., 2006; Bonas, and Kay, 2009). Typical symptoms of the disease include lesions on the leaves, fruit, and stems as well as twig dieback. *Xanthomonas* colonies are normally yellow due to their presence of a particular carotenoid pigment. Reproduction in genus *Xanthomonas* is similar to other bacteria, occurring through asexual binary fission in which each dividing clonal daughter cell receives an identical copy of the parental genome. These bacteria are a heterotrophic bacterium that obtain nutrients from the plants tissue it's infects. This bacterium thrives in subtropical regions of warm, high humidity and heavy rainfall (Civerolo, 1984).

The distinct types of citrus canker disease can cause by various pathovars and variants of the *X. axonopodis*. The separation of these froms from each other is based on host range and other phenotypic and genotypic characteristics of the bacterial strains. The most important strain in Asia canker disease is Canker A which caused by the Asian strain *X. axonopodis* pv. *citri* (Syn. *X. citri, X. campestris* pv. *citri*: Schaad et al., 2006). It is the most widespread and severe form of the disease. This strain caused disease most often referred as "citrus canker". The B and C types are caused by *X. axonopodis* pv. *aurantifolia*. Pathotype B strains are most severe on lemons (*C. limon* (L.) Burm f.), while grapefruit (*C.paradisi* Macf.) and sweet orange (*C. sinensis* (L.) Osb.) are affected little only in the groves. This pathotype has been found only in Argentina, Uruguay, and Paraguay. Pathotype C strains or Mexican lime canker affects only Mexican lime (*C. aurantifolia* (Christm.) Swingle) which was found in Brazil (Gottwald, 1993).

Lime canker disease in Thailand are caused by X. axonopodis py. citri-A* (Bui Thi Ngoc et al., 2007). This strain has also been discovered in Oman, Saudi Arabia, Iran and India. This Xanthomonas produces canker A-like lesions only on Mexican lime and appear to be distinct from the common A strains (Verniere et al., 1998). A similar strain has been isolated from Florida and designated as A^w (Sun et al., 2000). This strain has a restricted host rang that includes 'Key' lime and alemow (C. macrophylla Wester). Various cultivars of limes are found in Thailand. The most favorable commercial cultivars are Pan lime (C. aurantifolia) which has good quality of fragrant, thin skin and high amount of juice. This lime is in the group of west Indian or Mexican lime that is highly sensitive to canker disease. In contrast, citrus lemon group such as Tahiti lime (C. aurantiun), Nam Hom lime (C. reticulata) and Hnung kunturee lime (C. limon) are more resistance to canker but have lower quality and are unfavorable commercially (Graham et al., 1992, Graham, 2001). Thus, the controlling of disease is essential for prevention of yield devastation in Pan limes. Copper compound are standard control of the bacteria disease but dangerous to the farmers and environment. Disadvantage of copper treatment after long term used are accumulation of copper metal in soils and runoff water (McManus and Stckwell, 2001).

2.3 Symptoms and infection of citrus canker bacteria

The infection of citrus canker bacteria are mostly found on the above of ground leaf of citrus during the first half of the expansion phase growth. This

pathogen infective events often contain very low number of bacterial populations. But they represent the primary source of inoculum for introduction of disease into subsequent crops (Timmer et al., 1996). The *Xanthomonas* bacteria infection is like many other bacteria disease, infected through stomata of host plant tissues and wounds. The earliest symptoms on leaves appear as tinge, slightly raised blister-like lesions about 4-7 days after inoculation under optimum condition. The optimum condition are as followed present of water film and temperature between 20-30°C. Then, they turn light tan to brown when the lesion aged. The lesions are often surrounded by a chlorotic halo with spongy or corky on the centre of lesion (Fig 2.1A). Fruit and twigs lesions are similar, raised corky lesions surrounded by an oily or water-soaked margin (Fig 1B and C). The twig lesion do not contain chlorosis surround the lesion as occur on fruit lesions (Graham et al., 2004).



Figure 2.1 Canker lesion characteristic on lime leave (A), fruit (B), twig (C).

The canker bacterium has been spread through the transport of fruit, plants and equipment and also greatly dispersal by wind and rain (Myung et al. 2003). High wind speeds during rain causes water-soaking and facilitate entry of bacteria through stomatal openings into leaves. The canker bacterium remains alive in the margins of the lesions in leaves and fruit until they fall. The bacterial population has been founded to be about 10^5 to 10^6 CFU/ml in rainwater under infected tree (Stall et al., 1980). However, under symptomless fruit tree, the bacterium has also been discovered in the range of 10^2 to 10^3 CFU/ml (Canteros et al., 2004). The bacteria can also survive for up to several years in lesions of woody branches. When lesions surface is free of moisture, bacteria are released from an extracellular polysaccharide matrix and dispersed to new growth by rain splash and force of windblown rain droplets (Goto and Hyodo, 1985).

2.4 Prevention and control of citrus canker

2.4.1 Screening tests of citrus canker bacterial

The spread of citrus canker to new countries can be prevented by screening the plant material and fresh fruit. Because, the pathogen has been spread through the transport of fruit and plant material. Moreover, the equipments for harvesting also the main cause of diseases spread. As of January 2006, more than 15 million commercial trees in groves and nurseries had been destroyed in Florida for citrus canker eradication (Bronson and Gaskolla, 2006). Recently, the transport of live citrus plants in and out of Florida is prohibited. Every citrus plant trees must purchase a certified symptom free citrus plant through nurseries registered with the state (FDACS, 2006)

The screening tests are necessary to prevent the citrus canker outbreak form plant material and fruit transported. Many methods for screening canker pathogen are available for diagnostic test. PCR screening test with specific primers is one of the reliable methods for rapid analysis of suspect samples. Since, high sensitivity of 10^2 -

 10^3 CFU/ml can be detected (Cubero et al., 2001, Cubero and Graham, 2002, Leon, 2008) via specific of primers complementary with only *X. axonopodis* pv. *citri* gene. Example of primer sets available for diagnosis of *X. axonopodis* pv. *citri* are primers XAC01 and XAC02 which amplified *rpf* gene encoded pathogenicity effector protein (Coletla-Filho, 2006) and XACF and XACR which amplified the *hrpW* gene (*hypersensitive reaction and pathogenicity;* Park et al., 2006). Several other primers have been reported and reviewed in the diagnostic of *X. axonopodis* pv. *citri* by Graham et al. (2004) and OEPP/EPPO (2005). However, PCR method required special reagents, equipments and skill for generating reliable diagnosis.

Serological or immune diagnostics are technique that uses antibodies to detect the presence of pathogens. Antibodies are produced in laboratory animals, typically mice (monoclonal antibodies) or rabbits (polyclonal antibodies), in response to plant pathogens. Polyclonal antibodies (pAbs) are produced by injecting extracts from the pathogen into animal, usually a rabbit. The serum collected from blood contains antibodies. These antibodies can be used directly or after further purification. Monoclonal antibodies (mAbs) are made by fusing antibody-producing cells (lymphocytes) from the spleens of an inoculated animal (usually mice or rats) with cultured myeloma cells. This generates many hybridoma cell lines which produce a different single (monoclonal) antibody in cell culture medium. mAbs are more specific but also more complicated and slow to produce. Moreover, it is more expensive to both produce and maintain. And occasionally the antibody hybridoma cell dies or stop producing the required antibody. A widely-used assay for detect antibody or antigen presences is enzyme-liked immunosorbent assay (ELISA). The principle aim of this assay is to detect or quantify the binding of the diagnostic

antibody with the target antigen. It involves an enzyme-mediated color change reaction to determine antibody binding against target antigen. This is usually done in a microtitre plate where the antigen or antibody is immobilized on. After the antigen formed a complex with the antigen, the specific antibody is added. The antibody binding can be determined by enzyme-mediated color change reaction between enzyme-linked antibody (e.g. p-nitrophenyl phosphate) and its substrate added. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample (Ward et al., 2004).





Figure 2.2 Schematic diagram illustrating of plate-trapped antigen (PTA-ELISA), here shown using an direct detection (A) and indirect detection systems (B) to detect coating antigen on wells (modified from Ward et al., 2004).

The simplest format of ELISA assay is the plate-trapped antigen ELISA (PTA-ELISA, Fig 2.2). In this assay, the test samples or the target antigens are directly coated to the microtitre plate wells. This is followed by incubation with specific antibody which binds to target antigen. In some assay, the specific antibody is conjugated to the enzyme (direct detection). In other, the specific antibody is detected by secondary antibody (such as anti-rabbit or anti-mouse) which is conjugated to the enzyme (indirect detection). This technique has been used as a diagnostic tool in several fields such as, medicine and plant pathology (Kevin, 1999; Ward et al., 2004; Kumar et al., 2008). The sensitivity of the pathogen detection of this method is about 10^5 - 10^6 CFU/ml (Jin et al. 2001; Alvarez, 2004; Leon et al., 2008; Kokoskova and Mraz, 2008).

2.4.2 Chemical and antibiotic control

In the regions where *X. axonopodis* pv. *citri* is endemic, copper based bactericides has been used as standard control of citrus canker world-wide. Copper based spray programs are effective when targeted to the spring leaf flush to protect leaves from the one-half to full expansion stage over a period of 2-4 weeks. While, fruit is susceptible as they grow from 2.0 to 6.0 mm in diameter for a period of 90-120 days, depending on citrus species. Thus, copper treatments are usually repeated during the summer months for continues the fruit expanded. However, the effectiveness of copper spray programmed is reduced by rain and wind. Moreover, copper bactericides are not available after long-term use, including resistance to copper in *Xanthomonas* populations and the accumulation of copper metal in soils and water can be phytotoxic and environmentally harmful.

The antibiotics in the group of streptomycin can also be effective with canker bacteria. However the possibility of antibiotic resistance development in *Xanthomonas* populations can occur (McManus and Stckwell, 2001). Therefore, the prevention and control of citrus canker by chemical and antibiotic treatments needed to be monitor carefully in dose and time used. However, early sprays in the season of slow bacterial growth and alternating with the treatment with copper might increase the effectiveness of citrus canker control.

2.5 Hybrid lime for canker disease resistance

Most commercial limes in Thailand are highly susceptible to canker disease especially, lime in the group of west Indian or Mexican lime, such as Pan lime, Egglike lime, Hnung lime (*C. aurantifolia*). While, citrus lemon group such as Tahiti lime (*C. aurantiun*), Namhom lime (*C. reticulata*) and Hnung kunturee lime (*C. limon*) are more resistance to canker but, lower fruit quality and are unfavorable for consumer.

Conventional breeding program has been used by Dangpium et al. (2003) at Phichit research center to develop canker resistant lime. The hybrid lime (M33; Fig 2.3 C) was the product of crossed between Pan lime (female; Fig 2.3 A), a canker susceptible and Nam Hom lime (male; Fig 2.3 B), a canker resistant lime. F1 lime seeding were grown and selected for desirable characters including canker resistant efficiency, qualitative and quantitative of yield. The results of M33 evaluation showed high efficiency of canker resistance on leaves, twigs and fruits. The shape of fruit is round and flat with thin skin and high amount of juice and vitamin C.



Figure 2.3 Pan lime (A) and Nam Hom lime trees (B) planted at Suranaree University of Technology for field test; Phichit or M33 lime tree (C) planted at Phichit research center, Thailand.

2.6 Disease resistance (*R*) genes in plant

Plants have defense mechanisms to prevent themselves against pathogen infection. These mechanisms are controlled by R genes which are the specificity determinants of the plant immune response. The plant immune response system involves an allele specific genetic interaction between host R gene and pathogen avirulence (*Avr*) gene. This genetic interaction has been termed the gene-for-gene model (Flor, 1971). The model predicted that plant resistance will occur only when a plant dominant R gene complementary with pathogen Avr gene. Whereas, an

alteration or loss of the plant R gene (R changing to r) or of the pathogen Avr gene (Avr changing to avr) leads to successful pathogen and disease (Fig 2.4) (Bonas and Ackerveken, 1999).



Figure 2.4 The gene-for-gene model of plant immunity (adapted from http://pseudomonas-syringae.org/outreach/Module_4_Lab.htm).

The defense response of genetic interaction is characterized by rapid calcium and ion fluxes, an extracellular oxidative burst, transcriptional reprogramming within and around the infection sites. In most cases, this response will be localized programmed cell death which is termed hypersensitive response (HR) (McDowell and Woffenden, 2003). However, plants also have basal defense response in the absence of specific recognition. These basal defense responses are triggered by plant-associated molecular patterns (PAMPs) such as flagellin and lipopolysaccharides (LPS) from the pathogen. The basal defense response does not prohibit pathogen colonization but does limit the extent of its spread. Thus, R-protein from the R gene apparently accelerates and amplifies the innate basal defense response (Chisholm et al., 2006, Grennan, 2006). *R* genes have been isolated and classified to several classes based on the structure of their predicted protein product. The largest group of *R* genes carries leucine-rich repeats (LRRs) and nucleotide-binding site (NBS) domains. The NBS-LRR class of *R* genes can be further subdivided based on their ability to code for other recognizable domains. One subclass codes for a TIR domain (homology to the *Drosophila* Toll and mammalian Interleukin-1 receptors) at the N-terminus of the protein. NBS-LRR protein without TIR domain typically code for a coiled-coils structure near their N-terminus, sometimes in the form of a leucine zipper. The NSB sequence of this protein have conserved domains including consensus kinase 1a (P-loop), kinase 2, kinase 3a and GLPL motifs (Fig 2.5) while LRR domains play an important role in protein-protein interaction (McHale et al., 2006).



Figure 2.5 The composition of sub-domains in NBS-LRR protein class.

These common domains are the characteristic of various proteins with ATP/GTP binding activity (Moffett et al., 2002). Moreover, motifs of these domains are well conserved in several R genes, including *Arabidopsis RPS2* (Bent et al. 1994; Mindrinos et al. 1994), *RPP5* (Noel et al., 1999), tobacco N (Whithum et al. 1994) and Flax *L6* (Lawrence et al. 1995). The presence of these conserved domains have enabled rapid isolation of resistance gene analogs (*RGAs*) from different plant species using degenerate primers designed from these domains. Currently, NBS-LRR type *RGAs* have been cloned from different plant species by this approach. For examples, NBS-LRR classes of *RGAs* sequence from potato (Leister et al. 1996), soybean (Yu et

al. 1996), rice (Mago et al. 1999), wheat (Seah et al. 2000), cotton (He et al. 2004) sunflower (Radwan et al. 2003, 2004, 2008) and peanut (Radwan et al. 2010). NBS-LRR type *RGAs* in citrus plant have also been cloned (Deng et al., 2000). Cleaved amplified polymorphic sequence (CAPs) markers for resistance gene tagging and mapping has been developed. Some markers in the NBS-LRR class from Deng et al., 2000 were closely linked to citrus tristeza virus resistance (*Ctv*) gene and citrus nematode resistance (*Tyr1*) gene which are two important genes in *Poncitus* (Deng et al. 2000).

CHAPTER III

MATERIALS AND METHODS

3.1 Antibody production for citrus canker detection

3.1.1 Bacteria isolation

Bacteria were isolated from infected lime leaves tissue by commonly used methods (OEPP/EPPO, 2005). Briefly, the infected leaves were washed with sterile water and surface sterilize by soaked in 1% sodium hypochlorite for 3 min. Then, the lesions were rinsed in sterile water several times and excised with scalpel. Bacteria isolated from Samut Sakhon and Nakhon Ratchasima were initially selected by streaked the water-soak tissue from the lesion margins on sterile semi-selective media (KCD medium, nutrient agar (NA) supplemented with Kasugamycin (16 μ g/ml), Cephalexin (16 μ g/ml) and Daconil (Chlorothalonil) (12 μ g/ml) prior to enrichment on NA media without antibiotics. The bacteria isolated from Phichit were initially grew on NA medium and later on KCD medium. The bacteria were grown at 28-30°C for 24-48 h.

The bacterial colonies were collected for Gram staining and further used for pathogenicity tests. Other *Xanthomonas* strains included *X. axonopodis* pv. *vesicatoria*, *X. campestris* pv. *campestris*, *X. axonopodis* pv. *phaseoli*, *X. axonopodis* pv. *glycine*, *X. oryzae* pv. *oryzae* were obtained from Dr. Suvit Loprasert, Chulabhorn Research Institute (Thailand). They were used as negative control in strain identification and cross-reaction tests.
3.1.2 Specific primers identification and full length 16S rDNA sequencing

Single colony of *Xanthomonas* isolated and other *Xanthomonas* strains were resuspened in 1 ml DI-water and boiled for 10 min. Then, the suspension was used as template for PCR reaction, with specific XAC01 and XAC02 primers for X. axonopodis pv. citri identification (Coletta-Filho, 2006). The PCR reaction were performed with 50µl reaction mixture, containing 1µl of boiled cell suspension, 1X reaction buffer (Promega GoTaq), 1.5mM MgCl₂, 0.2mM dNTPs, 0.2µM primers (XAC01: CGC CAT CCC CAC CAC CAC CAC GAC, XAC02: AAC CGC TCA ATG CCA TCC ACT TCA), 1.25U Taq DNA polymerase (Promega). Unrelated bacterial DNA included Eschericia coli, Sinorrhizobium and Agrobacterium were also subjected to PCR reaction as negative control. PCR conditions were as followed; an initial cycle of 94°C for 5 min followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec, and 72°C for 45 sec, with a final step of 72°C for 5 min. The PCR products were observed under UV light after electrophoresis through 1.0 % (w/v) agarose gels and stained with ethidium bromide. The sensitivity of PCR amplification was performed with X. axonopodis pv. citri (BP210) in tenfold dilution series $(10^8 - 10^1 \text{ CFU/ml in})$ 0.85% NaCl).

The full length 16S rDNA of BP104 and BP210 were sequenced by Macrogen (Korea) (<u>http://dna.macrogen.com/eng/</u>). The sequence results were applied to BLAST program for gene comparison with data in the GenBank (NCBI).

3.1.3 Pathogenicity test

The phenotype evaluation of the resistance and susceptible limes were performed by leaf inoculation. Half of M33, Nam Hom and Pan limes leaves were wounded with a needle and celite, the other halves were not wounded (healthy) and then sprayed with 1 ml bacterial isolate suspension (10^8 CFU/ml) . The plants were then covered with plastic bags overnight and leave in natural condition for 2-3 weeks. The virulent canker disease apparent on each leaf was evaluated.

3.1.4 Serological tests

3.1.4.1 Bacterial antigen preparation and antiserum production

The bacterial pathogen was cultured on NB (nutrient broth) at 28-30°C for 48 h. The bacterial cells were collected by low-speed centrifugation (1,430 xg) and washed once in steriled 0.85% NaCl. The bacterial suspension was adjusted spectrophotometrically to A_{600} 0.2-0.25 (about 10^8 CFU/ml) and heated to 85°C for 15 min then used as antigen for antiserum production in two New Zealand white female rabbits. One ml of antigen mixed with Freund's complete adjuvant (1:1 v/v) was immunized to rabbit by subcutaneous injection on the first day. Then, 1 ml of antigen (mixed with Freund's incomplete adjuvant (1:1 v/v)) was injected subcutaneously 7 day later. After that, 1 ml of bacterial antigen without adjuvant was injected intravenous on day 14 and day 21. One week after last injection, the blood was drawn from the central ear artery and the serum was use as antiserum. This antiserum was used for indirect ELISA assay to consider the antibody titer, the sensitivity and the cross reaction of antiserum. The pre-immune serum was bleed before immunization to use as negative control in ELISA assay.

