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Analysis of the genetic background of resistance in potato with special attention to late blight (*P. infestans*) resistance

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Table of Contents

ABSTRACT	8
KIVONAT	9
ABSTRAKT	10
ABBREVIATIONS	11
1. INTRODUCTION	13
Research objectives	15
2. LITERATURE REVIEW	16
2.1. Potato, an overview on origin, variation and production2.1.1. Potato production in Europe	16 17
2.1.2. Potato production in Hungary	18
2.2. Impact of late blight disease on potato and strategy of control2.2.1. Genetic diversity of <i>P. infestans</i>	16 19
2.2.2. Strategies for disease management	20
2.3. Physiological aspects of resistance2.3.1. Recognition of pathogen effectors by R-genes	21 24
2.4. Hypersensitive Reaction (HR) mediated defense in <i>Phytophthora infestans</i>challenged potato2.4.1. Role of reactive oxygen species in hypersensitive response to <i>P. infestans</i>	25 27
2.4.2. Proteinaceous compounds as inhibitors to P. infestans	28
2.4.3. Phytoalexins as anti-fungal compounds produced in potato against <i>P. infestans</i>	30
2.5. Role of NBS-LRR molecules in host defense aginst late blight disease2.5.1. Co-evolution of host-pathogen genes in late blight resistance	31 32
2.5.2. Evolution of different Rpi genes in potato	33
2.6. Characteristics and advantages of major <i>P. infestans</i> resistance genes in breeding 2.6.1. R-gene homologs	35 36
2.6.2. Distribution of R-gene hot spots in the potato genome	37
2.6.3. Utilization of R gene resources in breeding programs	39
2.7. Field resistance	40
2.8. Marker assisted selection in potato resistance breeding 2.8.1. Intron Targeting Markers	42 44

2.9. Sequencing of transcriptome	44 45
2.9.2. Gene quantification by NGS technique	
2.10. Quantitative analysis by real-time PCR	48 49
2.10.2. Quantitative analysis of <i>Rpi</i> genes in potato	51
3. MATERIALS AND METHODS	52
3.1. Plant materials and pathogen isolates	
3.1.2. Pathogen isolates	52
3.1.3. Preparation of inoculums of <i>P. infestans</i>	53
3.1.4. Inoculations in greenhouse	
3.1.5. Detached leaf assay	
3.2. Detection of <i>R</i>-genes in White Lady by specific primers3.3. Bulked analysis of transcriptomes captured in multiple time points by next	55
generation sequencing	
3.4.1. Sequence alignment	
3.4.2. Applied phylogenetic analyzing approaches	59
3.4.1.1. Parsimony analysis	59
3.4.2.2. Maximum Likelihood Analysis	60
3.4.3. Selection test of homologues <i>P. infestans</i> resistance genes in TC database	
 3.4.4. NBS-LRR motifs in <i>R</i>-gene homologs alignments	
3.5.1. PCR procedures for the detection of <i>P. infestans</i> resistance genes and	
homologs	
3.5.2. Cloning and sequencing of the amplified fragments	63
3.6. Quantitative analysis of HR-mediated late blight resistance genes	
2.6.2 aDNA sumthasis by reverse transprintion reaction	
2.6.2. Designing the DTDCD primer pairs	03
2.6.4. OPCD assertises	
3.6.4. QPCK reaction	
3.6.5. Gel electrophoresis, cloning and sequencing of qPCR amplified fragments3.7. Analysis of the effect of <i>P. infestans</i> inoculation on the protein profile of White	
Lady leaves	

4. RESULTS	
 4.1. The R-gene content of White Lady according to inoculation tests	
4.2.2. Results of transcriptome (TC) analysis	73
4.2.3. Copy number and gene regulation	74
4.2.4. Heat map analysis	75
4.2.5. Identification of resistance genes to P. infestans	
4.2.6. Selection test of different <i>R</i> -gene homologs	77
4.2.7. Phylogenetic analyses of the <i>Rpi</i> homologs	
4.2.8. NBS-LRR alignments of <i>R</i> -gene homologs of the TC dataset	
4.3. Development of intron-targeting primers for the detection of the R1 gene4.3.1. Analysis of IT-amplified fragment in gene databases	
4.4. Quantitative analysis	
4.4.1. Protein analysis of <i>P. infestans</i> inoculated potato leaves	
4.4.2.1. Expression analysis of protease inhibitor genes	
4.4.2.2. Expression analysis of reactive oxygen species	
4.4.2.3. Expression analysis of PR proteins and immune receptor genes	
4.4.2.4. Expression analysis of NB-LRR genes	
4.4.3. Gel electrophoresis and sequence analysis of qPCR amplified fragments	
5. DISCUSSION	
5.1. <i>Rpi</i>-genes, importance and struggle for resistance against <i>P. infestans</i>5.2. Phylogenetic relationship of <i>P. infestans</i> resistance genes and gene homologs	
identified in the TC dataset	
5.3. Intron targeting marker development for the detection of the <i>R1</i> gene	
5.4.1. Expression pattern of non-specific resistance genes to <i>P. infestans</i>	101
infestans	
LIST OF NEW FINDINGS	105
ACKNOWLEDGEMENTS	106
PUBLICATION LIST	108
Referred articles related to thesis	

Conference abstracts related to the thesis	
Other publications	109
REFERENCES	
Appendix 1	
Appendix 2	
Appendix 3	
Appendix 4	
Appendix 5	

ABSTRACT

Potato is the third most important food crop worldwide. However, cultivated potato has many diseases there is a large reservoir of different resistance genes. Sources of resistances are mainly in wild *Solanum* species, which have been partially utilized in breeding. Potato is affected among others by different viruses and possibly the most dangerous disease of it is late blight caused by the oomycota *Phytophthora infestans*. The present study is part of a larger program that aims the detection and characterization of biotic stress response genes of potato for future utilization in breeding. To this end by RNA-sequencing a whole genomic transcriptome (TC) dataset of the cultivar White Lady was generated and different analyses of biotic stress response genes were performed. This cultivar was chosen, since it possesses important resistance genes among others to the PVX and PVY viruses and to P. infestans. In the present study we focused on the examination of the genetic background of late blight resistance in this cultivar. By infection tests with different isolates and comparison to the Mastenbroek differential Rlines it was found that White Lady possesses the R1, R2, and R3 race-specific P. infestans resistance genes from among the cloned R-genes that derive from S. demissum. With published sequence specific primers the presence of the R2, R3a and R3b genes could be revealed, while for the R1 gene a set of primers based on transcript sequences of the TC analysis were designed here, from among with the R1L333 intron-targeting primer pair a highly similar gene could be detected. It is suggested that this gene is either an allelic version or a homolog of R1. For the identification of further late blight resistance genes and homologs potentially present in White Lady the transcriptome dataset was analysed. In total 142 P. infestans resistance gene homologs could be identified from among 82 could be used in a phylogenetic analysis. The remaining genes were excluded from the analysis, since their sequence similarity to cloned late blight resistance genes was either too low, or their sequence was too short. In the phylogenetic analysis 21 cloned genes were also used. Results of the analysis revealed that not only S. demissum derived R-gene homologs are present in White Lady, but also homologs of broad spectrum resistance (Rpi) genes of such species which are not present in the genetic background of this cultivar. This indicates the common ancestral origin of P. infestans resistance genes in potato and sheds light on their evolution. Further, from the TC dataset 16 genes of four gene families have been chosen which are known to be active in biotic stress response in plants. By quantitation analysis with qPCR for eleven of these genes it was found that they are up-regulated by the P. infestans inoculation. The expression of these genes was characterized in seven different time points during the early period of the successful resistance response. Besides all the results of this study contribute to our understanding of the genomic background of biotic stress response in potato, it is believed that these results can be utilized in future development of molecular tools to enhance the effectivity of potato breeding.