3.1.4.2 Indirect ELISA assay

The blood was kept overnight at 4°C and the serum was collected after centrifugation at low speed for 15 min. This antiserum was aliquoted to several tubes and stored at -20°C for future use. The bacterial concentrations were adjusted from $10^3 - 10^8$ CFU/ml in 0.05 M carbonate coating buffer (1.59 g/L Na₂CO₃, 2.93 g/L NaHCO₃, 0.2 g/L NaN₃; pH 9.6). Half volume of each diluted bacterial suspension were treated at 85°C for 15 min to kill the cells and used as dead cells antigen. One hundred µl of each diluted bacterial suspensions (both live and dead cells antigen) were coated in microtiter plate well at 4°C overnight. The plate was washed three times with PBS-0.05% Tween20 (PBST) and 200 µl of 2% skim milk were added and incubated for 1 h at 37°C. Then, the plates were washed 3 times as above and 100 μ l of diluted antiserum were added to the wells. The ELISA plates were incubated at 37°C for 1 h. After that, three times washing were performed and 100 µl of dilution Anti-rabbit IgG Alkaline Phosphatase (ALP) conjugate (Sigma) (1:10,000 v/v) were added in each well and incubated for 1 h at 37°C. The wells were repeatedly washed and 100 µl of p-Nitrophenyl-phosphate (PNPP) substrate (Thermo Scientific) were added in each well. The reaction were stopped by adding equal volume of 0.75 M NaOH and the O.D. at 405 nm were measured after 15-30 min incubation in the dark at room temperature. The live and dead cells of E. coli (10^6) CFU/ml) and pre-immune serum were used as negative control.

3.1.4.3 Antibody titer and sensitivity tests

The antiserum was diluted to difference dilution (1:1,000, 1:2,000, 1:4,000, 1:8,000 and 1:1,600 v/v) and tested with both live and dead cells of bacterial antigen $(10^4-10^8 \text{ CFU/ml})$ by ELISA assay to test the titer of the antibody. For sensitivity testing, the 1:2,000 (v/v) diluted antiserum was used to test both live and dead cells of bacterial antigen $(10^3-10^8 \text{ CFU/ml})$ by indirect ELISA assay as described above. All experiments were done in duplicated and repeated at least 3 times.

3.1.4.4 Cross-reaction test

The diluted antiserum 1:2,000 (v/v) was used for *X. axonopodis* pv. *citri* (10⁶ CFU/ml) and other *Xanthomonas* species (10⁶ CFU/ml) detection (as mention in 3.1.1) by indirect ELISA assay as described above. The percent of crossreaction was calculated by;{[A₄₀₅ of individual other *Xanthomonas* species - A₄₀₅ of negative control] / [A₄₀₅ of positive control - A₄₀₅ negative control]} x 100.

3.1.5 Detached leaf assay

Full expanded young leaves of lime were washed in running tap water and surface sterilized in 1% sodium hypochlorite for 1-4 min. The leaves were aseptically rinsed thoroughly with sterile distilled water. Each leaf was divided into 4 parts for treatment separation, included 10^5 , 10^6 and 10^7 CFU/ml infected parts and one uninfected part. Each part of the leaf was wounded by puncturing with a small needle, through the lower surface (5 needle punctured wounds per part). Each wounded leaf was placed on 1% water agar in a Petri dish, with the back of the leaf up. Twenty µl of the different diluted bacterial suspension (10^5 - 10^7 CFU/ml *X. axonopodis* pv. *citri*) were dropped on wounds. The part of uninfected leaf, 20 µl of sterile 0.85% NaCl was dropped to used as negative control. The infected leaves were maintained in a lighted incubator at 25- 30° C.

The canker pathogen on each part of infected leaf was detected by ELISA method. Each part of the infected leaf was ground in 150 μ l coating buffer. One hundred μ l of leaf extract was coated in microtiter plate well and incubated at 4°C for overnight before detection with 1:2000 (v/v) diluted antiserum. The steps were done as mention in the ELISA protocol described in 3.1.4.2. One μ l of the leaf extract was

also performed in XAC specific PCR amplification as described in 3.1.2. The detection results were demonstrated as percentage (% detection), calculated by; [number of sample that can detect by XAC primers/total samples were tested] x 100.

3.1.6 Canker detection from field samples

Symptom and non-symptom plant materials (leaf and twig) from Pan, Nam Hom and M33 limes were taken from the field and washed in 25 ml 0.85% NaCl shaking for 30 min. Then, the plant material was removed, and the washing solution was boiled for 10 min. One µl of the boiled washing solution were used as template for specific primers (XAC01 and XAC02) amplification. The PCR condition was performed as described in 3.1.2. *Xanthomonas* BP210 isolate was used as positive control.

3.2 Canker resistance gene analogs in Thai hybrid lime M33

3.2.1 Bacterial isolation

The bacteria were isolated using the same method as in 3.1.1

3.2.2 Canker pathogen inoculation

The phenotype evaluation of the resistance and susceptible limes were performed by leaf inoculation as described in 3.1.3.

3.2.3 Plant samples

M33 limes (highly resistant to canker) were from Phichit research center, Samut Sakorn and Prachinburi (Thailand), Nam Hom limes (highly resistant to canker) were from Phichit research center and Pan limes (susceptible to canker) were from Samut Sakorn. These lime plants were planted in the test field at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

3.2.4 DNA isolation

Genomic DNAs were isolated from young leaflets using the modified 2.0% (w/v) cetyltrimethyl ammonium bromide (CTAB) protocol (Chen et al., 2003). Breifly, 2 gram of grounded leaves were add to 720 μ l of warm (65°C) CTAB extraction buffer (2% (w/v) CTAB, 1.4 M NaCI, 20 mM, EDTA, 100 mM Tris-Cl; pH 8.0) and incubated at 60°C for 30 min with occasional gentle swirling. Then, 1 V of phenol: chloroform: isoamylalcohol (25:24:1) were added to the samples and rock gently to mix for 5-10 min. The top aqueous layer was transferred to a new tube after centrifugation at 16,000 xg for 10 min. Then, the genomic DNA was precipitated by added 1/10 V of 3M sodium acetate followed by 1 V of cool isoamyl alcohol and mix gently. DNA pellet was collected after centrifugation and washed with 300 μ l of 70% ethanol. Then, DNA pellet was dried and resuspended in 50 μ l TE buffer (100 mM Tris-Cl and 1 mM EDTA; pH 8.0) and stored at -20°C. The quality and concentration of DNA were checked by agarose gel electrophorosis and nanodrop-spectrophotometer, respectively.

3.2.5 PCR amplification combination with restriction enzymes

Resistance gene candidates of Pan, Nam Hom and M33 limes were screened by PCR amplification using 12 specific primers (table 3.1). The PCR amplifications were performed in 25 μ l reaction volume; containing 1X reaction buffer (Promega GoTaq), 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M primers, 1.25U of *Taq* polymerase (Promega) and 100~150 ng genomic DNA. The initial denaturation was done at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at 47-67°C (depended on primers; described in table 3.1) and 30 sec at 72°C. The final step was performed at 72°C for 5 min for the last extension. One μ l each of PCR products were digested in 10 μ l reaction volume with 1U of *EcoRI*, *Tru1I*, *Bfa1*, *HinfI*, *AluI* or *TaqI*, separately. The digested incubation time and condition were described in table 3.2. The PCR products and their digested products were separated on 2% agarose gels, visualized by staining with ethidium bromide and UV illumination.

3.2.6 DNA sequencing and analysis

The PCR products of resistance gene analogs of *Pt9*, *Pt14* and *16R1-19* from each plant were cloned into Promega pGEM T @ -Easy Vector and transformed to DH5 α *E. coli* cells. The DNA sequencing was performed by Macrogen (Korea) (http://dna.macrogen.com/eng/) using SP6 and T7 primers. Resulting sequences were applied to BLAST program (blast.ncbi.nlm.nih.gov) for gene comparison with data in GenBank (NCBI). The alignment and phylogenetic tree were created by DNA analysis program (CLC Main Workbench 5).

RGAs primers ^a	Forward primers ^b	Reverse primers ^b	Ta (°C)
Pt6	GACTTGATCTCTCATGAATTTGAA	CACCGACATACCGTAGAACACC	59
Pt14	GACAATATCTCTTATCAGTTTGATG	GAGCTCAAAATAACCATCTGTAG	47
11P31	TGCTCGGGAGGTCTACAATGACAG	CCTCCGCACTTTCCATCAATCTT	63
Pt3	CCACAACAATGATGTCAAGAATAA	GTCCCTTTTCAGCCTTAGAGTTAC	59
Pt7	TTACGGCGACAAAGATGTCAG	TCCCAACTTCTCCAATCCTTTATTA	49
Pt18	TAAGCATAAATTCGATTGTTGTG	TCCAACTATTATCTGGCCTTAGAA	55
Pt8	ATTCGCGGAAAGATGATTTTGA	ACACTCTTTCGTCACGGTTTCAG	55
Pt9	AGCTTCTTGGTGCACCAAATGGTT	CCCTTTAGCTACACTTCTGGCTAGTTCA	65
18P33	AAGTCAACAACAACTTCCGCTATCA	GGTTTCGGCTAGCTCTGGAATACT	67
Pt19	AGGAAATTCAGAAGCAAGCAAAAG	ATCCGTCAGCCACCTCTCTT	67
16R1-19	TCTGCAGTGAGGAGCATGATTTTGAT	ATGACACTGACGTGCCACATGCT	67
11P33	GCAAGCTGCAGGTTGTGGTGTTTA	AGGCCGACCTGGTTGAGTTTG	63

Table 3.1 Primers name, sequence of forward and reverse primers and annealing temperature (Ta) used for canker RGAs screening.

^a: *RGAs* primers sequence derived from Deng et al. (2000); ^b: primer sequences were arranged from 5'- to 3'-end.

Restriction enzyme	Restriction site	Optimal temperature	Incubation time	Company
EcoRI	G↓AATTC	37°C	Overnight	Fermentas Ltd.
Tru1I	T↓TAA	65°C	Overnight	Fermentas Ltd.
FspBI (BfaI)	C↓TAG	37°C	Overnight	Fermentas Ltd.
HinfI	G↓ANTC	37°C	Overnight	Fermentas Ltd.
AluI	AG↓CT	37°C	Overnight	Sib Enzyme Ltd.
TaqI	T↓CGA	65°C	Overnight	Sib Enzyme Ltd.

Table 3.2 Restriction enzymes used in the experiment.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Antibody production for citrus canker (X. axonopodis pv. citri) detection

4.1.1 Bacteria isolation

Nineteen bacterial isolates from three different regions in Thailand; Samut Sakhon, Nakhon Ratchasima and Phichit were isolated. The bacteria isolated from Samut Sakhon and Nakhon Ratchasima were initially selected on KCD semi-selective medium. The bacteria isolated from Phichit were initially grew on NA medium and later on KCD medium.

The results showed that, 3 isolates from Phichit (PJ02, PJ04 and PJ05) did not grow on KCD medium. The colony morphology of bacteria isolated from Samut Sakhon on NA medium were circular in shape, convex, mucoid and shiny with yellow color (Fig. 4.1A). After long incubation period, barely visible small white colony appeared spontaneously which is a general colony characteristic of *Xanthomonas* bacteria similar to the report of Dai et al., 1991. The colonies on KCD medium were smaller and have less color (Fig. 4.1B), especially colony of PJ01 and PJ03 from Phichit were white on KCD medium. Bacteria isolated from Nakhon Ratchasima (SUT02, SUT06, K01 and K02) showed orange-yellow colony on both NA and KCD media but the colonies size on KCD medium were smaller than on the NA medium (Figs. 4.1C and D).



Figure 4.1 Colony morphology of bacteria isolated from lime leaves. A: yellow colony of *Xanthomanas* (BP102) on NA media, B: light-yellow colony of *Xanthomonas* (BP102) on KCD medium, C: orange-yellow colony of bacteria (SUT02) on NA medium, D: yellow colony of bacteria (SUT02) on KCD medium.



Figure 4.2 Gram staining of *Xanthomonas* bacteria (BP210).

The cell morphology of the bacteria were also demonstrated by Gram staining and observed under microscope. The Gram stain indicated that, all isolates were Gram-negative rod (Fig. 4.2) bacteria.

4.1.2 Specific primers identification and full length 16S rDNA sequencing

The isolated bacteria were identified by XAC specific primers (Coletta-

Filho, 2006). The results indicated that, all isolated from Samut Sakhon and 2 isolated from Phichit contain 581 bp Xac *rpf* gene (Fig. 4.3 lanes 2-10, 16 and 17). But this gene was not detected in samples from Nakhon Ratchasima, other Xanthomonas species and unrelated bacteria, such as *E. coli, Sinorhizobium* and *Agrobacterium* that were used as negative control (Figs. 4.3 and 4.4). Non-target sizes DNA bands were detected in isolated from Nakhon Ratchasima and some from Phichit. Two pathogenic *Xanthomonas* strains isolated from Samut Sakhon (BP104 and BP210) were identified by 16S rDNA sequencing. The 16S rDNA sequence confirmed that both strains were *X. axonopodis* pv. *citri* (Appendix A Figs. 2a and b).



Figure 4.3 Specific amplification of XAC target (581 bp fragment) by XAC01 and XAC02 specific primers. Lane 1 and 25: 100 bp marker; Lane 2-11: bacterial isolated from Samut Sakhon; Lane 12-15: Nakhon Ratchasima; Lane 16-20: Phichit; Lane 21-23 unrelated bacteria consist of *E. coli, Sinorhizobium* and *Agrobacterium*, respectively. Lane 24: negative control.

4.1.3 Pathogenicity tests

All bacterial isolates (19) from different regions were inoculated to leaves of

Pan, Nam Hom and M33 limes for canker resistance evaluation. The results showed that all isolates from Samut Sakhon with yellow colonies on NA medium can infect wounded lime leaves. However, isolates from Nakhon Ratchasima (SUT02, SUT06, K01, K02) with orange yellow colonies cannot infect neither wound or unwounded leaves (Appendix A table 1). The 581 bp XAC specific PCR products were also not detected in these samples (Fig. 4.3 lanes 12-15).



Figure 4.4 Specific amplification of XAC target by PCR. Lane 1 and 9: 100 bp marker; lance 2: X. axonopodis pv. citri (BP210); Lane 3: X. axonopodis pv. vesicatoria; Lane 4: X. axonopodis pv. phaseoli; Lane 5: X. campestris pv. campestris; Lane 6: X. axonopodis pv. glycine; Lane 7: X. oryae pv. oryzae, respectively. Lane 8: negative control.

This indicated that they might not be *Xanthomonas*, even if they can grow on KCD medium. Some isolates from Phichit (PJ02, PJ04 and PJ05) also have similar phenotype and the XAC specific PCR product were not detected as the isolates from Nakhon Ratchasima. However, isolate PJ01 and PJ03 should be *Xanthomonas* species since XAC specific genes product were seen (Fig. 4.3 lanes 16 and 18) and similar colonies morphology as the Samut Sakhon isolates were observed. Pathogenic bacteria isolates can infect wounded and unwounded lime leaves. The earliest symptoms on Pan lime leaves were characterized by watersoaked slightly raised blister-like lesions surrounded by chlorotic halo. Subsequently, the spongy or cocky on the centre of lesions appeared and turned brown 3 weeks postinfection. Whereas, the lesion on Namhom and M33 lime leaves were smaller and turned brown within 2 weeks post-infection (Fig. 4.5). This is the characteristic of hypersensitive response of resistant lime.



Figure 4.5 Canker lesion on upper and under leaf of Pan (A and B), Nam Hom (C and D) and M33 limes (E and F) by bacterial isolate BP109 inoculation.

The lesion caused by isolated BP104 and BP210 on both susceptible (Pan) and resistance limes (Nam Hom and M33) did not turn brown after 3 week post-infection (Fig. 4.6). Thus, isolated BP104 and BP210 could be virulence pathogen for citrus plant. These results demonstrated that *Xanthomonas* BP104 and BP210 isolate is most virulent bacteria that are pathogenic to the resistant lime. In this experiment, *Xanthomonas* BP210 isolate was used as bacterial antigen for further antibody production.



Figure 4.6 Canker lesion on upper and under leaf of Pan (A and B), Nam Hom (C and D) and M33 limes (E and F) by bacterial isolate BP210 inoculation.

4.1.4 Antiserum titer test

Antiserum were produced for *X. axonopodis* pv. *citri* (XAC) detection by rabbit injection. The XAC isolated from Banpaew, Samut Sakhon were used as antigen to produce antiserum in duplicated (pAb1 and pAb2). Titer of antiserum were tested using indirect ELISA assay. The results showed that, 1:16,000 or lower dilution of both antiserum (pAb1 and pAb2) were able to detected dead cells of XAC at 10⁵ CFU/ml or more. Whereas, 1:4,000 diluted antiserum were reactive against live cells of 10⁶ CFU/ml and dead cells at 10⁵ CFU/ml or more. The highly concentrate antiserum (1:2,000 and 1:1,000 dilution) strongly reacted against both live and dead cells (10⁵ CFU/ml) of XAC. However, pAb1 showed stronger reaction than pAb2 (Figs. 4.7 and 4.8 or Appendix A table 2). These results indicated that, at least 1:2,000 dilution of these pAbs were able to detected both live and dead cell of XAC at 10⁵ CFU/ml. Nevertheless, dead cells of bacteria showed stronger reaction with pAbs than live cells. Since these pAbs were produced from injection with heat killing cells might have broken some cells.



Figure 4.7 ELISA for pAb1 titer test. Different pAb1 dilutions (1:16,000, 1:8,000, 1:4,000, 1:2,000 and 1:1,000) were used to detect live and dead cells of *X*. *axonopodis* pv. *citri* suspension at different concentrations (10^4 , 10^5 and 10^6 CFU/ml). A ₄₀₅ data was indicated in Appendix A table 2.



Figure 4.8 ELISA for pAb2 titer test. Different pAb2 dilutions (1:16,000, 1:8,000, 1:4,000, 1:2,000 and 1:1,000) were used to detect live and dead cells of *X*. *axonopodis* pv. *citri* suspension at different concentrations (10^4 , 10^5 and 10^6 CFU/ml). A ₄₀₅ data was indicated in Appendix A table 2.

4.1.5 Sensitivity of X. axonopodis pv. citri detection

pAb1 and pAb2 at the dilution of 1:2,000 were tested for the sensitivity with live and dead cells of *X. axonopodis* pv. *citri* at different concentrations (10^3-10^8)

CFU/ml). The positive result is the number which is at least double the reaction intensity of negative control (E. coli and pre-immune serum reaction).

Table 4.1 Sensitivity of ELISA in detecting live and dead cells of X. axonopodis pv.

Antiserum	Cell	X. axonopodis pv. citri antigen (CFU/ml)						
	status	10 ⁸	10 ⁷	10 ⁶	10⁵	10 ⁴	10 ³	<i>E. coli</i> (10 ⁶ CFU/ml)
pAb1 (1:2,000)	Live cell	++ ^a	++	++				
	Dead cell	++	++	++	++			
pAb2 (1:2,000)	Live cell	++	++	++				
	Dead cell	++	++	++	++			

citri.

^a: The result was taken as positive (+) when A ₄₀₅ was twice greater than that of the negative (-). The A $_{405}$ data was indicated in Appendix A table 3.