KIVONAT

A burgonya a harmadik legfontosabb élelmiszernövény a világban. Noha a burgonyának számos betegsége van, a különféle rezisztenciagének széles repertoár-ja áll rendelkezésre. A rezisztenciaforrások leginkább a vad *Solanum* fajokban fordulnak elő, melyek részben már hasznosításra kerültek a nemesítés során. A burgonyát többek között különféle vírusok támadják meg, és a valószínűleg legveszélyesebb kórokozója a burgonyavészt előidéző petespórás gomba (oomycota), a *Phytophthora infestans*. A jelen tanulmány egy átfogó program része, melynek célja a burgonya biotikus stressz-válasz génjeinek jellemzése és hasznosítása a nemesítésben. E célból, ún. RNS-szekvenálással egy teljes genomi transzkriptom (TC) adatbázist hoztunk létre a White Lady fajtából, és a biotikus stressz-válasz gének különböző vizsgálatát végeztük el. E fajta számos értékes rezisztenciagénnel bír, többek között rezisztens a PVX és PVY vírusokkal, illetve a P. infestans hazánkban elterjedt rasszaival szemben. A jelen tanulmányban e fajta burgonyavész rezisztenciájának genetikai hátterét vizsgáltuk. Különböző izolátumokkal történő fertőzésekkel és a Mastenbroek differenciáló R-vonalakhoz történő hasonlítással megállapítottuk, hogy a White Lady az R1, R2 és R3 rassz-specifikus P. infestans rezisztenciagéneket tartalmazza a S. demissum származású klónozott gének közül. Publikált, szekvencia-specifikus primerekkel igazolni tudtuk a R2, R3a és R3b gének jelenléte, míg az R1 génre a TC adatbázis alapján különböző primereket terveztünk, melyek segítségével a R1L333 intron-targeting primer pár az R1 génnel nagyfokú hasonlóságot mutató szekvenciát detektált. Feltételezzük, hogy az a gén egy allélikus verziója vagy egy homológia az R1 génnek. További lehetséges burgonyavész rezisztenciagének és homológok azonosítása céljából a TC adatbázist elemzésével 142 P. infestans rezisztenciagén homológot találtunk, melyek közül 82-t filogenetikai vizsgálatát végeztük el. A többi gént kizártuk a vizsgálatból, mivel vagy a szekvencia-hasonlóságuk volt túl alacsony ismert rezisztenciagénekkel, vagy túl rövidek voltak. A vizsgálatba bevontunk még 21 klónozott P. infestans rezisztenciagént. Eredményeink azt mutatják, hogy nemcsak S. demissum eredetű R-gén homológok vannak jelen a White Lady fajtában, hanem horizontális rezisztenciát biztosító (*Rpi*) gének is olyan fajokból, melyek egyébként nincsenek jelenek e fajta genetikai hátterében. Ez a P. infestans rezisztenciagének közös őstől való eredtére utal a burgonyában és fényt vet azok evolúciójára. A továbbiakban a biotikus stressz-válaszban szerepet játszó 4 géncsalád 16 génjét választottuk ki a TC adatbázisból. E gének kifejeződését a fertőzés korai szakaszában hét különböző időpontban vizsgáltuk. E gének közül qPCR vizsgálattal 11 esetében mutattunk ki a P. infestans fertőzés hatására bekövetkező aktivációt. A jelen munka eredményei bővítik ismereteinket a burgonya biotikus stressz-válasz genetikai hátteréről, és úgy gondoljuk, hogy eredményeink a továbbiakban hasznosíthatóak a burgonyanemesítés hatékonyságát növelő molekuláris eszközök fejlesztésében.

ABSTRAKT

Kartoffel ist die drittwichtigste Nahrungspflanze weltweit. Jedoch hat Kartoffel viele Krankheiten, der besteht ein großes Reservoir an verschiedenen Resistenzgene. Quellen der Widerstände sind vor allem in der wilden Solanum-Arten, die teilweise in der Zucht verwendet worden sind. Kartoffel wird unter anderem von verschiedenen Viren und möglicherweise von der gefährlichste Krankheit, Krautfäule der durch die Oomycota Phytophthora infestans verursacht ist, angegriffen. Die vorliegende Studie ist Teil eines größeren Programms, das die Detektion und Charakterisierung von biotischen Stressantwort Gene der Kartoffel für die zukünftige Nutzung in Zucht anzielt. Zu diesem Zweck wurde durch RNA-Sequenzierung eine ganze genomische Transkriptom (TC) Datensatz von der Sorte White Lady generiert und unterschiedliche Analysen der biotischen Stressantwort-Gene wurde durchgeführt. Diese Sorte wurde gewählt, da es besitzt wichtige Resistenzgene unter anderem zu den PVX und PVY Viren und gegen P. infestans. In der vorliegenden Studie haben wir des genetischen Hintergrund der Kraut-und Knollenfäule Widerstand in diesem Sorte untergesucht. Von Infektionstests mit verschiedenen Isolaten und Vergleich mit den Differenz Mastenbroek R Linien wurde festgestellt, daß White Lady besitzt die R1, R2, R3 und rassenspezifische P. infestans Resistenzgene. Mit veröffentlichten Sequenz-spezifischen Primern das Vorliegen der R2, R3a und R3b Gene wurde erschlossen, während für die R1-Gen ein Primer-Set von die Transkript Sequenzen entworfen wurde, und hier aus mit der R1L333 Intron-targeting marker ein hoch ähnliche Gen nachgewiesen werden konnte. Es wird vorgeschlagen, dass dieses Gen entweder eine allele Variante oder ein Homolog von R1 sei. Für die Identifizierung von weiteren Krautfäule-Resistenz-Gene und Homologen die potentiell in White Lady vorhanden sein sollen, wurde das Transkriptom-Datensatz analysiert. Insgesamt aus 142 P. infestans Gen-Homologe 82 konnten in einem phylogenetischen Analyse verwendet werden. Die restlichen Gene wurden aus der Analyse ausgeschlossen, da ihre Sequenzähnlichkeit zu kloniert Krautfäule Resistenzgene entweder zu niedrig oder in ihrer Reihenfolge zu kurz war. In der phylogenetischen Analyse wurden 21 geklonten Gene verwendet. Die Ergebnisse der Analyse zeigten, dass nicht nur S. demissum abgeleitet R-Gen-Homologen liegen in White Lady, aber auch Homologen von Breitspektrum-Widerstand (RPI) Gene für solche Arten, die nicht in den genetischen Hintergrund dieser Sorte vorhanden sind. Dies zeigt die gemeinsame Herkunft der Vorfahren der P. infestans Resistenzgene in Kartoffeln und wirft Licht auf ihre Entwicklung. Ferner, es wurden aus der TC-Datensatz 16 Gene aus vier Genfamilien ausgewählt, die als in biotische Stressantwort Gene in Pflanzen bekannt sind. Durch quantitative Analyse mit qPCR für elf von dieser Gene es wurde festgestellt, dass sie durch der P. infestans Impfung hochreguliert geworden sind. Die Expression dieser Gene wurde in sieben verschiedenen Zeitpunkten während der frühen Periode des erfolgreichen Resistenzreaktion gekennzeichnet. Neben all die Ergebnisse dieser Studie tragen zum Verständnis der genomischen Hintergrund der biotischen Stressantwort in Kartoffel, und es wird angenommen, dass diese Ergebnisse in zukünftigen Entwicklung von molekularen Werkzeugen verwendet werden können, um die Effektivität der Kartoffelzucht zu verbessern.