The bacterial suspension of live cells at 10^6 CFU/ml and dead cells at 10^5 CFU/ml showed positive reaction in ELISA (table 4.1). The reaction intensities of serological reaction increased with the increased bacterial densities in both pAbs tested. These results indicated that, both pAb1 and pAb2 have the sensitivity of detection at 10^6 CFU/ml for live cell and 10^5 CFU/ml for dead cells. The sensitivity of ELISA for bacterial pathogen has been reported to be at the level of 10^{5} - 10^{6} CFU/ml (Jin et al. 2001; Alvarez, 2004; Leon et al., 2008; Kokoskova and Mraz, 2008). The detection efficiency of ELISA is limited by the level of the pathogen population and dependent upon the immunological properties of antiserum used. Anyhow, the pAbs produced in this study have the sensitivity as high as pAb produced in other reports.

4.1.6 Cross-reaction of antiserum to other *Xanthomonas sp.*

Antiserum dilutions (1:1,000 and 1:2,000) were tested for cross-reacted against live cells of other five *Xanthomonas sp.* and *E. coli* at 10^6 CFU/ml. The results showed that, dilution of 1:1,000 antiserum (both pAb1 and pAb2) strongly cross-reacted against *X. axonopodis* pv. *vesicatoria* and weakly reacted against *X. axonopodis* pv. *vesicatoria* and weakly reacted against *X. axonopodis* pv. *phaseoli* and *X. campestris* pv. *campestris*. While, more diluted antiserum (1:2,000) were cross-reacted with only *X. axonopodis* pv. *vesicatoria* but not with others (table 4.2). These results suggested that, antiserum diluted to 1:2,000 is the suitable condition for detection of target bacteria of at least 10^6 CFU/ml. Although, these antiserum showed cross-reaction with *X. axonopodis* pv. *vesicatoria* but this bacteria does not infect citrus plant. They only infect tomato and *Capsicum* pathogen (Kim et al., 2010).

Therefore, the cross-reaction detection should not be alarm. Bacterial cell surfaces display а varietv of antigenic molecules. including protein. lipopolysaccharides and extracellular polysaccharides. Thus, polyclonal antibodies (or antisera) from bacterial species induces are mixtures of antibodies with multiple specificities. Moreover, these antibodies are obtained from different B cell resources. Although the dominant antibodies showed acceptable specificity for a given pathogen but cross-reactions with unrelated species can be detected. On the other hand, monoclonal antibodies (mAbs) can be more specific than pAbs because it provides a single epitope to recognized target epitope which performed from individual hybrid cell line (hybridoma cell). But mAbs are time-consuming to produce. The entire process of producing mAbs takes 3-4 months for each fusion experiment. Moreover, monoclonals against conformational epitopes on native proteins may lose reactivity with antigens. Due to these limitations, mAbs might miss the important crossreactive determinants.

 Table 4.2 Cross-reaction of ELISA to other Xanthomonas species and unrelated microorganism.

	Diluted antiserum						
Bacterial strain tested	pA	.b1	pAb2				
(10 ⁶ CFU/ml)	(1:1,000)	(1:2,000)	(1:1,000)	(1:2,000)			
	(%)	(%)	(%)	(%)			
<i>X. axonopodis</i> pv. <i>citri</i> (positive control)	100 ^a	100	100	100			
X. axonopodis pv. vesicatoria	100	91	80	85			
X. axonopodis pv. phaseoli	33	0	39	0			
X. campestris pv. campestris	47	0	27	0			
X. axonopodis pv. glycine	0	0	0	0			
X. oryzae pv. oryzae	0	0	0	0			
E. coli (negative control)	0	0	0	0			

^a: The percent of reaction was calculated from formula in 3.1.4.4.

However, mAbs also used ELISA assay as detection procedures to determine. Many studies reported that, the ELISA sensitivity 10^5 - 10^6 CFU/ml is sufficient for identification of bacteria pathogens from symptomatic plants and colonies on selective media (Jin et al. 2001; Alvarez, 2004; Leon et al., 2008; Kokoskova and Mraz, 2008). However, boiling the bacteria samples cloud improved the sensitivity of detection (Figs. 4.7 and 4.8). Jones et al. (1997) have reported that, the sensitivity of ELISA can be increased tenfold by using an extraction buffer

containg EDTA (ethylenediaminetetraacetic acid) and lysozyme. This buffer extracted lipopolysaccharide (LPS) into solution, thereby enhancing the antibody– antigen reaction without increasing background readings.

4.1.7 Detach leaf assay

Different concentrations of canker bacteria $(10^7, 10^6 \text{ and } 10^5 \text{ CFU/ml})$ were inoculated to Pan lime leaves using detach leaf assay. The pathogen on the infected leaves were detected by ELISA method every 2 days after inoculation. The results showed that, infected leaves detection on first day of inoculation (day zero) showed negative reactions in ELISA whether the bacterial cells were killed by heat treatment or not (table 4.3). The inoculation of this experiment used 20 μ l bacterial suspensions dropped on leaves. Thus, bacteria population on leaves has lower 100 fold than bacterial suspension used, 10⁷, 10⁶ and 10⁵ CFU/ml inoculation indicated 10⁵, 10⁴ and 10³ CFU on the inoculated leaves. Moreover, the sensitivity of ELISA (10⁵ CFU/ml or 10^4 CFU/well for dead cells) should be sufficient for dead cells detection but protein molecules from crushing leaf may influenced to bacteria binding to the plate which caused low detection of target bacteria. Infected leaves on day two showed positive reaction with infected leaves that was inoculated with 10⁵ CFU of bacteria. Consequently, infected leaves after 4 days inoculation showed positive reaction in all bacteria concentration $(10^5, 10^4 \text{ and } 10^3 \text{ CFU})$ whether the cells were treated with boiling water or not. The boiled bacteria from infected leaves showed higher reaction in ELISA test than living cells. These results were in accordance with the results of antibody titer and sensitivity test (4.1.4 and 4.1.5). After day 4 of inoculation, symptom on the infected areas was observed as slightly raised blister-like lesions

(Fig. 4.9). Thus, these results indicated that, ELISA assay is a well-established method for identification of bacterial pathogens from symptomatic plants.

Day after	Initial bacteria density (CFU)	Cell st	atus	PCR detection
inoculation		Live	Dead	(XAC specific primers)
0	10 ⁵	^a		+
	10^{4}			+
	10^{3}			+
2	10 ⁵	+++	+++	+
	10^{4}			+
	10 ³			+
4	10 ⁵	+++	+++	+
	10^4	-++	+ + +	+
	10^{3}	- + +	+++	+
6	10 ⁵	+ + +	+++	+
	10^{4}	+++	+++	+
	10^{3}	+ + +	+++	+
8	10 ⁵	+++	+++	+
	10^{4}	+ + +	+++	+
	10^{3}	+ + +	+++	+
10	10^{5}	+ + +	+++	+
	10^4	+ + +	+++	+
	10^{3}	+ + +	+ + +	+

Table 4.3 ELISA detection of bacteria on infected leaf using detached leaf assay.

^a: The positive result (+) was indicated when A ₄₀₅ was twice greater than that of the negative (-).

However, enrichment techniques can enhance ability of pathogen infection on natural samples but more time is needed for the culturing period (Jin et al., 2001).



Figure 4.9 Canker lesions on lime leaf infection using detached leaf assay. Lesion on under (A) and upper leaf (B) showed callus-like lesion with water-soaked margins.

4.1.8 Amplification of XAC from canker lesions

Canker pathogen on symptom and non-symptom plant materials (leaf and twig) in infected areas were detected by PCR amplification using XAC specific primers. The results showed that, the XAC specific PCR products were detect in all samples of symptom plant materials and some non-symptom plant materials (table 4.4).

These specific primers were also detected canker pathogen on infected leaves from detach leaf experiment. The results showed that, canker pathogen were detected by these specific primers on first day inoculation while ELISA was not available (table 4.3). Since, Amplification assay using specific primers allowed detecting at low population of pathogen $(10^2-10^3 \text{ CFU/ml})$ (Cubero et al., 2001, Cubero and Graham, 2002, Coletla-Filho, 2006, Leon, 2008, Park et al., 2006). The sensitivity of PCR amplification of this study showed high sensitivity at 10^3 CFU/ml

or 1 CFU (Appendix A Fig. 3). Moreover, this methodology was not cross-reacted with other *Xanthonomas* species (Fig. 4.4). However, PCR method required laboratory equipment, special reagents and skill for preformed.

 Table 4.4 XAC specific amplification of canker pathogen from symptom and nonsymptom plant materials.

Plant materials	Plant status ^a		PCR detection ^b (% detection)	
	Symptom	Non-symptom	Symptom	Non-symptom
Leaf	10	10	100%	40%
Twig	6	6	100%	33%

^a: Number of plant material samples that used in this study.

^b: The percentage of detection was calculated from formula in 3.1.6.

4.2 Canker resistance gene analogs in Thai hybrid lime M33

4.2.1 Response to X. axonopodis pv. citri inoculation

M33 and its parents (Pan and Nam Hom) were inoculated with citrus canker bacteria on leaves. Blushed-like lesions surrounded by chlorotic halo with water-soaked were seen on both wounded and unwounded Pan leaves. Whereas, the lesions on M33 and Nam Hom leaves were smaller and turned brown within 2 weeks post infection (table 4.5) which is the characteristic of hypersensitive response of resistance lime. From these responses, we can hypothesize that Nam Hom and M33 could have defense response mechanisms to prevent themselves from XAC infection.

 Table 4.5 The host-pathogen interaction of virulent bacteria with susceptible and resistance plant.

Pathogen	Effectors interact with specific host targets				
	• Virulent bacterial isolates				
Plant	Susceptible	Resistance			
	(Pan lime)	(Nam Hom and M33 lime)			
Plant	Compatible	• Incompatible			
defense	• Basal defense only	• Basal defense and			
response		hypersensitive response			
Out come	Image: Constraint of the second se	Image: symplectic symple			
	chlorotic halo	× 1 ′			

Defense responses in resistance plants can be divided into 2 types, including basal defense response and hypersensitive response. Basal defense response can be activate within minutes of attack through PAMP-triggered immunity (PTI) and induced by Pathogen-Associated Molecular Patterns (PAMPs) which are common molecules expressed by all bacteria such as flagellin or bacterial lipopolysaccharides (LPS) (Chisholm et al., 2006; Reina-Pinto and Yephremov, 2009). The perception of PAMPs by plant extracellular receptors activated signal-transduction cascades that turn on basal defenses, including cellulose and silicone deposition to reinforce the cell wall, production of reactive oxygen species and ethylene, transcriptional induction of a large suite of defense genes, including pathogenesis-related genes (*PR*) and posttranscriptional suppression of the auxin-signalling pathway (Alfano and Collmer, 1996). Basal defense does not prohibit pathogen colonization but it does limit the extent of its spread (Glazebrook et al., 1997). However, hypersensitive response (HR) or programmed cell death activation is a second layer of defense that can overcome pathogen invasion. HR is activated by incompatibility between dominant 'resistance gene' (*R*) of plant and dominant 'avirulence gene' (*Avr*) of pathogen which produce effector proteins for infection. This interaction is known as the 'gene-for-gene model.

The infected plant cell used programmed cell death to create a protective zone of dead cells (browning) around the site of pathogen invasion (as seen in the infection of virulent bacteria to Namhom and M33 limes (table 4.5). This R proteinmediated resistance is referred to as effector-triggered immunity (ETI) (Ade and Innes, 2007). Nevertheless, some *Xanthomonas* isolated from this experiment can infect the lime known to be resistant to canker disease (M33 and Namhom limes). This may be due to the compatibility of the *Xanthomonas Avr* gene with the *R* gene (Hammond-Kosack and Jones, 1997) of the resistance plant that caused the susceptibility of M33 and Namhom limes with virulence bacteria (BP210).

4.2.2 Citrus canker resistance gene analogous discovery

The hybrid lime (M33) and it's parents (Pan and Nam Hom) were screened using 12 NBS-LRR genes specific primers in combination with restriction enzymes (Appendix B table1). The results indicated that, only three specific amplicons combination with three different restriction enzymes were closely linked to canker resistance gene within M33 and Nam Hom limes, including Pt9/Alu1, Pt14/Bfa1 and 16R1-19/Tru1I (Appendix B table 2 and 3). These results correspond with the resistance phenotype (hypersensitive respond) of M33 and Nam Hom limes after inoculation with *Xanthomonas*.

4.2.2.1 *Pt9/Alu*1 marker gene discovery

The *Pt9* NBS-LRR gene fragment combination with *Alu*1 showed 2 bands linked with the canker resistance of M33 and Nam Hom limes (Fig. 4.10 lanes 6-9). The PCR products of *Pt9* amplification from all limes cultivars were approximately to 450 bp (Fig. 4.10 lanes 1-3). After the PCR products were digested with *Alu*1, 2 smaller bands of 320 and 130 bp were seen from DNA of M33 and Nam Hom limes (Fig. 4.10 lanes 6-9). Whereas, PCR products from Pan lime followed by *Alu*1 digestion showed three bands consisted, 320 and 130 bp as seen from M33 and Nam Hom limes digestion results and another band of 250 bp (Fig. 4.10 lane 5).

These results indicated that, the *Pt9* amplicon from Pan lime contains only one allele with the *Alu*1 restriction site similar to the *Pt9* amplicon from M33 and Nam Hom. Another allele of *Pt9* amplicon from Pan lime can be digested with *Alu*1 at different position therefore, different band pattern was observed (Fig 4.10 lane 5 and Fig. 4.11). Since citrus plant are diploid (2n), these results can assumed that, R_{Pt9} gene on the chromosomes of M33 and Nam Hom genomes should be two dominant *R* gene (*RR*_{*Pt9*}) that can be completely digested by *Alu*1 enzyme and generated two specific bands of 320 and 130 bp. Whereas, *Rr*_{*Pt9*} gene on Pan lime

chromosomes should be one dominant *R* gene (R_{Pt9}) and one recessive *r* gene (r_{Pt9}) that contains different position of restriction site of *Alu1* (Fig. 4.11). This RR_{Pt9} gene were also founded on other resistance lime (Puang) (Fig. 4.10 lane 10) which confirmed the result from M33 and Nam Hom that the *Pt9/Alu1* genotype is linked to the *Xanthomonas* resistant phenotype in limes.



Figure 4.10 Pt9/Alu1 marker gene screened on M33 and their parents (Pan and Nam Hom); M: 100 bp marker; Lane 1: negative control; Lane 2-4: PCR product of Pt9 from Pan, Nam Hom and M33, Alu1 digested Pt9 amplicon from Pan (lane 5), Nam Hom (lane 6), M33 from Phichit (lane 7), M33 from Samut Sakhon (lane 8), M33 from Prachinburi (lane 9), Puang resistant lime (lane 10) and Giant resistant lime (lane 11).

The *Pt9* fragments from Pan, Nam Hom and M33 were cloned and sequenced (Appendix B table 2). The sequencing results confirmed the *Alu*1 restriction sites on R_{Pt9} and r_{Pt9} from Pan, Nam Hom and M33 genomes (Appendix B table 2). *Alu*1 restriction sites on the *Pt9* sequences gave the fragment size similar to what were observed on the agarose gel. The digestions by these enzymes also resulted to some small pieces that were not observed on the gel (Appendix B table 2 and Fig. 4.10).



Figure 4.11 Alu1 restriction enzyme map of R_{Pt9} and r_{Pt9} gene on Pan, Nam Hom and M33 amplicons. Rr_{Pt9} of Pan crossing with RR_{Pt9} of Nam Hom, resulted in RR_{Pt9} on M33 genome.

The *Pt9* sequences could be translated to polypeptide without any stop codons. Using the BLASTX program to search the GenBank database to the results indicated that, all Pt9 clones showed strong overall similarities (>90% identity) to *C. grandis* x *P. trifoliate* Pt9, Pt11 (Deng et al., 2000) and RGA 24 sequence recently cloned from *C. triloliata* (Shen et al., 2004).

Multiple alignment was performed with the translated sequence of Pt9 and the 3 most similar *RGAs* gene sequences and the partial resistance protein of *Arabidopsis* RPS2 (Bent et al. 1994; Mindrinos et al. 1994), RPP5 (Noel et al., 1999), tobacco N (Witham et al. 1994), and flax L6 (Lawrence et al. 1995). The similarity was especially high at the two NBS motifs (kinase-2 and kinase-3a) (Appendix B Fig. 1). Deng et al. (2000) and Meyers et al. (1999) had performed extensive analyses of the NBS domains of the plant NBS-LRR class *R* genes and a vast number of *RGAs*. They found that, *RGAs* could be classified into either TIR (Toll/Interleukin-1

receptor) or non-TIR groups. In their analysis, N and L6 belong to the TIR group, while RPS2 fall into the non-TIR group. The translated Pt9 peptide sequences from this study indicated that, the Pt9 clones formed a major cluster with RPS2. A Tryptophan residues (W) were found at the end of kinase 2 motif which often seen in the non-TIR-LRR class proteins. Thus, Pt9 proteins from our limes belong to the non-TIR-LRR class (Appendix B Fig. 1). Two differential amino acid sequences between R_{Pt9} and r_{Pt9} were founded, valine (V) replaced alanine (A) and glutamate (E) replaced lysine (K) (Appendix B Fig. 1). These 2 amino acid mutations might be involved in the malfunction of r_{Pt9} on susceptible lime.

4.2.2.2 Pt14/Bfa1 marker gene discovery

Pt14 NBS-LRR gene amplification in combination with *Bfa*1restriction digest showed 2 bands linked with the canker resistance of M33 and Nam Hom limes (Fig. 4.12 lanes 6-9). The PCR products of *Pt14* amplification were 430 bp in all lime cultivars (Fig. 4.12 lanes 2-4). After the PCR products were digested with *Bfa*1, 2 smaller bands of 300 and 130 bp were seen from DNA of M33 and Nam Hom limes (Fig. 4.12 lanes 6-9) indicating that both alleles of *Pt14* can be digested with *Bfa*1. These results correspond with the resistance phenotype (hypersensitive respond) of M33 and Nam Hom limes after inoculation with *Xanthomonas*. Whereas, the results of *Pt14* gene from Pan lime followed by *Bfa*1 digestion showed 3 bands of 430 bp, 300 bp and 130 bp (Fig. 4.12 lane 5).

These results indicated that, only one allele the *Pt14* amplicon from Pan lime can be digested with *Bfa1* and the other allele does not contain the *Bfa1* restriction site. Since citrus plant are diploid (2n), these results can assumed that, R_{Pt14} gene on the chromosomes of M33 and Nam Hom genomes should be two dominant R gene (RR_{Pt14}) that can be completely digested by Bfa1 enzyme. Whereas, R_{Pt14} gene on Pan lime chromosomes should be one dominant R gene (R_{Pt14}) and one resessive r gene (r_{pt14}) that cannot be cut with Bfa1 (Fig. 4.13).



Figure 4.12 *Pt14/Bfa1* marker gene screened on M33 and their parents (Pan and Nam Hom); M: 100 bp marker; Lane 1: negative control; Lane 2-4: PCR product of *Pt14* from Pan, Nam Hom and M33, Lane 5-10: *Bfa1* digestion of *Pt14* from Pan (5), Nam Hom (6), M33 from Phichit (7), M33 from Samut Sakhon (8), M33 from Prachinburi (9) and Puang resistant lime (10).

This RR_{Pt14} gene were also founded on other resistance lime (Puang) (Fig. 4.13 lane 10) which confirmed the result from M33 and Nam Hom that the *Pt14/Bfa1* genotype is linked to the *Xanthomonas* resistant phenotype in lime. The *Pt14* fragments from each plant were cloned and sequenced. The sequencing results confirmed the *Bfa1* restriction sites on R_{Pt14} from Pan, Nam Hom and M33 genomes (Appendix B table 2).



Figure 4.13 *Bfa*¹ restriction enzyme map of R_{Pt14} and r_{Pt14} gene on Pan, Nam Hom and M33 amplicons. Rr_{Pt14} of Pan crossing with RR_{Pt14} of Nam Hom. resulted in RR_{Pt14} on M33 genome.

The sequence results also confirmed that, r_{Pt14} from Pan did not contain *Bfa*1 restriction site in the sequence. The *Bfa*1 digestion in the R_{Pt14} sequences were close to the fragment size seen on the gel. The *Pt14* sequences could be translated to polypeptide without any stop codons. The BLASTX program search indicated that, all *Pt14* clones showed strong overall similarities (>90% identity) to *C*. *grandis* x *P. trifoliate* Pt14 cloned by Deng et al. (2000).

Multiple alignment with the translated Pt14 and the most similar RGAs peptide sequences (*C. grandis* x *P. trifoliate* Pt14) were performed with the partial sequence of the resistance protein of *Arabidopsis* RPS2 and RPP5, tobacco N, and flax L6. The similarity was especially high at the two NBS motifs (kinase-2 and kinase-3a) class (Appendix B Fig. 2). Pt14 peptide sequences from this study indicate

that, the Pt14 protein formed a major cluster with *Arabidopsis* RPP5, N and L6. They all have an aspartic acid residue (D) at the final residue position of the kinase-2 motif that is often seen in the TIR group (Appendix B Fig. 2). Thus, Pt14 protein should belong to the TIR-LRR class. One different amino acid was found between R_{pt14} and an r_{Pt14} protein, which is asparagine (N) replaced the threonine (T) at the internal kinase-3a motif (Appendix B Fig. 2). This amino acid mutation might be responsible for the malfunction of r_{Pt14} on the Pan susceptible lime.

4.2.2.3 16R1/Tru1I marker gene discovery

16R1-19 NBS-LRR gene amplification in combination with Tru1I restriction digest showed 2 bands links with the canker resistance of M33 and Nam Hom limes (Fig. 4.14 lanes 6-9). The PCR products of *16R1-19* amplification were 450 bp in all lime cultivars (Fig. 4.14 lanes 2-4) whiles, M33 showed one smaller band about 420 bp. However, when the *16R1-19* amplicons were digested with Tru1I, 2 dominant bands of 2x200 and 50 bp were seen from DNA of Pan, M33 and Nam Hom limes (Fig. 4.14 lanes 5-9). Whereas, *16R1-19/Tru1I* from Pan lime showed a bigger band of 250 bp above the 2 dominant bands (200x2 and 50 bp).

These results indicating that, the 16R1-19 amplicon from Pan lime contains two different allele. One is similar to the amplicon of 16R1-19 from M33 and Nam Hom. The other allele of 16R1-19 amplicon from Pan lime can be digested with Tru1I that gave different band patterns on gel. These results can assumed that, $R_{16R1-19}$ gene on the chromosomes of M33 and Nam Hom genomes should be two dominant *R* gene ($RR_{16R1-19}$) that can be completely digested by Tru1I enzyme and generated specific bands of 2x200 and 50 bp on gel. Whereas, $R_{16R1-19}$ gene on Pan lime chromosomes contain one dominant *R* gene ($R_{16R1-19}$) and one recessive *r* gene ($r_{16R1-19}$) that contains different restriction site of *Tru*1I (may be 250 and 200 bp) (Fig. 4.15).



Figure 4.14 16R1-19/Trul1 marker gene screened on M33 and their parents (Pan and Nam Hom); M: 100 bp marker; Lane 1: negative control; Lane 2-4: PCR product of 16R1-19 from Pan, Nam Hom and M33, Lane 5-10: Trul1 digestion of 16R1-19 from Pan (5), Nam Hom (6), M33 from Phichit (7), M33 from Samut Sakhon (8), M33 from Prachinburi (9) Puang resistant lime (10) and Giant resistance lime (11).

This $RR_{16R1-19}$ gene were also founded on other resistance lime included Puang and Giant limes (Fig. 4.14 lanes 10 and 11) which confirmed the result from M33 and Nam Hom that the 16R1-19/Tru1I genotype is linked to the *Xanthomonas* resistant phenotype in lime.

The amplicon of 16R1-19 from 3 lime cultivars were cloned and sequenced. The sequencing results confirmed the present of Tru11 restriction sites on $R_{16R1-19}$ and $r_{16R1-19}$ from Pan, Nam Hom and M33 (Appendix B table 3).

*Tru*1I restriction site on $R_{16R1-19}$ and $r_{16R1-19}$ sequences were similar to the fragment size seen on the gel. The *16R1-19* sequences could be translated to polypeptide without any stop codons.



Figure 4.15 *Tru*1I restriction enzyme map of $R_{16R1-19}$ and $r_{16R1-19}$ gene on Pan, Nam Hom and M33 amplicons. $Rr_{16R1-19}$ of Pan crossing with $RR_{16R1-19}$ of Nam Hom, resulted in $RR_{16R1-19}$ on M33 genome.

The BLASTX search program of NCBI indicated that, all *16R1-19* peptides showed strong overall similarities (>90% identity) to *C. grandis* x *P. trifoliate* 16R1-19, 16R1-13 (Deng et al., 2000) and many RGA sequences recently cloned from *C. triloliata* included, RGA 12, RGA 20 and RGA 21 (Shen et al., 2004). Multiple sequences alignment with the translated 16R1-19 and the five most similar RGAs peptide sequences were performed with the resistance protein of *Arabidopsis* RPS2 and RPP5, tobacco N, and flax L6. The similarity was especially high at the two NBS motifs (kinase-2 and kinase-3a) (Appendix B Fig. 3). The 16R1-19 peptide sequences from this study indicate that, 16R1-19 fragments formed a

major cluster with RPS2 and they all have a tryptophan residue (W) at the final residue position of the kinase-2 motif that is often seen in the non-TIR group. Thus, 16R1-19 protein should belong to the non-TIR-LRR class (Appendix B Fig. 3). Two different amino acids sequence between $R_{16R1-19}$ and $r_{16R1-19}$ proteins were founded. A methionine (M) replaced a leucine (L) and a serine (S) replaced an arginine (R) (Appendix B Fig. 3). These amino acid mutations might be responsible for the malfunction of the $r_{16R1-19}$ on susceptible Pan lime.



Figure 4.16 Phylogenetic analysis of Pt9, Pt14 and 16R1-19 peptide sequences from Pan Nam Hom and M33 limes, their most similar RGAs peptide sequences and the partial resistance protein of *Arabidopsis* RPS2, RPP5, tobacco N, and flax L6.

4.2.2.4 Phylogenetic tree analysis

All of the predicted Pt9, Pt14 and 16R1-19 peptides from this experiment, their most similar RGAs peptide sequences and the partial sequence of resistance protein of *Arabidopsis* RPS2 and RPP5, tobacco N, and flax L6 were used to create phylogenetic tree for sub-family classification. The results confirmed that, Pt9, 16R1-19 and their similar proteins were grouped with *Arabidopsis* RPS2 which classified to non-TIR-NBS-LRR class. While, Pt14 protein and their similar proteins were grouped within *Arabidopsis* RPP5, tobacco N, and flax L6 which classified to TIR-NBS-LRR class (Fig 4.16).
CHAPTER V

OVERALL CONCLUSSION

5.1 Antibody production for citrus canker detection

Nineteen canker bacteria isolated from three different provinces in Thailand; Samut Sakhon, Nakhon Ratchasima and Phichit. Some of the bacteria isolates identified as *X. axonopodis* pv. *citri* could infect both sensitive (Pan) and resistant (Namhom and M33) limes but with different symptom indicating that the resistant limes have some defense response mechanisms to prevent themselves against *Xanthomonas* infection. Polyclonal antibody specific to virulence *X. axonopodis* pv. *citri* (BP210) can detect live target bacteria at 10⁶ CFU/ml and 10⁵ CFU/ml for dead cells. Antibody dilution of 1:2,000 is the suitable concentration for *Xanthomonas* BP210 detection which cross–react with only *X. axonopodis* pv. *vesicatoria*. The sensitivity of this antibody was not sufficient for canker bacteria detection before the symptom can be observed but was able to detect on day 4 post infection which the

5.2 Canker resistance gene analogs in Thai hybrid lime M33

The citrus canker resistance (R) marker genes within M33 and its parents were screened utilized the Nucleotide binding site (NBS) Leucine-rich repeat (LRR) genes

by PCR amplification in combination with restriction enzymes digestions. The resistant phenotype evaluation showed that, the marker Pt9/Alu1, Pt14/Bfa1 and 16R1-19/Tru1I were closely linked to the citrus canker resistance genes in M33 and Nam Hom lime. These R genes were founded as two dominant genes on the resistance lime $(RR_{Pt9}, RR_{Pt14} \text{ and } RR_{16R1-19})$. Whereas, only one single dominant R gene and one recessive r gene (Rr_{Pt9} , Rr_{Pt14} and $Rr_{16R1-19}$) were found in Pan limes which give sensitive phenotype. These RR_{Pt9} , RR_{Pt14} and $RR_{16R1-19}$ genotype was also observed in Puang lime which is another resistance lime. The predicted Pt9 and 16R1-19 protein can be classified in to the non-TIR-NBS-LRR subfamily. The Pt9 protein belongs to the family of resistance protein Pt9 and Pt11 from C. grandis x P. trifoliate and RGA 24 from C. triloliata. While, 16R1-19 protein belongs to the family of resistance protein RGC20 and RGC2 from C. trifoliata and 16R1-19 and 16R1-13 from C. grandis x P. trifoliate. Predicted Pt14 protein can be classified to the TIR-NBS-LRR subfamily and belongs to the resistance protein Pt14 family from C. grandis x P. trifoliate. The Pt9 and 16R1-19 and their similar proteins were clustered with the Arabidodsis RPS2 protein. While, Pt14 protein and the most similar RGAs peptide sequences (C. grandis x P. trifoliate pt14) belong to a major cluster of the resistance protein of Arabidopsis RPP5, tobacco N, and flax L6. Some amino acid mutation on recessive r protein might be involved in the malfunction of R protein in the susceptible limes.

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APPENDICES

APPENDIX A

Figure 1 Alignment 16S rDNA sequence from bacteria BP104 and BP210 isolates with *X. axonopodis* pv. *citri* strain 306 (GenBank: AE008923