ABBREVIATIONS

AFLP - Amplified Fragment Length Polymorphism AHP- Apoplastic hydrophobic protein Avr - Avirulence gene **BLAST** - Basic local alignment search tool **BPB** - Brome phenol blue **CAPS** - Cleaved amplified polymorphic sequence CC - Coiled coil domain **4CL**-4 coumarate ligase **CDPK**- Ca²⁺-dependent protein kinase **cDNA** - Complementary deoxyribonucleic acid **ChIP** - chromatin immunoprecipitation **CNL** - CC-NB–LRR **CRN**- crinkling and necrosis **DGE-** Digital gene expression **DNA** - Deoxyribonucleic acid **EU**- European union **EBN** - endosperm balance number **ER** - Extreme Resistance **EST**- Expressed sequence tag **ETI** - Effector triggered immunity GMO - Genetically modified organism **GSPs** - Gene specific primers **GWAS**-Genome wide association studies **H0**- Null hypothesis **HA**- Alternative hypothesis HR - Hypersensitive reaction **IPTG** - Isopropyl β-D-1thiogalactopyranoside **IT** - Intron targeting

JA - Jasmonic acid LRR - Leucine-rich repeat MAPK - Mitogen-activated protein kinase MAS - Marker assisted selection **MPSS**- Massively parallel signature sequencing ML- Maximum likelihood mRNA - Messenger ribonucleic acid **NADPH**-Nicotinamide Adenine Dinucleotide Phosphate Hydrogen **NBS** - Nucleotide-binding site **NBS-LRR** -Nucleotide-binding site leucine reach repeat **NCBI** - National Center for **Biotechnology Information** NGS - Next generation sequencing **NO**⁻ - Nitric oxide **ORFs**- Open reading frames **PAL-** phenylalanine ammonia-lyase **PAMPs** - Pathogen-associated molecular patterns **PIs-** Proteinase inhibitors **PCR** - Polymerase chain reaction Potato-DM - Solanum tuberosum group *Phureja* DM1-3 5116R44 **PR** - Pathogenesis-related genes **PTI** - PAMP triggered immunity Pto - tomato serine-threonine protein kinase **PVX-** Potato virus X **PVY** - Potato virus Y **OTL** - Qualitative trait loci qPCR - quantitative- PCR

RBOHs - Respiratory burst oxidase inhibitors RFLP - Restriction fragment length polymorphism **RNA** - Ribonucleic acid **RNA-seq** - RNA-sequencing **ROS**- Reactive oxygen species Rpi- Phytophthora infestans -Resistance genes **RPKM**- Reads per kilobase of exon per million mapped reads **RT-PCR**- Real-time polymerase chain reaction SA - Salicylic acid SAGE - Serial analysis of gene expression SAR - Systemic acquired resistance **SCAR** - Sequence characterized amplified region

SDS-PAGE- Sodium dodecil sulphatepolyacrylamide gel SGN- SOL genomics network StCDPK-S. tuberosum calciumdependent protein kinase **SNP** - Single nucleotide polymorphism **SOLiD** - Sequencing by oligonucleotide ligation and detection SSR - Simple sequence repeat TC- Transcriptome **TDF-** Transcripts derived fragment TE - Tris-HCL, EDTA buffer **TEMED** - Tetramethylethylenediamine TIR - Toll interleukin-1 receptor domain UK - United Kingdom USA - United States of America **UHTS-** Ultra High-Throughput Sequencing

1. INTRODUCTION

Worldwide, cultivated potato belongs overwhelmingly to Solanum tuberosum L. Wild potato species can be found throughout the Americas, but the primary center is the Andean mountain of Peru and Bolivia where about 7000-10,000 years ago potato was domesticated (Spooner et al., 2005). During the domestication process on the Titicaca plateau the Aymara Indians developed more than 200 potato varieties at 3000 to 4600 meters above the sea level (Sleper and Poehlman, 2006). The importance of potato in the societies of the of origin were documented by many representations of potato on ceramic artworks collected from these area (Bamberg and Del Rio, 2005). This crop was unknown to the rest of the world until the 1500's, but afterward its spread was accelerated all over the world so that nowadays it is accounted as one of the most important food crops in the world along with rice and wheat (Haverkort et al., 2009). The tubers of this plant are carbohydrate rich, are a good source of microelements and vitamins, and are highly popular worldwide, prepared and served in very different kinds and methods. Potato is an unrivalled crop among economically important plants, because a diverse pool of wild species with various ploidy levels is at hand which can be utilized in breeding (Carputo and Barone, 2005). Two hundred ninety wild tuber-bearing Solanum species were recognized which distributed at wide geographic zones from the southwestern United States to central Argentina and southern Chile (Hawkes, 1990). They have different polyploidity from diploid (2n = 2x = 24) to hexaploid (2n = 6x = 72). Cultivated potato, S. tuberosum is a tetraploid (2n = 4x = 48) non-inbred crop species displaying tetrasomic inheritance. To avoid inbreeding depression bred potato should be highly heterozygous, although that complicates the process of improving and makes conventional breeding time consuming.

Potato is vegetatively propagated by tubers. Compared to seeds, with tubers much more diseases and even pests can be transmitted, which then may affect the leaves, stems, roots and the tuber yield. The pathogens which could attack potato belong to different groups

of fungi, oomycota, bacteria, viruses, viroids, phytoplasmas. Besides them also nematodes can be transmitted by tubers and decrease the quantity and quality of yield. Among the pathogens *Phytophthora infestans* that can cause late blight and some viruses like PVY and PVX pose a considerable threat to the crop in potato production areas all around the world. In the twentieth century, shortly after discovery of Mendel's laws of inheritance, a source of genetic resistance to P. infestans was discovered in a tuber bearing wild Solanum species (Gebhardt and Valkonen, 2001). Afterwards many wild Solanum species and accessions of cultivated potato were found to have late blight resistance genes which could be used in classical breeding and in cis-genetic molecular breeding for resistance (Park et al., 2009). In this aspect, localization of traits on the chromosomes, functional characterization of genes and analysis of gene variations have special importance. Nowadays, molecular markers are used as valuable and reliable tools for crop improvement, due to their usefulness in characterizing and mapping genetic loci responsible for monogenic and polygenic resistance traits. The molecular markers can effectively be employed in marker assisted selection (MAS) when they co-segregate with the target gene, they have a high polymorphic resolution, when their use is cost effective, simple and are applicable in high-throughput genotyping systems (Xu et al., 2003; Mohler and Singrün, 2005).

The mechanism of resistance in plants to biotic stresses is complicated and is not completely understood. Several physiological procedures in cells are involved to prevent progression of pathogen invasion locally and systematically through hypersensitive responses which is mediated mostly by major R genes. These R genes encode intracellular nucleotide binding – leucine rich repeat (NB-LRR) molecules which are assumed to regulate the production of biomolecules in signal transduction pathways (Leipe et al., 2004). In order to understand in details the resistance response, it is essential to figure out the role of defensive mechanisms. The quantitative (real-time) PCR technology allows to measure the relative expression level of a particular transcript in a given tissue or cell type and determine the fold change expression of it after being exposed to a specific alteration (Bookout and Mangelsdorf, 2003). More recently transcriptome based analysis of genes and signaling pathways help to better understand biological processes like organogenesis, fertilization or responses to biotic and abiotic

stresses (Yoo and Wendel, 2014). For many years, microarray and serial analysis of gene expression (SAGE) were the primary tools for transcriptome analysis, but recently a promising new ultra high-throughput sequencing (UHTS) technology called next generation sequencing (NGS) with multifunctional purposes was developed. NGS is used for RNA-sequencing (RNA-seq) for assessing the copy number of transcripts and to elucidate more details about any kinds of a transcriptome (Wang et al., 2012). This technique make millions number of reads of genes thereby provide rapid genome-wide expression profiling (Marguerat et al., 2008). In order for screening and selection of the gene homologs which are involved in resistance against *P. infestans*, and for the detection of R-genes with transcript derived markers, a bulked transcriptome analysis of the highly late blight resistant potato cultivar White Lady was performed in the current research. The cumulated dataset obtained by RNA-sequencing was analyzed by different bioinformatics software and stress induced expressional changes of some genes in probable role in stress response to *P. infestans* were examined by qPCR.

Research objectives

The research objectives of the present study are the followings:

- 1) Exploring race-specific resistance genes to *Phytophthora infestans* in White Lady, a Hungarian potato variety with high late blight resistance.
- Evaluation of biotic stress induced expressional changes in White Lady by analysis of RNA-sequencing generated transcriptome dataset.
- Phylogenetic analysis of the *P. infestans* resistance gene homologs of White Lady.
- Based on the transcriptome data of White Lady, development of introntargeting (IT) primers for the detection of R-gene homologs.
- QPCR analysis of the expressional profile of some selected genes known to be involved in biotic stress response.

2. LITERATURE REVIEW

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world after rice and wheat (Haverkort et al., 2009). This crop is rich in carbohydrates, microelements and vitamins, and is highly popular worldwide. Nevertheless, potato is the host of many pathogens, including fungi, bacteria, phytoplasmas, viruses, viroids and nematodes, which cause reductions in yield quantity and quality.

Among the fungal diseases, *Phytophthora infestans* (Mont.) de Bary causing late blight is one of the most important and destructive diseases of potato. In the 1840s it caused the Irish potato famine. Recently, new strains with capability to reproduce sexually are spreading that is associated with increased genetic diversity and survival in many parts of the world (Fry, 2008).