		20		40		60		80	
BP104	CATGGCTCAG	AGTGAACGCT	GGCGGCAGGC	CTAACAGAGC	TTGCTCTTAT	GGGTGGCGAG	TGGCGGACGG	GTGAGGAATA	80
BP210	CGCTCAG	AGTGAACGCT	GGCGGCAGGC	CTAACAGAGC	TTGCTCTTAT	GGGTGGCGAG	TGGCGGACGG	GTGAGGAATA	77
X.axonopodis pv.citri str.306	GCTCAG	AGTGAACGCT	GGCGGCAGGC	CTAACAGAGC	TTGCTCTTAT	GGGTGGCGAG	TGGCGGACGG	GTGAGGAATA	76
Consensus	C GCTCAG	AGTGAACGCT	GGCGGCAGGC	CTAACAGAGC	TTGCTCTTAT	GGGTGGCGAG	TGGCGGACGG	GTGAGGAATA	
		100		120		140		160	
BP104	CATCGG - AAT	CTACTCTTTC	GTGGGGG-AT	AACGTAGGG -	AAACTTACGC	TAATACCGCA	TACGACCTAC	GGGTGAAAGC	157
BP210	CATCGGGAAT	CTACTCTTTC	GTGGGGGGAT	AACGTAGGGG	AAACTTACGC	TAATACCGCA	TACGACCTAC	GGGTGAAAGC	157
X.axonopodis pv.citri str.306	CATCGG - AAT	CTACTCTTTC	GTGGGGG · AT	AACGTAGGG -	AAACTTACGC	TAATACCGCA	TACGACCTAC	GGGTGAAAGC	153
Consensus	CATCGG-AAT	CTACTCTTTC	GTGGGGG-AT	AACGTAGGG -	AAACTTACGC	TAATACCGCA	TACGACCTAC	GGGTGAAAGC	
		180		200		220		240	
BP104	GGAGGACCTT	COOCTICOC	GCGGTTGAAT	GAGCCGATGT	CGGATTAGCT	AGTTOGCOCO	GTAAAGGCCC	ACCAAGGCGA	237
BP210	GGAGGACCTT	CGGGCTTCGC	GCGGTTGAAT	GAGCCGATGT	CGGATTAGCT	AGTTGGCGGG	GTAAAGGCCCC	ACCAAGGCGA	237
X.axonopodis pv.citri str.306	GGAGGACCTT	CGGGCTTCGC	GCGGTTGAAT	GAGCCGATGT	CGGATTAGCT	AGTTGGCGGG	GTAAAGGCCC	ACCAAGGCGA	233
Consensus	GGAGGACCTT	CGGGCTTCGC	GCGGTTGAAT	GAGCCGATGT	CGGATTAGCT	AGTTGGCGGG	GTAAAGGCCC	ACCAAGGCGA	
		260		290		300		320	
BD104	CONTROCTAG	CTOCTOTO	ACCATCATCA	CCCACACTOC	AACTGAGACA	CONTROLO	TCCTACCCCA	GGCAGCAGTG	217
BP104 BP210	CGATCCGTAG	CTGGTCTGAG	AGGATGATCA	GCCACACTGG	AACTGAGACA	CGGTCCAGAC	TCCTACGGGA	GGCAGCAGTG	317
X axonopodis pv citri str 306	CGATCCGTAG	CTGGTCTGAG	AGGATGATCA	GCCACACTGG	AACTGAGACA	CGGTCCAGAC	TCCTACGGGA	GGCAGCAGTG	313
Consensus	CGATCCGTAG	CTGGTCTGAG	AGGATGATCA	GCCACACTGG	AACTGAGACA	CGGTCCAGAC	TCCTACGGGA	GGCAGCAGTG	
		340		360		380		400	
		1		T		T		1	
BP104	GGGAATATTG	GACAATGGGC	GCAAGCCTGA	TCCAGCCATG	CCGCGTGGGT	GAAGAAGGCC	TTCGGGTTGT	AAAGCC . CTT	396
X avananadie nu citri etr 306	GGGAATATTG	GACAATGGGC	GCAAGCCTGA	TCCAGCCATG	CCGCGTGGGT	GAAGAAGGCC	TICCCCTTCT	AAAGCC .CTT	390
Consecution	GGGAATATTG	GACAATGGGC	GCAAGCCTGA	TCCAGCCATG	CCGCGTGGGT	GAAGAAGGCC	TTCCCCTTCT	AAAGCC .CTT	995
Guiserisus	OUGAATATTO	630 KCAA10000	GUARGUUTUR	440	000010001	annonnoocc	1100001101	450	
5.00000		ĩ	n Cuarta a real constante	- T		Ĩ		ĩ	
BP104	TTGTTGGGAA	AGAAAAGCAG	TCGGTTAATA	CCCGATTGTT	CTGACGGTAC	CCAAAGAATA	AGCACCGGCT	AACTTCGTGC	476
BP210	TTGTTGGGAA	AGAAAAGCAG	TCGGTTAATA	CCCGATIGTT	CTGACGGTAC	CCAAAGAATA	AGCACCGGCT	AACTTCGTGC	476
Alaxonopodis pv.citri str.306	TIGTIGGGAA	AGAAAAGCAG	TEGGTTAATA	CCCGATIGIT	CIGACGGIAC	CCAAAGAATA	AGCACCGGCT	AACTICGIGC	4/2
Consensus	TIGTIGGGAA	AGAAAAGCAG	TCGGTTAATA	CCCGATIGIT	CIGACGGIAC	CCAAAGAATA	AGCACCGGCT	AACTICGIGC	
		1		T.		1		L.	
BP104	CAGCAGCCGC	GGTAATACGA	AGGGTGCAAG	CGTTACTCGG	AATTACTGGG	CGTAAAGCGT	GCGTAGGTGG	TGGT - TTAAG	555
BP210	CAGCAGCCGC	GGTAATACGA	AGGGTGCAAG	CGTTACTCGG	AATTACTGGG	CGTAAAGCGT	GCGTAGGTGG	TGGT - TTAAG	555
X.axonopodis pv.citri str.306	CAGCAGCCGC	GGTAATACGA	AGGGTGCAAG	CGTTACTCGG	AATTACTGGG	CGTAAAGCGT	GCGTAGGTGG	IGGI-IIAAG	001
Consensus	CAPCAPPPP	COTAATACCA		C C T T A C T C C C C			0 0 0 T + 0 0 T 0 0	TREE TTAKE	
	CAGCAGECGE	GGTAATACGA	AGGGIGCAAG	CGITACICGG	AATTACTGGG	CGTAAAGCGT	GCGTAGGTGG	IGGI-ITAAG	
	CAGCAGCCGC	BBD I	AGGGTGCAAG	600	AATTACTGGG	620	GCGTAGGTGG	640	
BP104	TCTGTTGTGA	AAGCCCTGGG	CTCAACCTGG	GAATTGCAGT	GGATACTGGG	CGTAAAGCGT	GTGGTAGAGG	GTAGCGGAAT	635
BP104 BP210	TCTGTTGTGA	AAGCCCTGGG	CTCAACCTGG	GAATTGCAGT GAATTGCAGT	GGATACTGGG GGATACTGGG	CGTAAAGCGT 620 TCACTAGAGT TCACTAGAGT	GTGGTAGAGG	GTAGCGGAAT GTAGCGGAAT	635 635
BP104 BP210 X.axonopodis pv.citri str.306	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG	CTCAACCTGG CTCAACCTGG CTCAACCTGG	GAATTGCAGT GAATTGCAGT GAATTGCAGT	GGATACTGGG GGATACTGGG GGATACTGGG	TCACTAGAGT TCACTAGAGT TCACTAGAGT	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT	635 635 631
BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT	635 635 631
BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT	635 635 631
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GGCAGTGAAAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT 720 CACTGACACT	635 635 631 715
BP104 BP210 X.axonopodis pv.citi at:1306 Consensus BP104 BP210	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AGCCCTGGG GCAGTGAAAT GCAGTGAAAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT GCGTAGAGAT	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG	CGTAAAGCG E20 TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT 700 1 AAGGCGGCTA AAGGCGGCTA	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT	635 635 631 715 715
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GAGTGAAAT GCAGTGAAAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT GCGTAGAGAT	GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG	CACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT	635 635 631 715 715 711
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT	GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GGAGGAGGAAC CGGGAGGAAC CGGGAGGAAC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG	CACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGGCTA	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT 720 CACTGACACT CACTGACACT CACTGACACT	635 635 631 715 715 711
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT 71	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGGCTA AAGGCGGCTA 780	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT 720 CACTGACACA CACTGACACA CACTGACACT CACTGACACT CACTGACACT	635 635 631 715 715 711
BP104 BP210 X axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA	AGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT AGCGTGGGGA	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT GCGAAACAGGA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA	CGCCCTAAAGCG	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT	635 635 631 715 715 711 795
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT 740 AGCCTGGGGA	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACCTGG GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT GCCAACAGGA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GGGAGGAACCGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CTTAGGATACCCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA	CGCCCTAAACCG TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AGGCGGCTA CGCCCTAAAC CGCCCTAAAC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG	635 635 631 715 715 715 711 795 795
BP104 BP210 X.axonopodis pv.chri str.306 Consensus BP104 BP210 X.axonopodis pv.chri str.306 Consensus BP104 BP210 X.axonopodis pv.chri str.306	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGAAAT AGCGTGGGGA AGCGTGGGGA	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAGAGAT GCGTAGAGAT GCGTAGAGAT GCGTAGAGAT GCGAACAGGA GCAAACAGGA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CTAGATACCC TTAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA	CGCCCTAAAC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG	635 635 631 715 715 711 795 795 791
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACCTGG CCTAACCTGG CCTAACACTGG CCTAAGAAT GCGTAGAAAT GCGTAAGAA CCAAACAGGA GCAAACAGGA GCAAACAGGA	GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CTTAGATACCC TTAGATACCC TTAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGCTAAAC CGCCCTAAAC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT T20 1 CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG	635 635 631 715 715 711 795 795 791
BP104 BP20 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA	AAGCCTGGG AAGCCTGGG AAGCCTGGG AAGCCTGGG GCAGTGAACT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACCTGG CCTAACACTGG CCTAGAGAT GCGTAGAGAT GCAAACAGGA GCAAACAGGA GCAAACAGGA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GGGGGGGGACC CGGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC TTAGATACCC TTAGATACCC TTAGATACCC TTAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA	CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GATGCGAACT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG	635 635 631 715 715 711 795 795 791
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA TGCAATTTGG	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAACT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGAAA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA CACCGAGTAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAAGCAT GCGTAGAGAT GCGTAGAGAT GCAAACAGGA GCAAACAGGA GCAAACAGGA GCAAACAGGA GCAAACAGGA	GATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC TTAGATACCC TTAGATACCC TTAGATACCC TAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA	CGTARAGCG TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGGCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GATGCGAACT GATGCGAACT GTCGCAAGAC	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG	635 635 631 715 715 715 711 795 795 795 795
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 BP210 BP210 BP210 Str.305 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA TGCAATTTGG	AAGCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAACT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA BCC CACCCAGTAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACCTGG CCTAACCTGG CCTAACACTGG CCTAGACAT GCGTAGACAT GCGTAGACAT GCAAACAGGA GCAAACAGGA CCAAACAGGA CCAACAGGA CCAACCAGGA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CTTAGATACCC TTAGATACCC TTAGATACCC TAGATACCC ACGCGTTAAG	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTCGCCGCCT TTCGCCGCCT	CGCCTAAACGCTAAACGCCTAAACGCCCTAAACGCCCCTAAACGCCCCCAAACCCGCCCCAAACCCGCCCCAAACCGCCCCCAAACCGCCCCCAAACGCGCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCAAACCGCCCCCAAACCGCCCCCACCGCCCCCC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GATGCGAAGAC GTCGCAAGAC	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTCGACTCAA	635 635 631 715 715 715 711 795 795 795 791 873 873
BP104 BP20 Staxonopodis pv.chri str.306 Consensus BP104 BP20 X.axonopodis pv.chri str.306 Consensus BP104 BP210 X.axonopodis pv.chri str.306 Consensus BP104 BP20 X.axonopodis pv.chri str.306 Consensus BP104 BP20 X.axonopodis pv.chri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA TGCAATTTGG TCCAATTTGG	AAGCCTGGG AAGCCTGGG AAGCCTGGG AAGCCTGGG GCAGTGAACT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA CACCGAGTAT CACCCACTAT	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAGAGAT GCGTAGAGAT GCGTAGAGAT GCAAACAGGA GCAAACAGGA GCAAACAGGA GCAAACAGGA CGAAGCTA CGAAGCTA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC TAGATACCC TTAGATACCC TTAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTCGCCGCCT TTCGCCGCCCT	CGCCTAAACCGCTAAACCGCCTAAACCGCCCTAAACCGCCCTAAACCGCCCTAAACCGCCCTAAACCGCCCTAAACCGCCCTAAACCGGCCCTAAACCGGCCCTAAACCGGCCCTAAACCGGCCCTAAACCGGCCCTAAACCGGCGCTAAACCGGCGCTAAACCGGCGCTAAACCGGCGCTAAACCGGCGCAAAACCGGCGCAAAACCGGGGAGTACGGGGGAGTACGGGGGAGTACG	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GATGCGAACT GTCGCAAGAC GTCGCAAGAC	GATAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT T20 CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG CGAACTCAA TGAAACTCAA TCAACTCAA	635 635 631 715 715 715 715 795 795 795 795 791 873 873 873 869
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BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCCGTGTGTG TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGA TGCAATTTGG AGGAATTGAC AGGAATTGAC AGGAATTGAC CATCCACGGA CATCCACGGA CATCCACGGA	AAGCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAACT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA AGCCTGGGGA GGGGCCGGC GGGGGCCGGC GGGGGCCGGC GGGGGCCGC GGGGCCGC GGGGGCCGC GGGGCCGC GGGGCCGC GGGGCCGC GGGGCCGC GGGGCCGCC	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACACTGG CGTAGAGAT GCGTAGAGAT GCGTAGAGAT GCGTAGACAGGA CGAACCAGGA CGAACCAGGA CGAACC-TA CGAAGC-TA CGAAGC-TA CGAAGC-TA CGAAGCGTG ACCAACGGTG ACCAACGGTG ACCAACGGTG GATGGATTGG GATGGATTGG CATGGATTGG CATGGATTGG CATGGATTGG CATGGATTGG	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAGC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGAAC CGGGAGAAC CTTAGATACCC TTAGATACCC TTAGATACCC TAGATACCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTGCCGCCT TTCGCCGCCT TTACCCGCCT TTTAATTCGA AACCGTGAGA AACCGTGAGA AACCGTGAGA	CGCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGGCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCCACAC CGCCCCACAC CGCCCCACAC CCCCCCACAC CCCCCCCC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC CTCCCAAGAC GTCGCAGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGG GGATGTTGG CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTGCACC	635 635 631 715 715 795 795 795 795 795 795 795 795 795 79
BP104 BP210 X axonopodis pv.citri str.306 Consensus BP104 BP210 X axonopodis pv.citri str.306 Consensus	TCCGGTGTGG TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA TGCAATTTGG GCAATTTGC CGGAATTGAC AGGAATTGAC CATCCACGGA CATCCACGGA CATCCACGGA CATCCACGGA TCGTGTCGTG	AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA CACCAGTAT CACCAGTAT CACCAGTAT GGGGGCCCGC GGGGGCCGCC	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAAGCTGG CCTAAGACT GCGTAGAGAT GCGTAGAGAT GCGTAGACACAGGA GCAAACAGGA GCAAACAGGA GCAACAGGA CGAAGC - TA CGAAGC - TA CACAGCGGTG CACAGCGTG CACAGCGTG CACAGCGTTG CATGGATTGG CATGGATTGG CATGGATTGG TTAAGTCCCC	GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT GAATIGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGAAC CGGGTAAG ACGCGTTAAG ACGCGTTAAG ACGCGTTAAG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG GAGTATGTGG CACCAGAGCGC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTCGCCGCCT TTTAATTCGA AACCGTGAGA AACCGTGAGA AACCCTGGCG	TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGGCTA AAGGCGGCTA AAGGCGGCTAAAC CGCCCTAAC CGCCCTAAC CGCCCCTAAC CGCCCCTAAC CGCCCCTAAC CGCCCCTAAC CCCCCTAAC CCCCCTAAC CCCCCTAAC CCCCCCCAAC CCCCCCCAAC CCCCCCCAAC CCCCCC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC AGCACGTAAT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGA TGAACTCAA TGAACTCAA GAACTCAA GGATGCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA GGTGGGAACT	635 635 631 715 715 795 795 795 795 795 795 795 795 795 79
BP104 BP210 X axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCCGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TGCAATTTGG TGCAATTTGG AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC CATCCACGGA CATCCACGGA CATCCACGGA CATCCACGGA	AAGCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCTGGG CAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA CAGCAGTAT CACCAGTAT CACCAGTAT GGGGGCCCGC GGGGCCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCGCC	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAACCTGG CCTAACACTGG CGTAGACAT GCGTAGACAT GCGTAGACAT GCGTAGACACGA GCAACAGGA CGAACC-TA CGAAGC-TA CGAAGC-TA CGAAGC-TA CGAAGC-TA CGAAGC-TA CGAAGCGTG ACAACCGGTG ACAACCGGTG ACAACCGGTG ACAACCGGTG CATGGATTGG GATGGATTGG GATGGATTGG TTAAGTCCCG TTAAGTCCCG TTAAGTCCCG	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGAGGAAC CGGGATAAGTACCC TTAGATACCC TTAGATACCC TTAGATACCC TTAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGATACCC TAGACCCC CAACGAGCCC CAACGAGCCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTGGCGCCT TTCGCCGCCT TTTAATTCGA AACCGTGAGA AACCGTGAGA AACCGTGAGA AACCCTTGTC AACCCTTGTC	CGCCTAAACCGA CGCCTAACAGT CGCCTAGAGT CGCCTAGAGT CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCTAAC CGCCCCAAC CGCCCTAAC CGCCCTAAC CCCCCCAAC CGCCCTAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCAAC CCCCCCCAAC CCCCCCCC	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAGGAC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC AGCACGTAAT AGCACGTAAT	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGGG TGAAACTCAA TGAAACTCAA TGAAACTCAA GTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA GTGGGGAACT	635 635 631 715 715 717 795 795 795 791 873 873 869 953 949 1021 102 102 102 102 102
BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus BP104 BP210 X.axonopodis pv.citri str.306 Consensus	TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCTGTTGTGA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA TCCCGGTGTA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA GAGGCACGAA TGCAATTTGC GGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC AGGAATTGAC CATCCACGGA CATCCACGGA TCGTGTCGTG	AAGCCTGGG AAGCCCTGGG AAGCCCTGGG AAGCCCTGGG GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGAAAT GCAGTGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA AGCGTGGGGA GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGCCCGC GGGGGCCCGC GGGCCCGC GGGGCCCGC GGGCCCGC	CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CTCAACCTGG CCTAAGACT GCGTAGAGAT GCGTAGAGAT GCGTAGACATGG GCAACAGGA GCAAACAGGA GCAAACAGGA GCAACAGGA GCAACAGGA GCAACAGGA CGAAGC - TA CGAAGC - TA	GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GAATTGCAGT GGATGCAGT CGGGAGGAGAC CGGGAGGAGAC CGGGAGGAGAC CGGGAGGAGAC CGGGAGGAAC CGGGATAACCC TTAGATACCC TTAGATACCC TTAGATACCC TAGACCACCACCC TACCACCACCC TACCACCACCC TACCACCACCCC	GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG GGATACTGGG ATCCGTGGCG ATCCGTGGCG TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TGGTAGTCCA TTGCCGCCT TTCGCCGCCT TTCGCCGCCT TTTAATTCGA AACCGTGAGA AACCGTGAGA AACCGTGAGA AACCGTGAGA AACCCTTGTC AACCCTTGTC	CACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT TCACTAGAGT AAGGCGCGCTA AAGGCGCGCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAAC CGCCCTAAC CGCCCTAAC TGCAACGCGA TCCAAGCGCA TCCAAGCGCA TCCAAGCGCA TCCAAGCCCGA TCCAAGCCGA TCCAAGCGCA TCCAAGCCGA TCCAAGCGCA TCCAAGCCGA TCCAAGCGCA TCCAAGCCGA TCCAAGCGCA TCCAAGCGCA TCCAAGCGCA TCCAAGCGCA TCCAAGCGCA TCCAAGCGCA TCCAAGCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCGA TCCAAGCCCAACCCAA	GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG GTGGTAGAGG CCTGGACCAA CCTGGACCAA GATGCGAACT GATGCGAACT GATGCGAACT GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GTCGCAAGAC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC GCTGCATGGC	GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT GTAGCGGAAT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT CACTGACACT GGATGTTGGG GGATGTTGGG GGATGTTGGG GGATGTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA CTGGTCTTGA	635 635 631 715 715 795 795 791 873 873 873 860 953 949 1022 1022 1022 1022

Figure 2 Blastn results of 16S rDNA from bacteria BP104 (A) and BP210 (B) isolate.

_	Sequences pro	oducing significant alignments:						
a	Accession	Description	<u>Max</u> score	<u>Total</u> <u>score</u>	<u>Query</u> <u>coverage</u>	${\displaystyle \bigtriangleup} \frac{\underline{E}}{\underline{value}}$	<u>Max</u> ident	Links
	<u>AE008923.1</u>	Xanthomonas axonopodis pv. citri str. 306, complete genome	<u>2579</u>	5158	98%	0.0	98%	
	DQ991194.1	Xanthomonas axonopodis pv. citri strain XCW 16S ribosomal RNA gene,	<u>2579</u>	2579	98%	0.0	98%	
	HQ264097.1	Uncultured Xanthomonas sp. clone SL37 16S ribosomal RNA gene, parti:	<u>2573</u>	2573	98%	0.0	98%	

	Sequences pro	oducing significant alignments:						
b	Accession	Description	<u>Max</u> score	<u>Total</u> <u>score</u>	<u>Query</u> <u>coverage</u>	$\Delta \frac{\underline{E}}{\underline{value}}$	<u>Max</u> ident	Links
	AE008923.1	Xanthomonas axonopodis pv. citri str. 306, complete genome	<u>2676</u>	5353	98%	0.0	99%	
	<u>DQ991194.1</u>	Xanthomonas axonopodis pv. citri strain XCW 16S ribosomal RNA gene,	<u>2676</u>	2676	98%	0.0	99%	
	HQ264097.1	Uncultured Xanthomonas sp. clone SL37 16S ribosomal RNA gene, parti:	<u>2671</u>	2671	98%	0.0	99%	

Figure 3 XAC specific PCR amplification from a tenfold dilution series of X. axonopodis pv. citri cultured cells. M: 100 bp marker; Lane 1: negative control; Lanes 2-7: 10⁵ CFU, 10⁴ CFU, 10³ CFU, 10² CFU, 10¹ CFU and 1 CFU; lanes 8-9: <1 CFU respectively.</p>



Bactertial isolates	Colony mo	orphology ^a	XAC specific	P	an	Nam	Hom	Μ	33
	NA	KCD	products (581 bp)	wound	Not	wound	Not	wound	Not
	medium	medium	Fig. 4.3^b		wound		wound		wound
BP 102	Y, C	LY, C	+	$+^{c}$	_ d	+	+	+	-
BP 104	Y, C	LY, C	+	+	+	+	-	+	-
BP 105	Y, C	LY, C	+	+	+	+	-	+	+
BP 107	Y, C	LY, C	+	+	-	-	-	+	-
BP 109	Y, C	LY, C	+	+	+	+	-	+	-
BP 201	Y, C	LY, C	+	+	-	+	-	+	-
BP 202	Y, C	LY, C	+	+	+	+	-	+	-
BP 203	Y, C	LY, C	+	+	-	+	-	+	-
BP 205	Y, C	LY, C	+	+	-	-	-	+	-
BP 210	Y, C	LY, C	+	+	+	+	-	+	-
SUT02	OY, C	OY, C	-	-	-	-	-	-	-
SUT06	OY, C	OY, C	-	-	-	-	-	-	-

Table 1 Colony morphology of bacterial isolates from canker lesion on lime, present and absent of XAC specific PCR product and diseasesreaction on Pan, Namhom and M33 limes after inoculation.

Bactertial	Colony mo	orphology ^a	^a XAC specific products Pan		an	Nan	nhom	M33	
isolates			(581 bp) Fig. 3 ^b	(581 bp) Fig. 3 ^b					
	NA	KCD		wound	Not	wound	Not	wound	Not
	medium	medium			wound		wound		wound
K01	OY, C	OY, C	-	-	-	-	-	-	-
K02	OY, C	OY, C	-	-	-	-	-	-	-
PJ01	Y, C	W	+	+	-	+	-	+	-
PJ02	OY, C	-	-	-	-	-	-	-	-
PJ03	Y, C	W	+	+	-	+	-	+	-
PJ04	OY, C	-	-	-	-	-	-	-	-
PJ05	OY, C	-	-	-	-	-	-	-	-

^a: Colony morphologies were indicated as Yellow (Y), Ligt-yellow (LY), Orange yellow (OY), White (W) in color and Circular (C) in shape; ^b: The results of XAC specific amplification were taken as positive (+) with 581bp detected and negative (-) with non detected or non-target detected; The present of symptoms after inoculation was taken as positive (^c+), whereas, symptomless was be negative (^d-); The name of the bacterial isolate are from the acronym of the location isolated; Banpaew, Samut Sakhon (BP), Phichit (PJ), Suranaree University of Technology, Nakhon Ratchasima (SUT), Kokkruad, Nakhon Ratchasima (K)

pAb2 pAb1 Density Antiserum Cell dilution status (CFU/ml) Reaction Reaction A_{405} A405 $+^{\mathbf{a}}$ 10^{6} 0.199 0.249 + 10^{5} Live 0.198 0.183 ++ 10^{4} _c 0.098 0.121 _ 1:1,000 10^{6} 0.743 0.689 ++ 10^{5} Dead 0.236 0.220 ++ 10^{4} 0.123 0.137 _ _ 10^{6} 0.198 0.180 ++ 10^{5} Live 0.150 0.123 _ 10^{4} 0.101 0.110 -1:2,000 10^{6} 0.748 0.644 ++ 10^{5} Dead 0.205 +0.174 + 10^{4} 0.110 0.115 -_ 10^{6} Live 0.158 +0.120 - 10^{5} 0.121 0.112 _ 10^{4} 0.094 0.110 _ 1:4,000 10^{6} 0.674 0.605 Dead ++ 10^{5} 0.191 0.156 + 10^{4} 0.110 0.105 _ -

Table 2 ELISA for pAb1 and pAb2 titer test. Different antiserum dilutions (1:16,000, 1:8,000, 1:4,000, 1:2,000 and 1:1,000) were used to detect live and dead cells of XAC suspension at different concentrations (10⁴, 10⁵ and 10⁶CFU/ml).

Table 2 (Continued)

Antiserum	Cell	Density	р	Ab1	ł	oAb2
dilution	status	(CFU/ml)	A ₄₀₅	Reaction	A ₄₀₅	Reaction
		10 ⁶	0.123	-	0.103	-
	Live	10 ⁵	0.110	-	0.102	-
1.8 000		10^{4}	0.090	-	0.102	-
1:8,000	Dead	10^{6}	0.525	+	0.511	+
		10 ⁵	0.173	-	0.143	-
		10^{4}	0.100	-	0.103	-
		10 ⁶	0.103	-	0.094	-
	Live	10 ⁵	0.095	-	0.094	-
1.16 000		10^{4}	0.090	-	0.090	-
1.10,000		10^{6}	0.434	+	0.429	+
	Dead	10 ⁵	0.156	-	0.133	-
		10^{4}	0.105	-	0.093	-
	Control			0.085 ^b		

^a: The positive reaction (+) was indicated when A ₄₀₅ was twice greater than that of the negative control (^b).