2.1. Potato, an overview on origin, variation and production

The Inca Indians in Peru were the first people who domesticated the potato around 8,000 B.C to 5,000 B.C. After the Spanish conquered the Inca empire, they introduced the potato to Europe in the second half of the 16th century. Since then, it was spread around the world and became as a staple crop in many countries (Hawkes and Francisco-Ortega, 1993).

The genus *Solanum* includes more than 2000 species which is distributed throughout the Americas from the United States to central Argentina and southern Chile (Hijmans and Spooner, 2001). The tuber bearing potatoes are in a range of polyploidy from diploid (2n = 2x = 24) to hexaploid (2n = 6x = 72). The cultivated potato *Solanum tuberosum* L. is a tetraploid (2n = 4x = 48) that displays tetrasomic inheritance and is placed in the series of *tuberosa*. The tetraploid potato (*S. tuberosum*) arose from hybridization of *S. stenotomum* which is domesticated from wild prototype *S. leptophyes*, and a wild diploid species, *S. sparsipilum* (Hawkes, 1988). There are two genetically distinct population groups of *S.*

tuberosum, one is a short-day adapted landrace population of the Andes and the other is long-day adapted of coastal Chile. They have been classified as separate subspecies *S*. *tuberosum* subsp. *andigena* and *S. tuberosum* subsp. *tuberosum* which are referred to as Andigena and Chilean Tuberosum potatoes respectively (Raker and Spooner, 2002). Although, most of the current potato varieties are derived from Chilean lowland races potato, but root testing of potato varieties and wild species showed that they all from a single origin located in southern Peru and northwest of Bolivia (Innovation, 1989; Spooner et al., 2005).

Nowadays potato germplasm preservation in the world is confined to the countries which have one of the followings specificities: i) enriched sources of genetic variation of potato, ii) technologies of preservation, production of pathogen-free seedlings and seed tubers and improving potato by breeding programs (Kaczmarczyk et al., 2011).

According to the FAO statistics in 2012, the total amount of potato production was 364,808,768 tons from 19,202,082 hectares under cultivation (Fao, 2012). Considering 1990 as a base, it can be concluded that potato production dramatically increased until 2012 with about 98,000,000 tons, while the land used for production increased just with 1,546,000 hectares during this 22 years period. This can be due to progress in knowledge and using of new technologies in the fields of crop management and breeding. In the first decade of the 21st century, an average annual diet of a person was about 33 kg of potato. However, the local importance of potato is extremely variable and rapidly changing. It remains an essential crop in Europe (especially eastern and central Europe), where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in southern and eastern Asia (Hijmans and Spooner, 2001). China is now the largest potato-producing country in the world with nearly 24 percent of total production (Fao, 2012).

2.1.1. Potato production in Europe

The first report about cultivation of potato outside South America was in the Canary Island in 1567 and soon thereafter it was brought to Spain in 1573 (Hawkes, 1990; Hawkes and Francisco-Ortega, 1993). Afterwards potato was distributed to whole Europe

and subsequently was exported and cultivated in many other parts of the world and therefore potato is referred as a "European" crop (Hawkes and Francisco-Ortega, 1993).

There is a long controversy about the origin of potato in Europe. Juzepchuk and Bukasov (1929) propose that the European potato originally derived from landraces of Chile (Juzepchuk and Bukasov, 1929), while British investigators believed that it came originally from the Andes and persisted until the occurrence of the big European potato late blight epidemic in 1845 (Salaman, 1937; Salaman and Hawkes, 1949), after which it was replaced with Chilean germplasm through introductions and breeding efforts. Chronological studies with a plastid DNA deletion marker on 49 European herbarium specimens of *S. tuberosum* distinguished germplasms originating from the high Andes and from lowland Chile. Results of this study indicated that Andean potato was predominant in Europe in the 1700s, and the Chilean potato was introduced into Europe as early as 1811 and became predominant long before the late blight epidemics in the UK (Ames and Spooner, 2008).

2.1.2 Potato production in Hungary

Potato is the most consumed vegetable in Hungary. Production area in the Hungary dramatically decreased during the last 15 years from 50.000 to 22.000 ha. However, the average yield increased from 16 ton/ha to over 23 ton/ha during this period. After Hungary joined the EU, the seed potato production area also significantly decreased from 1500 ha to 350 ha. The total production reached 511,100 tons while 54,800 tons were only seed potatoes (Fao, 2012) which is less than 1% of EU's total potato production and could just cover the needs of the local market. Out of the total consumption less than 10% is consumed as processed food. The average consumption of potato is approximately 65 kg/year/capita in Hungary. According to FAO's report, in terms of production Hungary is in the 21st position in potato production and has the 23rd position in terms of production area in Europe. Hungarian varieties are produced on twenty percent of the total production area. All of these varieties were developed by the Potato Research Centre (PRC) of the University of Pannonia located in Keszthely which is the only institute dedicated to potato research and breeding in Hungary.

The Potato Research Centre has a more than 50 years long tradition on potato breeding and R&D on production technologies. The Centre due to its consistent resistancebreeding efforts has utilised germplasm partially originating from wild species and developed 12 varieties which are registered also on the EU list (Arany Chipke, Démon, Balatoni rózsa, Katica, Lorett, Góliát, Rioja, Hópehely, White Lady, Vénusz Gold, Luca XL and Kánkán). These varieties due to their complex resistance against major pests and pathogens, high yielding potential and outstanding consumption quality are unique in their kind. Some of them are especially advised for organic production.

2.2. Impact of late blight disease on potato and strategy of control

Undoubtedly late blight, caused by *Phytophthora infestans* is the most destructive disease of potato. The pathogen *P. infestans* belongs taxonomically to the oomycetes. This pathogen first made its impact outside of Mexico in the mid-1840s when severe epidemics swept through North America and Europe and resulted in the Irish potato famine (Large, 1940). Over 160 years later, still it remains a major and complicated threat for potato cultivation despite different strategies for controlling and holding its aggressiveness down in potato cultivation zones. Annual potato crop losses due to late blight are conservatively estimated about \$ 6.7 billion worldwide (Haverkort et al., 2008). This pathogen is equipped with genetic changes that can overcome the resistance in potato even though in potato cultivars with high level of partial resistance (Inglis et al., 1996; Tai, 1998).

2.2.1. Genetic diversity of P. infestans

P. infestans is heterothallic, requiring two mating types (designated as A1 and A2) for sexual reproduction and the production of oospores. The presence of both mating types allows sexual reproduction that contributes to the formation of resistant oospores in early infections and the adaptation of the pathogen to certain fungicides and also to host resistance. Generally sexual recombination leads to the generation of particularly fit lineages that have new combinations of troublesome traits (Smart and Fry, 2001;

Turkensteen et al., 2008). Another mechanism involved in genetic diversity in the agricultural zone is pathogen migration. This phenomenon appears to define population dynamics of *P. infestans*. Population displacement by genotypes with increased fitness is a recurrent event (Vleeshouwers et al., 2011b).

Before 1980, the worldwide population of *P. infestans* outside Mexico appeared to be asexual and consisted of a single clonal lineage (US-1) of A1 mating type characterized by this single genotype. In contrast, the population in the highlands of Mexico was sexual and consisted of both A1 and A2 mating types which were genotypically highly diverse (Grünwald and Flier, 2005). The global situation was disrupted by at least two different migrations from Mexico in the twentieth century. Due to these events the population genetics of *P. infestans* was dramatically altered and is now recognized as a second wave of introductions (Fry et al., 2009). The first migration of the A2 compatibility type was possibly to Europe and was detected in the early 1980's in Switzerland (Hohl and Iselin, 1984). It is widely believed that new strains migrated within consignments of ware potatoes imported into Europe in the dry summer of 1976 (Niederhauser, 1991). Since European producers sent tubers to many locations throughout the world, the fungal population was widely distributed to South America, North Africa and Asia. The second migration event of the A2 type was from Mexico to the United States and Canada (Lamour and Kamoun, 2009).

2.2.2. Strategies for disease management

Rapid changes in the population of *P. infestans* could be managed by two alternative strategies including application of more fungicides or use of potato cultivars with durable resistance to the pathogen. The second strategy could reduce fungicide applications also and bring less costs of crop production for farmers and less environmental pollutions. Therefore developing resistant cultivars is in the focus of modern breeding programs (Inglis et al., 1996; Peters et al., 1999).