 $^{c}:$ The negative reaction (-) was indicated when A_{405} was lower than twice of the negative control ($^{b}).$

Antiserum	Cell		X. axon	opodis j	pv. <i>citri</i> a	antigen (CFU/ml)
	status	10 ⁸	10 ⁷	10 ⁶	10 ⁵	10 ⁴	10 ³	E. coli ^b
pAb1	Live	1.300 ^a	0.692	0.231	0.154	0.102	0.100	0.090
(1:2,000)	Dead	1.990	1.630	0.739	0.212	0.123	0.102	0.090
pAb2	Live	0.543	0.472	0.183	0.120	0.101	0.098	0.090
(1:2,000)	Dead	0.803	0.763	0.633	0.180	0.100	0.096	0.090

 Table 3 Sensitivity of ELISA in detecting live and dead cells of X. axonopodis pv.

 citri.

^a: The results was indicated as A_{405} by the ELISA reader.

^b: *E. coli* at 10⁶ CFU/ml was used as negative control.

APPENDIX B

Table 1 Difference restriction enzymes digestion of 12 R gene analogs from Pan,Nam Hom and M33 limes.

PCC s	Amplicon		Digestion							
RGCS		EcoRI	Tru1I	BfaI	HinfI	AluI	TaqI			
Pt3	\sqrt{a}	X ^b		Х	Х	\checkmark				
Pt6		Х		X	X	X	Х			
Pt7	Х	_ d	-	-	-	-	-			
Pt8	\checkmark	Х	Х	Х		Х				
Pt9		Х		*** ^c		***	Х			
Pt14	\checkmark	X	Х	***						
Pt18		X	Х	X	X	Х				
Pt19	\checkmark	Х								
11P31	ν	Х		Х	Х		Х			
11P33	Х	-	-	-	-	-	-			
18P33		Х	\checkmark							
16R1-19		Х	***			X	X			

^a $\sqrt{\cdot}$ able to amplify and digest but similar results in all limes.

^b X: unable to amplify or digest.

^c***: was able to distringuish between susceptible and resistant limes.

^d-: was notperformed.

RGAs name	R clones	Sequences length (bp)	Digestion enzymes	Digested positions	Fragment size (bp) ^a	Observed band
	Pan-r	446		439, 316, 66, 3	<u>250, 123,</u> 63, 7, 3	- 313 - 123,126
	Pan-R	446		439, 316, 3	<u>313,126,</u> 7,3	_ ,
Pt9	Nam Hom- <i>R</i> 1	443	Alu1I	436, 316, 3	<u>313,120,</u> 7,3	- 313x2
	Nam Hom- <i>R</i> 2	446	(Fig. 4.10)	439, 316, 3	<u>313,123,</u> 7,3	120,123
	M33- <i>R</i> 1 and – <i>R</i> 2	443		436, 316, 3	<u>313,120,</u> 7,3	- 313 - 120
	Pan-r	426		None ^c	426	- 426
	Pan-R	426		288	<u>288, 138</u>	- 288 - 138
<i>Pt14</i>	Nam Hom- R1	425	Bfa1	288	<u>288, 137</u>	-288x2
	Nam Hom- R2	426	(Fig. 4.12)	288	<u>288, 138</u>	138, 137
	M33- <i>R</i> 1 and – <i>R</i> 2	426		288	<u>288, 138</u>	- 288 - 138

Table 2 Restriction sites of dominant R_{pt9} , R_{pt14} and recessive r_{pt9} , r_{pt14} clones from Pan, Nam Hom and M33 DNA used Alu11 andBfa1enzymes.

^a: the underline number is fragment size of observed band.

RGAs name	R clones	Sequences length (bp)	Digestion enzymes	Digested positions	Fragment size (bp) ^a	Observed band
	Pan-r	455		401, 223	<u>223, 178, 54</u>	-223
- 16R1-19	Pan-R	452	<i>Tru</i> 11 (Fig. 4.14)	163, 220, 398	<u>178, 163,54,</u> <u>57</u>	- 57, 54x2
	Nam Hom- <i>R</i> 1 and - <i>R</i> 2	455		166, 220, 401	<u>178, 166,54,</u> <u>57</u>	- 178, 166 - 57, 54
	M33- <i>R</i> 1 and – <i>R</i> 2	452	-	163, 220, 398	<u>178, 163, 54,</u> <u>57</u>	- 178, 163 - 54, 57

Table 3 Restriction sites of dominant $R_{16R1-19}$ and recessive $r_{16R1-19}$ clones from Pan, Nam Hom and M33 DNA used *Tru*11 enzymes.

^a: the underline number is fragment size of observed band.

		20		40		60	
Pt9 r[Pan]	LIGAR-NGED	VVIWVVVSKD			RRIGEIDESW	KNGSL - EDK	10
Pt0_P[Pan]		VVIWVVVSKD		IQEKIG	PRIGEIDESW	KNGSL-EDKA	10
Pt9 R1[Nam Hom]	LIGAP-NGED	VVIWVVVSRD	LOVEK	IOEKIG	RGIGELDESW	KNGSL-EDKA	40
Pt9_R2[Nam Hom]	LLGAP-NGED	VVIWVAVSKD	LOVEK	IOFKIG	RRIGELDESW	KNGSL - EDKA	40
Pt9 R1 and R2[M33]	LLGAP - NGED	VVIWVVVSRD	LOVEK	IQEKIG	RRIGELDESW	KNGSL - EDKA	49
PT9 Deng2000gil22947705	LIGAP-NGED	VVIWVVVSKD		IOFKIG	RRIGELDESW	KNGSL - EDKA	40
RGA24IC trifoliata]oi53680942	LIGAP-NGED	VVIWVVVSKD		IOFKIG	RRIGELDESW	KNGSL - EDKA	49
Pt11 Deng2000-gi22947710	LLGAP-NGED	VVIWVVVSKD		IOFKIG	RRIGELDESW	KNGSL - EDKA	40
RPS2		VIIWVOMSRE	FGECT	IODAVG	ARIGI	DEKETGENRA	41
16	FD	CCACELDNI	RETOEKDOVV	VLOKKIV	SELLBIDSGS	VGENNDSGGR	47
N	FD	GA CELKDI	KEN - KRGMH	S LONALL	SELLB	ANYNNEEDGK	42
BPP5	· · · · · · · ID	RAEVSYSRNI	YSGANPDDPN	MKLOLOGHEL	SELL	KDIKIDDPA-	47
Concensio	LLCAR NOED	VVIWVVVEKD	LOVEK	LOEKIC	PRICEIDESW	KNOSI EDKA	47
Consensus	LLGAP-NGPD	VVIVVVVSKD	LUXER	IQEKIG	RRIGFLDESW	KNGSL-EDKA	
Conservation	nnnn nn l				nn TenTell	Done Doe	
0%		1. 0.00				100	
		kinase-2 [®]		K	inase-sa	120	
Pt9_r[Pan]	SDILRILSKK	KFLLLLDDIW	E-RVDLTKVG	VPFPDPENKS	E) VFTTRFLE	ICGAMKAH-E	107
Pt9 R[Pan]	SDIFRILSKK	KFLLLLDDIW	E-HVHLTKVG	VPFPDPENKS	KIVFTTRFLE	I CGAMKAH - E	107
Pt9 R1[Nam Hom]	SDILRILSKK	KFLLLLDDIW	E-RVDLTTVG	VPFPDPENKS	KIVFTTRFLE	I CGAMKAH - E	107
Pt9 R2[Nam Hom]	SDVLRILSKK	KFLLLLDDIW	E-RVDLTKVG	VPFPDPEKKS	KIVFTTRFLE	I CGAMKAH - E	107
Pt9 R1 and R2[M33]	SDILRILSKK	KFLLLLDDIW	E-RVDLTTVG	VPFPDPENKS	KIVFTTRFLE	I CGAMKAH - E	107
PT9 Deng2000gi 22947705	SDILRILSKK	KFLLLLDDIW	E-RVDLTKVG	VPFPNLENKS	KIVFTTRFLE	ICGA KAH-E	107
RGA24[C.trifoliata]gi53680942	SDILRILSKK	KFLLLLDDIW	E-RVDLTKVG	VPFPNLENKS	KIVFTTRFLE	I CGAMKAH - E	107
Pt11 Deng2000-gi22947710	SDILRILSKK	KFLLLLDDIW	E-RVDLTKVG	VPFPNLENKS	KIVFTTRFLE	ICSAIKAH-E	10
RPS2	LKIYRALRQK	RFLLLLDDVW	E-EIDLEKTG	VPRPDRENKC	KVMFTTRSIA	LCNNMGA E	98
L6	KTIKERVSRF	KILVVLDDVD	EKFKFEDMLG	SPKDFISQ-S	RFIITSRSMR	VLGTLNENQC	100
N	HQMASRLRSK	KVLIVLDDID	NKDHYLEYLA	GDLDWFGNGS	RILITTROKH	L I EKND	98
RPP5	-ALEERLKHQ	KVLIIIDDLD	D-IMVLDTLV	GQTQWFGYGS	RIIVVTNDKH	FL IAHGID	103
Consensus	SDILBILSKK	KELLLDDIW	E-RVDLTKVG	VPEPDPENKS	KIVETTRELE	ICGAMKAH-E	
100%							
Conservation							
0%		140		160		190	
0%		140 I		160		180	
% Pt9_r[Pan]	F-LKVECLGP	140 EDAWRLFREN	LRRDV	160 I		180	13
% Pt9_r[Pan] Pt9_R[Pan]	F-LKVECLGP F-LKVECLGP	140 EDAWRLFREN EDTWRLFREN	LRRDV	160 		180 I	13 [.] 13 [.]
% Pt9_r[Pan] Pt9_R[Pan] Pt9_R1[Nam Hom]	F-LKVECLGP F-LKVECLGP -LKVECLRP	EDAWRLFREN EDTWRLFREN EDAWRLFREN	LRRDV	160 1		180 1	13 [.] 13 [.] 13(
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R1[Nam Hom] Pt9_R2[Nam Hom]	F - LKVECLGP F - LKVECLGP - LKVECLRP F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN	LRRDV LRRDV LRRDV LRRDV	160	·····	180 1	13 [.] 13 [.] 13(13 [.]
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R1[Nam Hom] Pt9_R2[Nam Hom] Pt9_R1 and R2[M33]	F-LKVECLGP F-LKVECLGP -LKVECLRP F-LKVECLGP -LKVECLRP	EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN	LRRDV LRRDV LRRDV LPRDV LRRDV	160 1		180 1	13 ⁻ 13 ⁻ 13(13 ⁻ 13(
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R1[Nam Hom] Pt9_R2[Nam Hom] Pt9_R1 and R2[M33] PT9_Deng2000;j12247705	F-LKVECLGP F-LKVECLGP -LKVECLRP F-LKVECLGP -LKVECLGP F-LKVECLGP	EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN	LRRDV LRRDV LRRDV LPRDV LRRDV LRRDV	160		180 1	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_Dang2000gi[22947705 RGA24[C.trifoliata]pf53680942	F-LKVECLGP F-LKVECLGP -LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN	LRRDV			180	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R1 and R2[M33] PT9_Deng2000gil22947705 RGA24[C.trifoliata]g53869942 Pt11_Deng2000-gil22947710	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV	160 1		180	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2Ram Hom] Pt9_Deng2000gi/22947705 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RF52	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP YKLRVEFLEK	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV			180	13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°]
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R1 and R2[M33] PT9_Deng2000gi[22947705 RGA24(C.trifoliata]pi53680942 Pt11_Deng2000-gi22947710 RPS2 L6	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP YKLRVEFLEK KLYEVGSMSK	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK FRSLELFSKH	LRRDV LRRDV LPRDV LRRDV LRRDV LRRDV LRRDV VWRKD AFKKNTPPSY	YETLANDVVD	TTAGLPLTLK	VIGSLLFKQE	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 12 ⁻ 16 ⁻
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_Deng2000gi/22947705 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RS2 L6 N	F-LKVECLGP F-LKVECLGP -LKVECLGP -LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP YKLRVEFLEK KLYEVGSMSK IIYEVTALPD	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK PRSLELFSKH HESIQLFKQH	LRRDV	YETLANDVVD FEKLSLEVVN	TTAGLPLTLK YAKGLPLALK	VIGSLLFKQE VWGSLLHNLR	13 13 13 13 13 13 13 13 13 13 13 13 15 16 15
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_Deng2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RF252 L6 N RP55	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP KLKVEFLEK KLYEVGSMSK KLYEVGSMSK HIYEVTALP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN HAUELFCSK PRSLELFSKH VHACQMLCQS	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AFKKNTPPSY AFGKEVPNEN AFKQNYAPKG	YET LANDVVD FEK LS LEVVN FED LVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHNLR LLGKYLRRD	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 15 ⁰ 15 ⁰ 15 ⁰ 15 ⁰
** Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R1 and R2[M33] PT9_Deng2000gi[22947705 RGA24[C.trifoliata]pI53680942 Pt11_Deng2000-gi22947710 RPS2 L6 N RP55 Consensus	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP YKLRVEFLEK KLYEVGSMSK IIYEVTALPD HIYEVSEPTD F-LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK PRSLELFSKH HESIQLFKQH VHACQMLCQS EDAWRLFREN	LRRDV LRRDV LPRDV LRRDV LRRDV LRRDV LRRDV AFKKNTPPSY AFKKNTPPSY AFKKNTPPSY AFKKNYAPKG LRRDV	YET LANDVVD FEK LSLEVVN FED LVVDVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	180 1 VIGSLLFKQE VWGSLLHNLR LLGKYLRRRD	13 13 13 13 13 13 13 13 13 13 13 15 16 15
Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_Deng2000gi/22947705 RGA24[C.trifoliata]pf53680942 Pt11_Deng2000-gi/22947770 RGA24[C.trifoliata]pf53680942 Pt11_Deng2000-gi/22947710 RR52 LG N RP55 Consensus	F - LKVECLGP F - LKVECLGP - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP KLRVEFLEK KLYEVGSMSK KLYEVGSMSK HIYEVSFPTD F - LKVECLGP	EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK PRSLELFSKH VHACQMLCQS EDAWRLFREN	LRRDV	YETLANDVVD FEKLSLVVN FEDLVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	180 VIGSLLFKQE VWGSLLHNLR LLGKYLRRRD	13 13 13 13 13 13 13 13 13 14 15 16 15 16
** Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[1]Nam Hom] Pt9_r[1]nd R2[M3] Pt9_beng2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RF25 Conservation Conservation	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP KLTVEFLEK KLYEVGSMSK KLYEVGSMSK HIYEVSFPTD F-LKVECLGP	EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN HASELFSKH VHACQMLCQS EDAWRLFREN	LRRDV	YET LAND VVD FEK LS LEVVN FED LVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKGE VWGSLLHKGE LGKYLRRD	13 13 13 13 13 13 13 13 13 12 16 15 16
۲۵۵ Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_Deng2000gi/22947705 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RPS2 LG N RPS5 Consensus Sconsensus Sconservation 100 100 100 100 100 100 100 10	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I I YEVTALPD HI YEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HASLELFSKH HESIQLFKQH VHACQMLCQS EDAWRLFREN	LRRDV	YET LANDVVD FEK LSLEVVN FED LVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHKQE UWGSLLHNLR LLGKYLRRRD	13' 13' 13' 13' 13' 13' 13' 13' 13' 15' 16' 15'
94 Pt9_r[Pan] Pt9_R[Pan] Pt9_R[Pan] Pt9_R[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_Deng2000gi[22947705 RG424[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947705 RG424[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947705 RF52 Conservation 100 100 100 100 100 100 100 10	F - LKVECLGP F - LKVECLGP KL IY EVTALPD HIYEVSFPTD F - LKVECLGP		LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AF KKNTPPSY AF KKNTPPSY AF KKNTPPSY AF KKNTPPSY AF KKNTPSY AF KKNTPSY		TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE LLGKYLRRRD	13' 13' 13' 13' 13' 13' 13' 13' 12' 16' 15' 16'
** Pi9_r[Pan] Pi9_R[Nam Hom] Pi9_R2[Nam Hom] Pi9_R2[Nam Hom] Pi9_R2[Nam Hom] Pi9_R1 and R2[M33] P19_Deng2000gi/22947710 RGA24[C.trifoliata]gi53860942 P11_Deng2000_i22947710 RFS2 L6 N RPP5 Consensus Conservation ************************************	F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP F-LKVECLGP YKLRVEFLEK KLYEVGSMSK IIYEVTALPD HIYEVSFPTD F-LKVECLGP	LDNHPD I PEL	LRRDV	YET LANDVVD FEK LS LEVVN FEE LVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKOE VWGSLLHNCR LGKYLRRD	13 ¹ 13 ¹
۲۹۹ _[Pan] Pig_R[Pan] Pig_R[Pan] Pig_R[Nam Hom] Pig_R2[Nam Hom] Pig_R2[Nam Hom] Pig_R1 and R2[M33] Pig_Deng2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947710 RPS2 Consensus RPS5 Consensus room Pig_R[Pan] Pig_R[Pan]	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK KLYEVGSMSK KLYEVGSMSK F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN HACQMLCQS EDAWRLFREN 200 200 LDNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKENNEN LRRDV	YETLANDVVD YETLANDVVD FEDLVVDVVR FEDLVVDVVR	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFRQE VIGSLLFRQE UWGSLLHNLR LLGKYLRRRD	13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 12 ¹ 16 ¹ 15 ¹ 16 ¹
*** Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Nam Hom] Pt9_r[2]Nam Hom] Pt9_r[2]Nam Hom] Pt9_t1_2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 RGA24[C	F - LKVECLGP F - LKVECLGP - LKVECLGP - LKVECLGP F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN UNHPDIPEL LDNHPDIPEL	LRRDV	160 1 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKGE VWGSLLFKGE	13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 14 ¹ 15 ¹ 16 ¹
*** Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_beng2000gi]22947705 RGA24[C.trifoliata]gi53680942 Pi11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pi11_beng2000-gi22947710 RPS2 LG6 N RPP5 Conservation Conservation Pi9_r[Pan] Pi9_r2[Pan] Pi9_r2[Pan] Pi9_r2[Pam] Pi9_r2[Pam] Pi9_r2[Pam]	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I I YEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HAWELFCSK HASILFSKH HESIQLFKQH VHACQMLCQS EDAWRLFREN 200 200 200 200 200 200 200 200 200 20	LRRDV	160 1 Y ET LAND VVD FEK LS LEVVN FED LVVD VVR 6 6 6 6 6 7 7 48 48 48 48 48	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHKQE	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 15 ⁻ 16 ⁻ 15 ⁻ 16 ⁻
Pi9_r[Pan] Pi9_R[Pan] Pi9_R[Pan] Pi9_R[Nam Hom] Pi9_R2[Nam Hom] Pi9_R2[Nam Hom] Pi9_Deng2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 RGA24[C.trifoliata]gi536809	F - LKVECLGP F - LKVECLGP - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVEFLEK KLYEVGSMSK KLYEVGSMSK F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN UDAWRLFCSK EDAWRLFREN 2000 1 UDNHPDIPEL LDNHPDIPEL LDNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AF KKNTPPSY AF KKNTPPSY AF KKNTPPSY AF KKNTPPSY AF KKNTPPSY AF KKNTPSY AF KKNTSY AF KKNTPSY AF KKNTPSY	160 1 YETLANDVVD YETLANDVVD FEDLVVDVVR FEDLVVDVVR 48 48 47	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE UWGSLLFHNLR LLGKYLRRRD	13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 15 ¹ 16 ¹
*** Pi9_r[Pan] Pi9_r[Pi0, Pi9_r[Pi0, Pi9_r[2]Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] RGA24[C.trifoliata]gl53680942 Pi11_Deng2000gl22947710 RF25 Conservation *** Pi9_r[Pan] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r22[Nam Hom] Pi9_r2[Nam Hom] Pi9_r	F - LKVECLGP F - LKVECLGP F - LKVECLRP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I YEVTALPD HIYEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HESIGLFKGH VHACQMLCQS EDAWRLFREN 200 DAWRLFREN LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AFGKEVPNEN AGGKEVPNEN AGGKEVP	160 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHNLR LLGKYLRRD	13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 12 [°] 16 [°] 15 [°] 16 [°]
Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r0ng2000gi[22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000-gi22947705 Consensus Consensus Pi9_r2[Nam Hom] Pi9_r2[Nam Ho	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK KLYEVGSMSK HIYEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK PRSLELFSKH VHACQMLCQS EDAWRLFREN 200 LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AF KK NTPPSY AF KK VPNEN AF KQNYAPKG LRRDV AF SVAKG - 1 ARSVAKG -	160 1 YETLANDVVD FEDLVVDVVR FEDLVVDVVR 6 48 48 48 47 48 48 48 47 48 50	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE UWGSLLFKNE LLGKYLRRRD	13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 13 [°] 15 [°] 16 [°] 16 [°]
Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi229477710 RGA24[C.trifoliata]gi53680942 NRPP5 Consensus Conservation Pt9_r[Pan] Pt1_beng2000-gi22247710	F - LKVECLGP F - LKVECLGP - LKVECLGP - LKVECLGP F - LKVECLGP	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN UNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFGKEVFNEN AF	160 1 YETLANDVVD FEKLSLEVVN FELVVDVVR FEDLVVDVVR 48 48 48 47 48 45 50 50	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKOE VWGSLLHNLR LIGKYLRRD	13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 13 ⁻ 15 ⁻ 16 ⁻ 15 ⁻ 16 ⁻
*** Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_beng2000gi]22947705 RGA24[C.trifoliata]gi53680942 Pi11_beng2000_i22947710 RPS2 LG N RPS5 Consensus ***********************************	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I I YEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HASIELFSKH HESIQLFKQH VHACQMLCQS EDAWRLFREN 200 1000000000000000000000000000000000	LRRDV	160 1 YETLANDVVD FELLANDVVD FELLEVVN FEDLVVDVVR 48 48 47 48 48 47 48 45 50 50 50 40	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHKQE VWGSLLHNLR LLGKYLRRRD	13 13 13 13 13 13 13 13 13 12 16 15 16
Pi9_r[Pan] Pi0_r[Pan] Pi9_r[Pan] Pi0_r[Pan]	F - LKVECLGP F - LKVECLGP HIYEVSFPTD F - LKVECLGP I - LKVECLGP HIYEVSFPTD F - LKVECLGP	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN UDAWRLFCSK PRSLELFSKH VHACQMLCQS EDAWRLFREN 2000 1 DNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AF SK VAKG 1 AF SK VAKG 1 ARSVAKG 1	160 160 160 160 160 160 160 160	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE LLGKYLRRRD	13 ¹ 13(13) 13(13) 13(13) 13(13) 13(14) 16(15) 16(
*** Pi9_r[Pan] Pi9_R[Nam Hom] Pi9_R2 AllNam Hom] Pi9_R2 and R2[M33] Pi9_Deng2000gi/22947705 RGA24[C.trifoliata]gl53680942 Pt11_Deng2000_i22947710 RF92 L6 N RP95 Conservation *** Pt9_r[Pan] Pt9_R2[Nam Hom] Pt9_R2[Nam Hom] Pt9_R2	F - LKVECLGP F - LKVECLGP F - LKVECLRP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I YEVTALPD HIYEVSEPTD F - LKVECLGP I - LKVECLGP	1400 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HASSELFSKH HESIGLFKGH VHACQMLCQS EDAWRLFREN 200 DAWRLFREN LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL	LRRDV	160 160 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHNLR LLGKYLRRD	13 ¹ 13(13) 13(13) 13(13) 13(13) 13(14) 16(15) 16(
Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_r[Pan] Pi9_peng2000gi]22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000_gi22947705 RGA24[C.trifoliata]gi53680942 Pi11_Deng2000gi]22947705 Consensus conservation Pi9_r[Pan] Pi9_r[Pan] Pi9_r2[Nam Hom] Pi9_r2[Nam Hom] Pi9_r2[Na	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I Y EVTALPD H I YEVSFPTD F - LKVECLGP - LKV	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK PRSLELFSKH VHACQMLCQS EDAWRLFREN 200 LONHPDIPEL LSSSIRRL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV RRDV RRDV AF KKNTPPSY AF KKVPNEN AF KQNYAPKG LRRDV AF SVAKG - 1 ARSVAKG -	160 160 160 160 160 160 160 160	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE WGSLLFKNE LLGKYLRRRD	13 ¹ 13 ¹
Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] RGA24[C.trifoliata]gi53680942 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Pan] Pt9_r[Nam Hom] Pt9_r[Nam Hom] Pt9_r[Nam Rom] Pt9_r[Nam Rom] Pt9_r[Nam Rom] Pt9_r[Nam Rom] Pt1_Deng2000-gi22947710 Pt11_Deng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 Pt11_beng2000-gi22947710 RGA24[C.trifoliata]gi53680942 RGA24[C.trifoliata]gi5368094	F - LKVECLGP F - L	140 EDAWRLFREN EDTWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFSK PRSLELFSKH VHACQMLCQS EDAWRLFREN LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LCNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV AF SK NTPPSY AF SK VAKG 1 AF SVAKG 1 ARSVAKG 1 AR	160 1 YETLANDVVD FEKLSLEVVN FELVVDVVR FEDLVVDVVR 48 48 47 48 45 50 50 50 50 50 50 50 50 50 5	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VIGSLLFKQE LLGKYLRRRD	13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 12 ¹ 16 ¹ 15 ¹ 16 ¹
Pt9_r[Pan] Pt9_r[Pan]	F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP F - LKVECLGP YKLRVEFLEK KLYEVGSMSK I YEVSFPTD F - LKVECLGP - LKVECLGP I Y EVSFPTD F - LKVECLGP - LKV	140 EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN EDAWRLFREN KHAWELFCSK HESIGLFKQH VHACQMLCQS EDAWRLFREN 200 DNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LDNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL LCNHPDIPEL	LRRDV LRRDV LRRDV LRRDV LRRDV LRRDV RRV AFGKEVPNEN AFGKEVPNE	160 1 Y ET LAND VVD F EK LS EVVN F ED L VVD VVR 48 48 47 48 48 47 48 48 49 1	TTAGLPLTLK YAKGLPLALK HAGNFPLGLN	VIGSLLFKQE VWGSLLHKQE	13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 13 ¹ 12 ¹ 16 ¹ 15 ¹ 16 ¹