The need for resistant cultivars was clear and an apparent breakthrough came in 1909 when Salaman recognized the Mexican wild species *S. demissum* as a source of extreme resistance that could be backcrossed into *S. tuberosum* (Müller and Black, 1952). Breeding for late blight resistance therefore concentrated on using *S. demissum*'s major

dominant R-genes, of which 11 were identified (Müller and Black, 1952; Malcolmson and Black, 1966; Malcolmson, 1969). Afterwards, some other wild potato species were identified to have one or more genes or allelic variants responsible for late blight resistance.

2.3. Physiological aspects of resistance

There are two important lines at the plant cell level which act as defense barrier against pathogenic organisms. The first is a line of surface-exposed pattern recognition receptors which mediate the recognition of highly conserved microbial molecules called PAMP-triggered immunity (PTI). PAMP stands for pathogen-associated molecular pattern that recognizes different components of the pathogens, like peptides derived from bacterial flagella, elongation factors, conserved secreted proteins from bacteria, fungi or oomycetes, polysaccharides like chitin and beta-glucans (Postel and Kemmerling, 2009). PTI is activated through receptor-like proteins or receptor-like kinases and the recognition is peripherally located on the plant cell surface. This line in plants is thought to be the main mediator of basal immunity against pathogen attack (Jones and Dangl, 2006). To cope with this, pathogens use effectors to block PTI and convert to virulence.

The second line of the defense barrier evolved to recognize effectors of the pathogen thus is called effector-triggered immunity (ETI). If pathogens block the first line and pass through the cell, they encounter ETI. This line is stronger and is more effective against the pathogen and evolved to produce resistance (R) proteins. The majority of *R*-genes contain nucleotide binding site - leucine rich repeat (NBS-LRR) receptors and are able to specifically recognize cytoplasmic effectors of the pathogen.

Although it is generally known that PTI and ETI share many signaling components, it has been proposed that immune responses in ETI occur more quickly, are more prolonged, and are more robust than those in PTI, suggesting that PTI is a weak variant of ETI (Tao et al., 2003; Jones and Dangl, 2006; Tsuda et al., 2009; Tsuda and Katagiri, 2010; Thomma et al., 2011). Typically, the propensity to trigger ETI is pathogen strain or race specific and is associated with a hypersensitive reaction (HR) and systemic acquired resistance (SAR), while PTI is not. Although, it is demonstrated that HR is not exclusively restricted to ETI but can also occur in PTI responses (Wei et al., 1992; Khatib et al., 2004; Ron and Avni, 2004; Thomma et al., 2011) and PAMP perception may also result in SAR (Mishina and Zeier, 2007). Finally, it should be noticed that accumulating evidence indicates that the separation between PAMPs and effectors, and between pattern recognition receptors and R proteins, and thus also between PTI and ETI, cannot strictly be maintained. Rather, there is a continuum between PTI and ETI (Thomma et al., 2011). The molecular interaction between plant cell and *P. infestans* during their encounter is schematized in Figure 1.



Fig.1. A schematic view of host interaction with *P. infestans*. In a susceptible cell pathogen effectors are not recognized by plant cell receptors and the disease may progress. In a resistant cell different receptors on the cell surface and in cytoplasm (resistance R protein) recognize the avirulence compound of the pathogen that triggers defense response in hypersensitive reaction (HR). Abbreviations: ROS: Reactive Oxygene Species; HR: Hypersensitive Reaction; Pr-proteins: Pathogenesis related proteins; PIs: Protein Inhibitors; PAMP: Pathogen Associated Molecular Patterns; C-effector: Cytoplasmic effectors.

2.3.1. Recognition of pathogen effectors by R-genes

Research has shown that oomycete plant pathogens, such as P. infestans, secrete an arsenal of effector proteins that modulate innate immunity of host and enable parasitic infection (Kamoun, 2007). Although these effectors primarily function as virulence factors, but it is possible that they are recognized by plant R proteins in particular host genotypes resulting in activation of effector-triggered immunity. In such cases, the effectors are said to have an avirulence (Avr) activity. In ETI the Avr proteins induced plant response in most cases is a HR, a form of programmed cell death, followed by restriction of the invading pathogen (Jones and Dangl, 2006; Torres, 2010). In the genefor-gene model (Flor, 1971), the presence of both the R gene in plant and the corresponding avirulence (Avr) gene from the pathogen results in resistance (incompatible interaction), whereas absence of either the R gene or the Avr gene results in disease (compatible interaction). In fact, HR is a part of plant innate immunity and its aim is to limit the invading pathogens to the infected area by depriving them from the source of nutrients. Combination between race of pathogen and R gene may be an important trigger to switch on some signal transduction pathways for production of defenseassociated compounds.

In nature, numerous races of P. *infestans* have evolved which are able to infect plants containing some R genes. On the other hand many different resistance genes evolved in potato, thus, late blight resistance proteins account as one of the largest group among devastating pathogens in this crop.

In potato many different types of signal molecules were found which trigger defense responses. In systemic acquired resistance (SAR) signals are transported from the infection site to other parts of the plant to produce molecules designated as components of the defense response pathways. Components which are pronounced in pathogen induced hypersensitive reaction belong to many different groups including reactive oxygen species (ROS), pathogenesis related proteins (PR), proteinase inhibitors and antimicrobial compounds among others (Pieterse et al., 1992; Vleeshouwers et al., 2000a; Yoshioka et al., 2003; Tian et al., 2004; Doke, 2005; Guevara et al., 2005; Fernández et al., 2012).

2.4. Hypersensitive Reaction (HR) mediated defense in *Phytophthora infestans* challenged potato

When potato and the pathogen of the late blight disease come into contact many changes in the metabolisms of the host occur. Molecular crosstalk between Phytophthora and plants involves a multitude of signal exchanges. The pathogen produces effectors which are molecules that manipulate host cell structure and function by facilitating infection (virulence factors) or triggering defense responses which is induced by avirulence (Avr) factors or specific elicitors. The Avr molecules induce expression of defense response genes and the production of antimicrobial compounds in host cells. During the initial stages of infection when the pathogen penetrate into the host, Avr factors of incompatible race of the pathogen activate corresponding R genes in the host plant (Flor, 1971; Dangl and Jones, 2001; Collier and Moffett, 2009) and consequently signals transfer from stressed exposed tissue to distal parts. So combination between race of pathogen and R gene may be an important trigger to switch on signal transduction pathways for the production of defense-associated compounds. Production of these compounds leads to the induction of hypersensitive reaction (HR) in which the pathogen is localized around the site of infection and cannot progress anymore. Recently, several candidate signaling molecules have been studied including SA (salicylic acid), JA (jasmonic acid), methyl salicylate, an as yet undefined glycerolipid-derived factor, and a group of peptides that are involved in cell-cell basal defense signaling and systematically acquired resistance (Vlot et al., 2008). Some of these signals induce defense responses in both susceptible and resistant cultivars but others can do it only in resistant ones (Huitema et al., 2004).

These signals are transported in the plant and stimulate the meristems or stems to produce molecules designated as components of the defense response pathways including resistance proteins like pathogenesis related (PR) proteins, proteinase inhibitors, reactive oxygen species, antimicrobial compounds or various other plant molecules involved in the hypersensitive reaction (HR).

Evidence on the process of hypersensitive reaction in plants suggest that in many aspects this is a genetically programmed and active process likewise to apoptosis in animals (Torres, 2010). This localized response at the site of pathogen attack displays as a programmed cell death and could contribute to limit the spread of the pathogens or be a source of signals for establishment of further defenses (Mur et al., 2008a).

As in animal cells, this process is regulated by proteolytic cleavage with a number of cellular proteins and different protease enzymes are involved. Plants used to apply many similar enzymes and proteins for developing HR after being attacked by pathogens (Fig. 2).



Fig.2. Proteins involved in resistance response in tomato against *P. syringae*. Pto is a tomato serine-threonine protein kinase. Pto is polymorphic and hence satisfies the genetic criteria for the definition of a disease resistance protein. Pto activity requires the NB-LRR protein Prf, and the proteins form a molecular complex. Prf is monomorphic, at least in the tomato species analysed to date. Pto is the direct target of two unrelated *P. syringae* effectors, AvrPto and AvrPtoB, each of which contributes to pathogen virulence in *pto* mutants (Jones and Dangl, 2006).

2.4.1. Role of reactive oxygen species in hypersensitive response to P. infestans

To control a large array of biological processes ranging from regulation of development, growth and response to biotic/abiotic stresses plants deploy reactive oxygen species (ROS) like superoxide or hydrogen peroxide (Mittler et al., 2011). In plant-pathogen interactions, ROS molecules are involved in hypersensitive response (Fig. 3) that is a common short term response in which cells surrounding the site of infection either are killed or signaled to undergo programmed cell death, in order to prevent the spread of the pathogen to other parts of the plant (Kiraly et al., 1972; Mur et al., 2008b). ROS generate lipid derivatives by non-enzymatic oxygenation that can produce membrane damage or they are functioning as signaling molecules (Montillet et al., 2005). By acting as signal molecules, ROS can mediate the generation of phytoalexins and secondary metabolites that inhibit further pathogen growth (Thoma et al., 2003). Different types of ROS as derivatives of superoxide including superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (HO) were known to be highly reactive and toxic, and can lead to the oxidative destruction of cells (Mittler et al., 2004).

Several studies have shown that biotic and abiotic stresses are accompanied by an oxidative burst mediated by NADPH oxidases called respiratory burst oxidase homologs (Rboh) (Cazalé et al., 1998; Miura et al., 1998; Fodor et al., 2001; Torres and Dangl, 2005; Suzuki et al., 2011). These molecules are assigned to produce the main source of ROS and are an essential intermediate step in plants to recognize effectors of the pathogen, both in PTI and ETI and other abiotic stimuli, as well as in the activation or amplification of defense responses.

In potato the *S. tuberosum* calcium-dependent protein kinase (*StCDPK5*) has been shown to phosphorylate the N-terminal region of plasma membrane Rboh proteins, and participate in *StrbohB*-mediated reactive oxygen species burst. By transgenic approaches it was proven that the constitutively active form, *StCDPK5VK*, provides resistance to *P. infestans* by ROS production at the infection sites (Kobayashi et al., 2012). Yamamizo et al. (2006) found that in potato the attack of *P. infestans* activates the mitogen-activated protein kinase (MAPK) cascade that induces a large array of defense genes, including the *StrbohC* and *StrbohD* NADPH oxidases. The strong induction of both genes indicates

that *StrbohC* and *StrbohD* may be responsible for the oxidative burst in response to the pathogen attack in the potato leaves and for the resulted hypersensitive response-like phenotype. These results indicate that Rboh-dependent ROS contribute in potato to basal defense against *P. infestans* (Yamamizo et al., 2006).





Double-head arrow: indicates the cross talk between ROS in these compartments.

Thick arrows: point to the functions of these ROS in relation to activation of plant defenses.

Blocked end line: indicates inhibitory effect (Torres, 2009).

2.4.2. Proteinaceous compounds as inhibitors to P. infestans

Among proteinaceous compounds the pathogenesis related (*PR*) proteins have an important role in plant defense elicited by environmental stress or by developmental stimuli (Edreva, 2005). The PRs which are able to inhibit the growth of pathogens, are divided into 17 subgroups (*PR1-PR17*) based on similarity in amino acid sequence data

and molecular masses (Van Loon and Van Strien, 1999; Okushima et al., 2000; Park et al., 2004). Moreover, some subgroups of PR-proteins are members of multi-gene families, or example up to six members has been detected in *PR-1* gene. The role of many different PR subgroups in resistance to late blight disease was determined and probably more genes of these groups will be identified and functionally characterized in future. (Niderman et al., 1995). They are accumulated locally at the site of infection, and are systematically transferred to the whole plant as a part of systemic-acquired resistance to control further infection. Many research works also indicate that these components are produced constitutively in different plant organs and in seeds, regardless to the stress conditions. These findings suggest a possible role of preformed defense barriers (Vigers et al., 1991; Buchel and Linthorst, 1999). An important common feature of most PRs is their antifungal effect but some PRs exhibited also antibacterial, insecticidal, nematicidal, and antiviral action. PRs target different cell organelles for instance PR-2, PR-3, PR-4, PR-8 and PR-11 target the cell wall of the pathogen, PR-1 and PR-5 attack the cell membrane, *PR-10*, *PR-6* and *PR-9* threaten RNA of the pathogen and further up-to-now undefined proteins of it (Gurr and Rushton, 2005). Toxicity of PR proteins could be due their role in hydrolytic, proteinase-inhibitory, peroxidase activator and to permeabilization reactions of membrane metabolisms (Woloshuk et al., 1991; Beerhues and Kombrink, 1994; Niderman et al., 1995; Edreva, 2005). Several studies have shed light on the role of pathogenesis related proteins in the major *R*-gene mediated resistance of potato to *P. infestans*.

Vleeshouwers et al. (2000a) studied if basal *PR* gene expression contributes to nonspecific resistance to *P. infestans*. Analyzing the *PR*-1, *PR*-2 and *PR*-5 mRNA levels in 13 wild *Solanum* clones (*Solanum berthaultii*, *S. arnezii x hondelmannii*, *S. circaeifolium* ssp. *circaeifolium*, *S. microdontum*, *S. sucrense*, *S. vernei*, ABPT hybrid, *S. nigrum*) and in five cultivars (Bintje (susceptible), Ehud (*R1* gene), Estima (*R10* gene), Premiere (*R10* gene) and Robijn (multiple *R* genes) they concluded that constitutive expression of *PR* genes may contribute to non-specific resistance to *P. infestans* in *Solanum*, and therefore, *PR* mRNAs could serve as molecular markers in potato breeding programs. (Vleeshouwers et al., 2000a). In potato members of the PR-1 family, PR-1b1 and PR-1b2 were identified to be involved in *P. infestans* resistance and it is suggested that PR-1b2 is a homologue of the PR-1 genes of tomato and tobacco (Evers et al., 2006). Strong accumulation of PR-1bmRNA and protein occurs in leaves in response to *P. infestans* infection. PR-1b mRNA and protein accumulation is initiated at the infection site, but a delayed and sustained accumulation can also be observed in neighbouring, uninfected leaves of potato plants (Hoegen et al., 2002). Homologs of osmotin as a PR-5 protein which is inducible by pathogens and osmotic stress in tomato and potato have been suggested to have anti oomycete activity against *P. infestans*, since *in vitro* and transgenic tobacco and potato plants have enhanced resistance against this pathogen (Singh et al., 2013)

Proteinaceous compounds also have a noticeable role in protection of plants against metabolites of microorganisms during infection. Biosynthesis of proteinase inhibitors in response to *P. infestans* was reported initially in tomato and a correlation between increased content of trypsin and chymotrypsin inhibitors and plant resistance to the pathogen was described (Peng and Black, 1976). Different kinds of protease inhibitors including the Kunitz-type protease inhibitor, aspartic protease inhibitor, Kazal-like serine and cysteine protease inhibitors as apoplastic hydrophobic proteins are known to be effective compounds in resistance against *P. infestans* in potato. These inhibitors may play a significant role in the natural defense mechanisms of the potato plant against insect and phytopathogen attack and have a high toxicity toward the pathogen by inhibiting the germination of hyphae and accelerating the destruction of fungal spores (Tian et al., 2004; Guevara et al., 2005; Fernández et al., 2012).

2.4.3. Phytoalexins as anti-fungal compounds produced in potato against *P. infestans* A heterogeneous group of low molecular mass secondary metabolites with antimicrobial activity that are induced by stress are collectively named phytoalexins (Hammerschmidt, 1999). Phytoalexins are an important part of the plant defense repertoire and are considered as molecular markers of disease resistance (Shinbo et al., 2006; Schmelz et al., 2011). These were first described by Müller and Börger (1939) during studies on *P. infestans - S. tuberosum* interactions(Mueller and Börger, 1939). Although, since then, the field has evolved extensively, the biosynthesis of most phytoalexins, the regulatory

networks involved in their induction by biotic and abiotic stress, and the molecular mechanisms behind their cytotoxicity are largely unknown. For most species and cultivars the phytoalexins have yet to be characterized (Ahuja et al., 2012).