Figure 1 Alignment of predicted Pt9 amino acid sequences from Pan, Nam Hom and M33 limes compared with the NBS domains of other R proteins: *C. grandis* x *P. trifoliate* Pt9, Pt11 (Deng et al., 2000), *C. triloliata* RGA 24 (Shen et al., 2004), RPP5 (Noel et al., 1999), tobacco N (Witham et al. 1994), *Arabidopsis* RPS2 (Bent et al. 1994; Mindrinos et al. 1994) and flax L6 (Lawrence et al. 1995). Consensus kinase-2 and kinase-3a motifs are square. The amino acid mutations in Pt9-r Pan are circled.



Figure 2 Alignment of predicted Pt14 amino acid sequences from Pan, Nam Hom and M33 limes compared with the NBS domains of other R proteins: C. grandis x P. trifoliate Pt14 (Deng et al., 2000), RPP5, tobacco N, Arabidopsis RPS2 and flax L6. Consensus kinase-2 and kinase-3a motifs are square. The mutated amino acid is circled.

		20		40		60	
16R1-19 r[Pan]	-cs	EE - HDEDV	VIWSVVSREP	MAROLOEDIG	KRIGESTDSW	ERKSE-EERA	48
16R1-19 R[Pan]	- CS	EE - HDFDV	K-GSVVSREP	NLKQIQEDVG	KRIGFSKNSW	QDKSF-EERA	47
16R1-19 R1 and R2[Nam Hom]	- C S	EE - HDFDV	VIWSVVSREP	NLNQIQEDIG	KRIGFSTDSW	ERKSF - EERA	48
16R1-19_R1 and R2[M33]	- c s	EE - HDFDV	K-GSVVSREP	NLNQIQEDVG	KRIGFSKNSW	QDKSF - EERA	47
RGA20[C.trifoliata]_gi 53680934	FCS	EE - HDFDV	VIWSVVSREP	NLMQIQEDIG	KRIGFSTDSW	QRKSL-EERA	49
RGA21[C.trifoliata]_gi 53680935	FCS	EE - HDFDV	VIWSVVSREP	NLMQIQEDIG	KRIGFSTDSW	QRKSL-EERA	49
16R1-19_Deng2000gi 22947604	FCS	EE - HDFDV	VIWSVVSREP	NLMQIQEDIG	KRIGFSTDSW	QRKSL-EERA	49
16R1-13_Deng2000gi 22947596	FCG	DEQHHFDV	VIRSVVSREP	NMKQIQEDIG	KRIGFSKNSW	QDKSF - EERA	50
RGA12[C.trifoliata]_gi 53680918	FCS	EE - HDFDV	VIWSVVSREP	NLMQIQEDIG	KRIGFSTDSW	QGKSF-EERA	49
RPS2		QYDV	LIWVQMSREF	GECTIQQAVG	ARLGLSW	DEKETGENRA	41
L6	FDCCCFTD	NIRETQEKDG	VVVLQKK	LVSEILRIDS	GSVGFNNDSG	GRKTIKER	53
N	FDGA CFLK	DIKENKRG	MHS LQNA	LLSELLK	CKKDIKIDDD	GKHQMASK	48
RPP3,	TURAFVSTSK	NITSGANPOD	PNWKLQLQGH	FLSEIL	GRADIAIDDP	AALEEK	52
Consensus 100%	FCS	EE-HDFDV	VIWSVVSREP	NLMQIQEDIG	KRIGFSIDSW	QRKSF-EERA	
Conservation							
16D1 10 (Dec)		KINASE-Z			DIVETTREEC	TOOKMOAHKN	100
16P1-19_[[Pan]	SDITNSIKHK		ESEIDLIKLG	VPLQTLDSGS	RIVETTREEG	TCGKMGAHKN	100
16R1-19_R[Fall]	SDITNSLKHK		ESEIDLIKLG	VPLQTLDSDS	RIVETTREEG	TCGKMGAHKN	107
16P1-19_P1 and P2[M33]	SDITNTIKHK	KEVILIDDIW	ESEIDITKIG	VPLOTIDSCS	RIVETTREEG	TCGKMGAHKN	107
RGA20[C trifoliata] dil53680934	SDITNSIKHK	KEVILIDDIW	ESEIDITKIG	VPLOTIDSGS	RIVETTREEG	TCGKMGAHKN	109
RGA21[C trifoliata]_gi[53680935	SDITNSLKHK	KEVLLDDIW	ESEIDLTKLG	VPLOTIDSGS	RIVETTREEG	TCGKMGAHKN	109
16R1-19 Deng2000gil22947604	SDITNSLKHK	KEVLLDDIW	ESEIDLTKLG	VPLQTLDSGS	RIVFTTREEG	TCGKMGAHKN	109
16R1-13 Deng2000gil22947596	SDITNTLKHK	KEVLLLDDIW	EFEIDLTKLG	VPLOTLDSGS	RIVFTTREEG	TCGKMGAHKN	110
RGA12[C.trifoliata] gi 53680918	SDITNTLKHK	KFVLLLDDIW	ESEIDLTKLG	VPLLTLDSGS	RIVFTTRFEG	TCGKMGADKN	109
RPS2	LKIYRALRQK	RFLLLLDDVW	E-EIDLEKTG	VPRPDRENKC	KVMFTTRSIA	LCNNMGA - EY	99
L6	VSRF	KILVVLDDVD	EKFKFEDMLG	SPKDFISQ-S	RFIITSRSMR	VLGTLNENQC	106
N	LRSK	KVLIVLDDID	NKDHYLEYLA	GDLDWFGNGS	RIIITTROKH	L I EKND	98
RPP5,	LKHQ	KVLIIIDDLD	D-IMVLDTLV	GQTQWFGYGS	RIIVVTNDKH	FL IAHGID	103
Consensus	SDITNSLKHK	KFVLLLDDIW	ESEIDLTKLG	VPLQTLDSGS	RIVFTTRFEG	TCGKMGAHKN	
100% Conservation							
001301444011							
0%							
0%		140		160		180	
% 16R1-19_r[Pan]	R-YKVFCLGD	140 I DDAWKLFE		160 I	G		136
% 16R1-19_r[Pan] 16R1-19_R[Pan]	R - YKVFCLGD R - YKVFCLGD	140 I DDAWKLFE DDAWKLFE		160 I	G		136 135
∾ 16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam_Hom]	R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD	140 1 DDAWKLFE DDAWKLFE DDAWKLFE		160 I	G G	180 VVCSVLNKH VIGRYVLNKH VVGRYVLNKH	136 135 136
** 16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam_Hom] 16R1-19_R1 and R2[M33]	R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD	140 T DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE		160 I	G G G	180 VVC VLNKH VIGRYVLNKH VVGRYVLNKH VIGRYVLNKH	136 135 136 135
™ 16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam_Hom] 16R1-19_R1 and R2[M33] RGA20[C.trifoliata]_gij53680934	R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD R - YKVFCLGD	140 DDAWKLFE DDAWKLFE DDAWKLFE - DDAWKLFE DDAWKLFE		180 1	G G G G	180 VVC VLNKH VIGRYVLNKH VVGRYVLNKH VIGRYVLNKH VVGSYVLNKH	136 135 136 135 137
** 16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[M33] RGA20[C.trifoliata]_gi[53680934 RGA21[C.trifoliata]_gi[53680935	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD	DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE	·····	160 I	G G G G	180 VVG VLNKH VIGRYVLNKH VVGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH	136 135 136 135 137 137
**************************************	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD	140 1 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE		160	G G G G G 	180 VVCS VLNKH VIGRYVLNKH VIGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH	136 135 136 135 137 137 137
16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Mam] 16R1-19_R1 and R2[Mam] RGA20[C.trifoliata_g]53680934 RGA221[C.trifoliata_g]53680935 16R1-19_peng2000g]22947504 16R1-13_Deng2000g]22947504 16R1-13_Deng2000g]22947504	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD	140 1 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE			G G G G G 	180 VVCSVLNKH VIGRVVLNKH VIGRVVLNKH VIGRVVLNKH VVGSVVLNKH VVGSVALNKH VVGSVVLNKH VVGRVVLNKH	136 135 136 135 137 137 137 138
16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[M33] RGA20[C.trifoliata]_gi[53680935 16R1-19_Deng2000gi]22947596 RGA12[C.trifoliata]_gi[53680918 RGA12[C.trifoliata]_gi[53680918	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE		160		180 VVCSVLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VVGRYVLDKL	136 135 136 135 137 137 137 138 137
16R1-19_[PR] 16R1-19_R[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Mar] RGA20[C.trifoliata_g]53680934 RGA21[C.trifoliata_g]515860936 16R1-19_Deng2000g]22947504 16R1-13_Deng2000g]22947504 16R1-13_Deng2000g]22947506 RGA12[C.trifoliata]_g]53680918 RP22	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVFYLGD K-EKVFYLGD	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE KHAWELFE KHAWELFE KHAWELFE				100 VVC VLNKH VIGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VVGRYVLNKL KWRKDLLES	136 135 136 135 137 137 137 138 137 127
16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_Deng2000gi]22947604 16R1-13_Deng2000gi]22947604 16R1-13_Deng2000gi]22947696 RGA12[C.trifoliata]_gi]53680918 RFS2 RGA12[C.trifoliata]_gi]53680918 RFS2 RFS2 NB	R - YKVFCLGD R - FKVFYLGD K - EVVFLEK K L YVEFLEK K L YVEFLEK K L YVEFLEK K L YVETLEN K L YVETLEN	140 DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- RSLELFSKH	AFKKNTPPSY	160 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	G G G G G G G G G G G G G G G G G G G	100 VVCSVLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYLLKK EVWCKLLES VIGSLLFKQE	136 135 136 137 137 137 137 138 137 127 166
" 16R1-19_[Pan] 16R1-19_[RPan] 16R1-19_R1 and R2[Nam_Hom] 16R1-19_R1 and R2[M33] RGA20[C.trifoliata]_gi53680934 RGA21[C.trifoliata]_gi53680918 RGA12[C.trifoliata]_gi53680918 RGA2[C.trifoliata]_gi53680918 RF25 RGA12[C.trifoliata]_gi53680918 RF25 L6 N RPP5	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-KVPYLGD K-FKVPYLG K-FKVPYLG K-FKVPYLG K-FKVPYLG K-FKVFYLG K-FKVFYLG K-FYFT	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE MHAUELFC PRSLELFSKH HESIQLFKQH	AFKKNTPPSY AFGKEVPNEN AFKONYAPKG	TET LANDVVD YET LANDVVD FEK LSLEVVN FED LVDVVP	G G G G G G G G G G G G G G G G G G G	180 VVCSVLNKH VIGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLDKL KVWRKDLLES VIGSLLFKQE VWGSLLHNLR LGKYLRRRD	136 135 136 135 137 137 137 138 137 127 166 158 163
16R1-19_[Pan] 16R1-19_R1-19_R[Pan] 16R1-19_R1 and R2[Mam] 16R1-19_R1 and R2[Mam] RGA20[C.trifoliatagi53680934 RGA21[C.trifoliatagi53680936 16R1-19_Deng2000gi22947504 16R1-13_Deng2000gi22947506 RGA12[C.trifoliata_gi53680918 RPS2 LG N RPS2 LG N N RPP55 Consensus	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVPYLGD K-LRVEFLEK KLYEVGSMSK KLYEVGSMSK R-YKVFCLGD	140 DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- RSLELFSKH HESIQLFSKH HESIQLFKQH VHACQMLCQS DDAWKLFE-	AFKKNTPPSY AFGKEVPNEN AFGKQNYAPKG	TET LANDVVD YET LANDVVD FEK LSLEVVN FED LVVDVVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH V GRYVLNKH VGRYVLNKH VGSYVLNKH VGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VGSYLLKL KVWRKDLLES V GSLLFKQE VWGSLLHNLR LLGKYLRRRD	136 135 136 135 137 137 137 137 127 166 158 163
*** 16R1-19_r[Pan] 16R1-19_R1 16R1-19_R[Pan] 16R1-19_R1 and R2[Mas] 16R1-19_R1 and R2[Mas] RGA20[C.trifoliata]_gi[53680934 RGA21[C.trifoliata]_gi[53680934 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-19_R1 16	R - YKVFCLGD R - FKVFYLGD K - FKVFYLGD K - FKVFYLGD H - YKVFCLGD H - YKVFCLGD R - YKVFCLGD	140 DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- DDAWKLFE- RSLELFSKH HESIQLFKQH VHACQMLCQS DDAWKLFE-	AFKKNTPPSY AFGKEVPNEN AFGKQNYAPKG	Teologian YETLANDVVD FEDLVVDVVR	G G G G G G G G G G G G G G G G G G G	100 VV VLNKH VIGRYVLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLDKL VVGSVLNKH VVGRVLDLES VIGSLLFKQE VMGSLLHNLR LLGKYLRRRD VVGSYVLNKH	136 135 136 135 137 137 137 138 137 127 166 158 163
" 16R1-19_[Pan] 16R1-19_[RPan] 16R1-19_[R1an] 16R1-19_R1 and R2[Nam_Hom] 16R1-19_R1 and R2[M33] RGA20[C.trifoliata]_gi53680935 RGA21[C.trifoliata]_gi53680918 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-13_Deng2000gi22947504 16R1-19_R1and 16R1	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-EKVPYLGD K-EKVPYLGD K-EKVFYLGD HIYEVTALPD HIYEVTALPD R-YKVFCLGD	100 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE KHAWELFC RSIGLFKQH HESIGLFKQH VHACQMLCQS DDAWKLFE	AFKKNTPPSY AFGKEVPNEN AFKONYAPKG	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSLLHNLR LLGKYLRRD VVGSVLNKH	136 135 136 135 137 137 137 138 137 127 166 158 163
" 16R1-19_[[Pan] 16R1-19_R1 and R2[Mar]. 16R1-19_R1 and R2[Mar]. 16R1-19_R1 and R2[Mar]. 16R1-19_R1 and R2[Mar]. 16R1-19_Deng2000jl22947504 16R1-13_Deng2000jl22947504 16R1-13_Deng2000jl22947506 RGA12[C.trifoliata]_gl53680918 RPS2 LG N RPS5 Consensus 1000 100	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVPYLGD K-FKVPYLGD K-FKVPYLGD R-YKVFCLGD	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE PRSLELFSKH HESIGLFKGH VHACQMLCQS DDAWKLFE DDAWKLFE 200	AFKKNTPPSY AFGKEVPNEN AFGKONYAPKG		G G G G G G G G G G G G G G G G G G G	100 VV VLNKH V GRYVLNKH VGRYVLNKH VGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VVGRYVLNKH LLGKYLRRRD VVGSYVLNKH	136 135 136 135 137 137 137 137 138 137 166 158 163
" 16R1-19_[Pan] 16R1-19_R1and R2[Nam, Horn] 16R1-19_R1and R2[M33] RGA20[C.trifoliata_gi]53680934 RGA21[C.trifoliata_gi]53680938 16R1-19_Deng2000gi]22947594 16R1-13_Deng2000gi]22947594 16R1-13_deng2000gi]22947594 16R1-13_deng2000gi]22947596 16R1-19_R080918 RGA12[C.trifoliata]_gi]53680918 RFS5 Consensus 16R1-19_R080918 100 100 100 100 100 100 100 1	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVFYLGD K-EKVFYLGD K-EKVFYLGD R-YKVFCLGD R-YKVFCLGD	10 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE KHAWELFC FRSLELFSKH HESIQLFKQH VACQMLCQS DDAWKLFE 2000 VAP	AFKKNTPPSY AFGKEVPNEN AFKONYAPKG	YETLANDYVD FEKLSLEVYN FEKLSLEVYN	G G G G G G G G G G G G G G G G G G G	100 VV VLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLDKL VVGSYLNKH VVGSYLNKH	136 135 136 137 137 137 137 138 137 127 166 158 163
" 16R1-19_[Pan] 16R1-19_R1 and R2[Mam, Horn] 16R1-19_R1 and R2[Mam, Horn] 16R1-19_R1 and R2[Mam, Horn] 16R1-19_R1 and R2[Mam, Horn] 16R1-13_Deng2000ji[22947504 16R1-13_Deng2000ji[22947504 16R1-13_0]53680918 RPS2 6 N RPP55, Conservation 0 16R1-19_[Ra] 16R1-19_[Ra] 16R1-19_[Ra] 16R1-19_[Ra] 16R1-19_[Ra]	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVPYLGD K-FKVPYLGD R-YKVFCLGD R-YKVFCLGD PDI-PKLAEH	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE PRSELFSKH HESIQLFKQH VHACQMLCQS DDAWKFE 200 VAR 200 VAR	AFKKNTPPSY AFGKEVPNEN AFKONYAPKG	YETLANDVVD FEKLSLEVVN FELLVVDVVR	G G G G G G G G G G G G G G G G G G G	100 VV VLNKH V GRYVLNKH VGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH EVWRKDLLES V GSLLFNLR LLGKYLRRRD VVGSYVLNKH	136 135 136 137 137 137 137 138 137 127 166 158 163