The production of phytoalexins is either induced by elicitors of the pathogens or by wounding when plant signal compounds like jasmonates, NO⁻ and ROS are released. (Pieterse et al., 1992). Hence, phytoalexins are involved not only in the short-term hypersensitive response, but also in the long-term response i.e.: the systemic acquired resistance.

Potatoes produce a number of antinutritional phytoalexins such as sesquiterpenoid compounds including rishitin, phytuberin, lubimin and solavetivone (Metlitskii et al., 1970; Kuc, 1982) or the steroid glycoalkaloids α -solanine and α -chaconine. Nevertheless, besides their beneficial role in plant defense these latter phytoalexins display a certain level of toxicity for humans. (Matthews et al., 2005) Therefore, their production in tubers should be avoided or kept at minimal level.

Here the phenylpropanoid pathway has a central role in rapid browning and hypersensitive cell death during incompatible interaction of potato leaves and tubers with *P. infestans*. Rapid increments of transcription rate of two genes encoding phenylalanine ammonia-lyase (*PAL*) and 4-coumarate: *CoA* ligase (4CL) were detected within a few hours post inoculation with the pathogen (Fritzemeier et al., 1987).

Further anti-fungal compounds which play major role against *P. infestans* infection in potato are phenolic compounds. Scanning-electron microscopy and staining light-microscopy revealed depositions of phenolic compounds as extracellular globules in hypersensitive cells of the epidermis and mesophyll layer in response to infection by the late blight pathogen (Vleeshouwers et al., 2000b).

2.5. Role of NBS-LRR molecules in host defense aginst late blight disease

The broad spectrum *R*-genes against *P. infestans* (*Rpi*) which provide non-racespecific resistance, typically encode immune receptor intracellular plant proteins (Ballvora et al., 2002; Huang et al., 2005; Lokossou et al., 2009; Pel et al., 2009). These proteins belong to the nucleotide binding site - leucine-rich repeat (NBS-LRR) class, and contain two important parts including nucleotide binding (nb) site which is central NB domain and leucine reach repeat (Lrr-domain) which have a C-terminal (Sacco and Moffett, 2009). More than 50 functional NB-LRR genes have been cloned from potato and related members of the *Solanaceae* (Hein et al., 2009). Recently, based on an amino acid motif based search of the annotated potato genome 438 NB-LRR type genes were identified among about 39,000 potato gene models. Of the predicted genes, 77 contain an N-terminal toll/interleukin 1 receptor (TIR)-like domain, and 107 contain an N-terminal coiled-coil (CC) domain (Jupe et al., 2012).

All homologs of the functionally characterized late blight R resistance genes including R1, R2, Rpi-bt1, Rpi-blb2, Rpi-blb3 and Rpi-vnt1 were CNL (CC-NB LRR) type (Jupe et al., 2012).

2.5.1. Co-evolution of host-pathogen genes in late blight resistance

The genome of several oomycota pathogen including P. infestans has been sequenced. The 240 Mbp genome of *P. infestans* is remarkable large in the genus. It is three to fourfold larger than the genome of two other analyzed species, P. soja and P. ramorum. This increase in the amount of DNA is mainly due to transposons and other repetitive sequences which account for 74% of the *P. infestans* genome. While most gene families are not expanded the RXLR (effectors carrying an N-terminal type signal peptide) and CRN (crinkling and necrosis) effector families which occupy repeat rich regions in the genome that accelerate effector evolution expanded twofold or more in P. infestans compared to that other two species (Thines and Kamoun, 2010). The RXLR effectors are secreted by *Phytophthora* species across the haustorial host-pathogen interface and target host proteins as well as cellular processes to enhance susceptibility. CRN proteins are another class of host translocated effectors of *Phytophthora* species and can be found also in other pathogenic oomycetes. CRNs target cytoplasmic host factors and induce death of the host cell. Dynamic evolution by non-allelic homologous recombination and tandem gene duplication characterizes these effector gene families (Lamour and Kamoun, 2009; Hardham and Cahill, 2010; Oliva et al., 2010). Subsequently, the pathogen may evolve to escape of being recognized by *Rpi* genes. This may occur in the host plant with different mechanisms including: i) alteration of binding site of the effectors; ii) by evolution to

overcome host defense; iii) or simply by entire gene deletion (Kamoun, 2006; Whisson et al., 2007; Lamour and Kamoun, 2009; Nowicki et al., 2012). The plant genome may in turn evolve fortuitous compensations that restore recognition of altered effectors (Friedman and Baker, 2007). The *Rpi* genes against *P. infestans* typically encode immune receptor proteins of the coiled coil - nucleotide binding - leucine rich repeat (CC-NB-LRR) class of intracellular plant proteins (Ballvora et al., 2002; Huang et al., 2005; Lokossou et al., 2009; Pel et al., 2009). Recognition of the pathogen effectors occurs in the LRR domain of R proteins. The LRR domain undergoes a higher rate of changes than the other parts of the gene to get the ability of recognition of effectors which may have been lost due to the evolution of the pathogen.

2.5.2. Evolution of different *Rpi* genes in potato

Rpi genes have been proposed to follow either of two distinct evolutionary patterns. Some of them are fast-evolving and others are slow-evolving and are designated as type I or type II R genes, respectively (Friedman and Baker, 2007). For both types, sequence exchanges mostly occur between clade members. However, the rate of sequence exchange between paralogs in clades of type I is higher, so they may have higher haplotypic diversity, whereas paralogs in clades of type II show infrequent sequence exchanges and keep orthologous relationships (Friedman and Baker, 2007). One of the most noticeable mechanism involved in rapid evolution of R genes is the unequal crossing over which results in local duplications (Kuang et al., 2004; Leister, 2004; McDowell and Simon, 2006).

Frequent sequence exchanges and conserved intron region characterize the R1 gene, a race specific resistance gene to *P. infestans* originating from the hexaploid *S. demissum* Lindl., that represents a type I gene with fast-evolution and has divergent homologs with typical chimeric structures (Kuang et al., 2005). An interesting finding in R1 gene clusters is the high rate of sequence exchanges confined to specific regions of this gene, while other regions show a normal pattern of evolution with slower rate of sequence exchanges (Friedman and Baker, 2007).

The R3~P. infestans resistance gene also from *S. demissum*, is representing the dynamic evolution of the potato genome regarding co-evolution with *P. infestans*. The genomic region of R3 is functionally diverse for *P. infestans* resistance. The R3 locus consists of two distinctly functional *R* genes, the R3a and R3b (Huang et al., 2004). Even it was shown that there is a large expansion in the R3a subfamily, with the capacity to recognize additional elicitors from *P. infestans* (Bos et al., 2006). It is suggested that the R3 locus might have passed through multiple rounds of gene duplication and diversifying selection to produce new specificities for *P. infestans* resistance (Huang et al., 2004).

Unlike the *R1* and *R3* resistance genes which were classified as type I resistance genes, no obvious sequence exchanges were found among paralogs of the RB gene, a P. infestans resistance gene that derives from the diploid species S. bulbocastanum (Song et al., 2003). Despite recognition of a large spectrum of *P. infestans* races, this gene shows a clear orthologous relationship in resistant and susceptible haplotypes and its evolutionary pattern is attributed to type II, i.e.: it is a slow-evolving gene. Current models for NB-LRR proteins suggest a dual role for the LRR domain, not only as recognition specificity determinants, but also as repressors of inappropriate nucleotide binding activation (Belkhadir et al., 2004). Furthermore, evolutionary analyses of R proteins have shown selection pressure on several domains within them especially in the LRR region and in the b-strand/b-turn motif of it. It is suggested that this region may undergo co-evolution with the pathogen to establish and maintain recognition of the effectors (Meyers et al., 1998; Ellis et al., 1999; McDowell and Simon, 2006). On the other hand new findings proposed a different theory which implies rather conservation in the LRR domain and more variation in the NBS domain as it was found in some *Rpi* genes. This may bring to mind the existence of a different signaling pathway or additional effectors being recognized by these later type of *Rpi* proteins (Lokossou et al., 2009).