*** 16R1-19_r[Pan] 16R1-19_R1 and R2[Mam] 16R1-19_R1 and R2[Mam] 16R1-19_R1 and R2[Mam] RGA20[C.trifoliata]_gi53680934 RGA21[C.trifoliata]_gi53680935 16R1-13_Deng2000[22947506 RGA12[C.trifoliata]_gi53680935 RGA12[C.trifoliata]_gi53680935 RGA12[C.trifoliata]_gi53680935 RGA12[C.trifoliata]_gi53680935 RGA12[C.trifoliata]_gi53680935 RGA12[C.trifoliata]_gi53680935 Conservation % 16R1-19_r[Pan] 16R1-19_R[Pan] 16R1-19_R[Pan] 16R1-19_R[Pan]	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVPYLGD R-FKVPYLGD K-EKVFYLGD HIYEVSFPTD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH	140 DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. KHAWELFC. PRSLELFSKH VHACQMLCQS DDAWKLFE. 200 VAR.	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFKONYAPKG 		G G G G G G G G G G G G G G G G G G G	100 VV () VLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLDKL VVGSLFKQE VIGSLLFKQE VVGSLFKQE	136 135 136 135 137 137 137 137 138 137 127 166 158 163
<pre>16R1-19_[Pan] 16R1-19_RPan] 16R1-19_RPan] 16R1-19_R1 and R2[Mam] 16R1-19_R1 and R2[Mam] RGA20[C.trifoliata_gi]53680934 RGA21[C.trifoliata_gi]53680938 RGA12[C.trifoliata]_gi]53680918 RGA2[C.trifoliata]_gi]53680918 RGA2[C.trifoliata]_gi]53680918 RR5 Consention 16R1-19_RPan] 16R1-19_R1 and R2[Mam_Hom] 16R1-19_R1 and R2[Mam]</pre>	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-EKVPYLGD K-EKVPYLGD K-EKVPYLGD HIYEVSFPTD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE MAWELFC PRSLELFSKH HESIGLFKGH VHACQMLCQS DDAWKLFE 200 201 VAR VAR VAR VAR VAR	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFKONYAPKG 	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VIGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH ELGKYLRRRD VVGSLLHNLR ELGKYLRRRD	136 135 136 135 137 137 137 137 137 138 137 127 166 158 163
*** 16R1-19_[Pan] 16R1-19_R1 and R2[Mam]Hom] 16R1-19_R1 and R2[Mam]Hom] 16R1-19_R1 and R2[Mam] RGA20[C.trifoliatagi53680934 RGA21[C.trifoliatagi53680918 RGA12[C.trifoliatagi53680918 RPS2 LG N RPP55 Consensus *** 16R1-19_R1 and R2[Mam]Hom] 16R1-19_R1 and R2[Mam]	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-LRVEFLEK KLYEVGSMSK IIYEVTALPD HIYEVSFPTD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH	140 DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - DDAWKLFE - PRSLELFSKH HESIGLFKGH VHACQMLCQS DDAWKLFE - 1 VAR - VAR - VAR - VAR - VAR -	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFGKUPNEN AFGKUPNEN AFKONYAPKG 		G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VVGRYVLNKH LIGKYLRRRD VVGXYVLNKH	136 135 136 135 137 137 137 137 137 166 158 163
16R1-19_[Pan] 16R1-19_[Pan] 16R1-19_[R1 and R2[Nam_Hom] 16R1-19_R1 and R2[Nam] RGA20[C.trifoliatagi]5360034 RGA21[C.trifoliatagi]5360034 16R1-19_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_deng2000gi]22947504 16R1-13_cherg2000gi]22947504 16R1-19_cherg2000gi]22947504 16R1-19_[R1 and R2PAS] 16R1-19_R1 and R2[Nam] 16R1-19_R1 and R2[Nam]Hom] 16R1-19_R1 and R2[Nam] 16R1-19_R1 and R2[Nam]Hom] 16R1-19_R1 and R2[Nam] 16R1-19_R1 and R2[Nam]Hom] 16R1-19_R1 and R2[Nam]Hom] 178_R1 and R2[Nam]Hom] 18_R1 and	R - YKVFCLGD R - YKVFCLGD K - EKVFYLGD K - EKVFYLGD K - EKVFYLGD R - YKVFCLGD PDI - PKLAEH PDI - PKLAEH PDI - PKLAEH PDI - PKLAEH PDI - PKLAEH PDI - PKLAEH	100 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE RHAWELFC PRSLELFSKH HESIQLFKQH VHACQMLCQS DDAWKLFE 2000 20 VAR 1 VAR 20 VAR 20 VAR 20 VAR	AFKKNTPPSY AFGKEVPNEN AFKQNYAPKG 	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VGRYVLNKH VGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH	136 135 136 135 137 137 137 137 138 137 127 166 158 163
16R1-19_[fPan] 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. RGA20[C.trifoliatagi53680935 16R1-19_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-13_Deng2000gi]22947504 16R1-19_R1 RPP55, Consensus 50 Conservation 16R1-19_[RPan]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVPYLGD K-FKVPYLGD R-YKVFCLD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH	140 DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. NHAWELFC. PRSLELFSKH HESIGLFKQH VHACQMLCQS DAWKLFE. 200 VAR 200	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFKONYAPKG OCH 150 	YETLANDVVD FEKLSLEVVN FELLVVDVVR	G G G G G G G G G G G G G G G G G G G	100 VV VLNKH V GRYVLNKH VGRYVLNKH VGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH ELGKYLRRRD LLGKYLRRRD	136 135 136 137 137 137 137 137 127 127 166 158 163
16R1-19_[fPan] 16R1-19_R1 and R2[Nam, Horn] 16R1-19_R1 and R2[Nam, Horn] 16R1-19_R1 and R2[Nam, Horn] 16R1-19_R1 and R2[Nam] RGA20[C.trifoliatal_gi]53680934 (RGA21[C.trifoliatal_gi]53680938 16R1-19_Deng2000gi]22947596 (Conservation) 16R1-19_R1 and R2[Nam, Horn] 16R1-19_R1 and R2[Nam] 16R1-19_R1 and R2[Nam] 17R1-19_R1 and R2[Nam] 18R1-19_R1 and R2[Na	R - YKVFCLGD R - YKVFCLGD K - KVYFLGD K - KVYFLGD K - KVYFLGD R - YKVFCLGD PDI - PKLAEH PDI - PKLAEH	10 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE NHACQMLCQS DDAWKLFE 2000 21 VAR	AFKKNTPPSY AFGKEVPNEN AFKONYAPKG GCH 150 	YETLANDVVD FEKLSLEVVN FEDLVVVVV	G G G G G G G G G G G G G G G G G G G	VV VUNKH VIGRYVLNKH VGRYVLNKH VGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYLLHNLR VVGSLLHNLR VVGSYLLNKH	136 135 136 137 137 137 137 137 137 166 158 163
16R1-19_[Pan] 16R1-19_RPan] 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. RGA20[C.trifoliatagi53680934 RGA21[C.trifoliatagi53680918 RGA12[C.trifoliata]_gij53680918 RGA2[C.trifoliata]_gij53680918 RPS Consensus Consensus 16R1-19_R1 and R2[Mam,Hom] 16R1-19_R1 and R2[Mam,Hom] 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 17R1-19_R1 a	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVYLGD K-EKVYLGD K-EKVFLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DAWKLFE DAWKLFE DAWKLFE DAWKLFE DAWKLFE NAR 200 200 200 200 200 200 200 20	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFKONYAPKG 	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH V GRYVLNKH VGRYVLNKH VGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH LGKYLRRRD LGKYLRRRD	136 135 136 135 137 137 137 137 138 137 166 158 163
16R1-19_[Plan] 16R1-19_[Plan] 16R1-19_[R1 and R2[Nar, Horn] 16R1-19_R1 and R2[Nar] 16R1-19_R1 and R2[Nar] RGA20[C.trifoliata_g]53680934 (RGA21[C.trifoliata_g]53680934 16R1-13_Deng2000g]22947596 16R1-13_Deng2000g]22947596 L6 N RCPES_ Consensus 16R1-19_[R1 and R2[Nar] 16R1-19_R1 and R2[Nar] 17R1-19_R1 and R2[Nar] 18R1-19_R1 and R2[Nar] 18R1-	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-YKVFCLGD K-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVPYLGD K-FKVPYLGD K-FKVPYLGD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH SDE-LTLAKD	10 DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. DDAWKLFE. RHAWELFC. NHACQMLCQS DDAWKLFE. 200 200 200 200 200 200 200 20	AFKKNTPPSY AFGKEVPNEN AFKQNYAPKG AFKQNYAPKG QCH 155 QCH 155 QC		G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGYVLNKH VVGYVLDKL VVGYVLDKL VVGYVLNKH	1365 1365 1377 1377 1377 1377 1377 1666 1588 163
16R1-19_[R]an] 16R1-19_[R]an] 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. RGA20[C.trifoliatagi53680934 RGA21[C.trifoliatagi53680938 RGA12[C.trifoliata]_gi53680938 RGA12[C.trifoliata]_gi53680938 RRP5, Consentation 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 17.10000000000000000000000000000000000	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-FKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAED SIF.TLAKD SIF.TLAKD	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE MAAWELFC PRSLELFSKH HESIQLFKQH VAR VA	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFGKEVPNEN AFKONYAPKG QCH 155 QCH 155	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VIGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSLLHNLR LLGKYLRRRD VVGSLLHNLR	136 135 136 137 137 137 138 137 127 166 158 163
<pre></pre>	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVPYLGD K-FKVPKLGD R-FKVFCLGD PDI-PKLAEH	140 DDAWKLFE KHAWELFC VAR	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFKONYAPKG 		G G G G G G G G G G G G G G G G G G G	VV VLNKH V GRYVLNKH VGRYVLNKH VGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGYVLNKH VVGYVLNKH VVGYVLNKH LGKYLRRRD VVGSYVLNKH	136 135 136 135 137 137 137 137 137 137 166 158 163
16R1-19_[Pan] 16R1-19_[R] 16R1-19_[R] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680934 RGA21[C.trifoliatal_gi]53680938 RGA21[C.trifoliatal_gi]53680918 RGA21[C.trifoliatal_gi]53680918 RFP5, Conservation 16R1-19_R1 16R1-19_R1 16R1-19_R1Pan] 16R1-19_R1 and R2[Nam, Hom] 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680934 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680935 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680936 RGA12[C:trifoliatal_gi]53680935 16R1-19_Deng2000gi]22947506 RGA12[C:trifoliatal_gi]53680935 16R1-19_Deng2000gi]22947506 RGA12[C:trifoliatal_gi]53680935 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680935 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] RGA20[C:trifoliatal_gi]53680934 16R1-19_R1 and R2[M33] 16R1-19_R1 and R2[M33] 17R1-19_R1 and R	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD K-EKVFLGD K-EKVFLGD R-YKVFCLGD R-	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE NHACQMCQS DDAWKLFE YARCALFSKH HESIQLFKQH VARCALFSKH VARCALFS	AFKKNTPPSY AFGKEVPNEN AFKQNYAPKG GCH 151 	YETLANDVVD FEKLSLEVVN FEDLVVDVVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH VIGRYVLNKH VGRYVLNKH VGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLDKL KVWRKDLLES VIGSLLHNLR LLGKYLRRD VVGSYVLNKH	136 135 136 135 137 137 137 137 137 138 137 127 168 163
16R1-19_[fPan] 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. 16R1-19_R1 and R2[Mam]. RGA20[C.trifoliatagi53680934 RGA21[C.trifoliatagi53680918 RGA12[C.trifoliata]_gi53680918 RGA2[C.trifoliata]_gi53680918 RPS, Consensus 16R1-19_R1 16R1-19_R1and R2[Mam]. 16R1-19_R1and 18R1-19_R1and 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1And 18R1-19_R1	R - YKVFCLGD R - YKVFCLGD K - EKVYLGD K - EKVYLGD K - EKVFLGD PDI - PKLAEH PDI - PKLAEH	140 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE NAR VAR	AFKKNTPPSY AFGKEVPNEN AFGKEVPNEN AFKONYAPKO GCH 151 	YETLANDVVD FEKLSLEVVN FEDLVVDVR	G G G G G G G G G G G G G G G G G G G	VV VLNKH V GRYVLNKH VGRYVLNKH VVGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH LGKYLRRRD VVGSYVLNKH	136 135 137 137 137 137 137 138 137 166 158 163
16R1-19_R[Pan] 16R1-19_R[Pan] 16R1-19_R1 and R2[Nar, Horn] 16R1-19_R1 and R2[Nar] RGA20[C.trifoliata_g]53680934 RGA21[C.trifoliata_g]53680938 RGA21[C.trifoliata_g]53680918 RGA12[C.trifoliata_g]53680918 RFS2 Consensus Conservation 16R1-19_R1 and R2[Nar] 16R1-19_R1 and R2[Nar] 17R1-19_R1 and R2[Nar] 18R1-19_R1 and R2[N	R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-YKVFCLGD R-FKVFYLGD K-EVFYLGD R-FKVFYLGD R-YKVFCLGD PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH PDI-PKLAEH	10 DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE DDAWKLFE RAWELFC NAWELFC 1000000000000000000000000000000000000	AFKKNTPPSY AFGKEVPNEN AFKQKYPNEN AFKQNYAPKG QCH 155 QCH 151 QCH 151 QC	YETLANDYVD FEKLSLEVVN FEKLSLEVVN	G G G G G G G G G G G G G G G G G G G	100 VV VLNKH VIGRYVLNKH VIGRYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGSYVLNKH VVGRYVLNKH VVGRYVLNKH LIGKYLRRRD VVGXYVLNKH	136 135 137 137 137 137 137 137 127 127 166 158 163

Figure 3 Alignment of predicted 16R1-19 amino acid sequences from Pan, Nam Hom and M33 limes compared with the NBS domains of other R proteins: *C. grandis* x *P. trifoliate* 16R1-19, 16R1-13 (Deng et al., 2000), *C. triloliata* RGA 12 RGA 20, RGA21(Shen et al., 2004), *Arabidopsis* RPS2 and RPP5, tobacco N, and flax L6. Consensus kinase-2 and kinase-3a motifs are square. Hypothesis amino acid mutation is circled.

BIOGRAPHY

Ms. Pongpan Songwattana was born on August 15th, 1985 in Samut Sakhon , Thailand. She graduated with a bachelor degree of Crop production of technology from Suranaree University of Technology in Year 2007. She applied to study Master degree course in school of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology whit Asst. Prof. Dr. Mariena Ketudat-Cairns. While studying, she received a Graduate scholarship from SUT to support her tuition and fee. Her research topic was production of polyclonal antibody for citrus canker detection and identification of resistance gene analogs in Thai hybrid lime. The results from some part of this study have been presented as poster and oral presentation at (1) The 15th National Graduated Research Conference, Rajaphat Nakhon Ratchasima 14-15th Dec 2009. (2) 8th International Symposium on Biocontrol and Biotechnology, King Mongkut's Institute of Technology Ladkrabang (KMITL) and Khon Kaen University, Nongkhai Campus, 4-6th October 2010. (3) The 3rd SUT Graduate Academic Seminar 2010 Suranaree University of Technology, Nakhon Ratchsima, 21-23th November 2010.