2.6. Characteristics and advantages of major *P. infestans* resistance genes in breeding

Since the organization of genes controlling disease resistance in the potato genome including late blight resistance (R) genes and quantitative trait loci (QTLs) were reviewed (Gebhardt and Valkonen, 2001), significant progress has been achieved at molecular level in mapping, cloning and marker assisted selection (MAS). Wild and primitive cultivated *Solanum* species are often used in potato breeding programs, especially for resistance breeding, because their extensive phenotypic diversity makes them valuable resources for high value traits (McCann et al., 2010). Two types of major R genes were found from wild *Solanum* species. R genes derived from *Solanum demissum* confer race-specific resistance whereas the identical Rpi genes from the wild potato species *Solanum bulbocastanum* confer high levels of resistance to a range of *P. infestans* isolates with complex race structures (Helgeson et al., 1998; Song et al., 2003; van der Vossenet al., 2003).

Currently grown potato cultivars lack adequate late blight tolerance. In earlier bred cultivars disease resistance genes were used which confer immunity only to some strains of the pathogen harboring the corresponding avirulence genes. Specific resistance genemediated immunity and also control chemicals are rapidly overcome in the field when new pathogen races arise through mutation, recombination, or migration from elsewhere. (Yamamizo et al., 2006) The most sustainable strategy to protect potato plants from late blight would be the introgression of multiple R genes especially broad-spectrum P. *infestans* resistance (Rpi) genes into cultivars (Pink and Puddephat, 1999; Jones, 2001; Park et al., 2009; Rietman et al., 2012). In addition to durability, pyramiding of resistance genes has the advantage that multiple R genes strongly delay the onset of late blight symptoms in the field (Tan et al., 2010). Allele stacking i.e.: the introgression of different alleles or same alleles of one gene (allele-dosage) and even defeated R genes may enhance resistance in multiple condition (Tan et al., 2010).

Sixty-three resistance genes to *P. infestans* have been identified from various *Solanum* species and out of them twenty-seven have already been cloned (Rodewald and Trognitz, 2013). Eleven major resistance genes (*R1-R11*) which derive from the hexaploid wild potato species *S. demissum* Lindl. (Black et al., 1953; Bonde et al., 1959; Malcolmson

and Black, 1966; Malcolmson, 1969) were identified and have been introgressed into cultivated potato (Gebhardt and Valkonen, 2001). These resistance genes are race-specific, thus they provide non-durable resistance and are rapidly overcome by virulent strains of the pathogen (Malcolmson and Black, 1966). From among the *S. demissum* derived *R* genes four have been cloned and analyzed. These are *R1* (Ballvora et al., 2002), *R2* (Li et al., 1998; Park et al., 2005a; Lokossou et al., 2009) *R3a* (El-Kharbotly et al., 1996; Huang et al., 2004; Huang et al., 2005) and *R3b* (Huang et al., 2004; Huang et al., 2005) and *R3b* (Huang et al., 2004; Huang et al., 2005).

Recently, *Rpi* genes were identified in the diploid wild potato species *S. bulbocastanum* Dunal, and some of them were cloned. These cloned genes are the *Rpi-blb1* (Van Der Vossen et al., 2003) also known as *RB* (Song et al., 2003), the *Rpi-blb2* (Vossen et al., 2005), the *Rpi-blb3* (Lokossou et al., 2009) and the *Rpi-bt1* (Oosumi et al., 2009). Another *Rpi* gene which possibly derives from *S. bulbocastanum* is the *Rpi-abpt* (Lokossou et al. 2009), that was isolated from a complex quadruple hybrid of *S. acaule* Bitter. *S. bulbocastanum, S. tuberosum* group Phureja and *S. tuberosum* L. (Park et al. 2005b). Several late blight resistance genes were cloned from *S. stoloniferum* Schltdl (Vleeshouwers et al., 2008), from *S. edinense* Berthault, *S. hjertingii* Hawkes, *S. schenckii* Bitter., (Champouret, 2010) and from *S. venturii* Hawkes & Hjert (Foster et al., 2009). Late blight resistance genes from *S. okadae* Hawkes & Hjert., and *S. mochiquense* Ochoa were isolated and patented. While the *R1-R11* genes which derive from *S. bulbocastanum* confer horizontal resistance against all known races of the pathogen.

2.6.1. *R* gene homologs

Whole-genome sequencing of the potato (Potato Genome Sequencing Consortium, 2011 –Xu et al., 2011), the Sol Genomics Network database (<u>http://solgenomics.net</u>), transcriptome analyses and data base mining (Vleeshouwers et al., 2011a) indicate that R gene homologs are abundant in potato. However, these homologs show high level of sequence similarity to late blight resistance genes, the structure based prediction of the function of these genes is challenging, since homologs proteins often have distinct and
sometimes multiple functions (Alberts, 2002). These homologs may reflect to the fast evolution of R genes, hence it would be important to determine the real function of them for potential utilization in marker assisted breeding.

High variation in length and homologue numbers of the R1 gene was found in three different haplotypes. Structural comparison of these R1 homologs showed three distinct groups with frequent sequence exchange among them indicating that this gene is under rapid evolution. (Kuang et al., 2005).

For the *R2* gene eleven orthologues from different *Solanum* species have been identified which confer resistance to *P. infestans* (Vleeshouwers et al., 2011b). These are: *R2* from *S. demissum, R2-like* from *S. edinense, Rpi-blb3* from *S. bulbocastanum, Rpi-abpt* from a quadruple hybrid (*S. acaule, S. bulbocastanum, S. tuberosum* group Phureja and *S. tuberosum*), *Rpi-mcd1.1* from *S. microdontum, Rpi-snk1.1* and *Rpi-snk1.2* from *S. schenckii, Rpi-edn1.1* from *S. edinense, Rpi-hjt1.1, Rpi-hjt1.2,* and *Rpi-hjt1.3* from *S. hjertingii*. These *R2* gene homologs derive not only from different potato species but also from different regions of South- and Central-America and show adaptation to their regional *P. infestans* populations, which may have led to distinct recognition spectra and degree of resistance (Lokossou, 2010; Vleeshouwers et al., 2011b). In spite of these differences of the *R2* gene homologs, they are all located in the same region on potato chromosome IV. Structural comparison of the homologs revealed high frequency of sequence exchanges among them (Lokossou et al., 2009). All these findings regarding the *R2* homologs suggest common ancestral origin and evolutionary changes during speciation.

For the R3 it was found that it is a complex late blight R locus where besides the two resistance genes, R3a and R3b paralogs are also known (Huang et al., 2004; Bos et al., 2006).

2.6.2. Distribution of *R* gene hot spots in the potato genome

In some cases, loci of R genes are conserved across genera and some alleles at these loci maintain similar function and specificity for the same or related pathogen taxa. Several Rpi genes are found in clusters of various R genes which sometimes co-localize with homologs of unknown function. This clustering is more pronounced when the R genes

and homologs of different *Solanaceous* crops like tomato, red pepper and potato are combined on a comprehensive map (Grube et al., 2000).

Several late blight resistance genes and also QTLs are located in resistance gene hotspots, that enables the effective transfer of different resistance characteristics and genes from the donor by marker assisted breeding.

As shown in figure 4, the short arm of potato chromosome IV is a hotspot for resistance, harboring several distinct R gene clusters with resistance specificities to different pathogens. Here, the cloned root knot nematode R gene Hero (Ganal et al., 1995) is part of an extensive R gene cluster that is located distal to Rpi-blb3. Also the cyst nematode R locus Gpa4 (Bradshaw et al., 1998), the virus R locus Ny_{tbr} (Celebi-Toprak et al., 2002), and quantitative trait loci for late blight resistance (Leonards-Schippers et al., 1994b; Oberhagemann et al., 1999; Sandbrink et al., 2000) are located here.

The *R1* gene is located within a hot spot for pathogen resistance on potato chromosome V (Birch et al., 2006). The *Rx2* resistance gene to the potato virus X (PVX) (Ritter et al., 1991; De Jong et al., 1997) and QTLs for root cyst nematodes (Kreike et al., 1994; Rouppe van der Voort et al., 2000), as well as major QTLs for late blight resistance (Leonards-Schippers et al., 1994a; Collins et al., 1999) are localized in this region also.

On potato chromosome VI besides *Rpi-blb2* which is located on distal part of the chromosome QTLs for resistance to *P. infestans* and *Erwinia carotovora* have also been described in the same region (Gebhardt and Valkonen, 2001).

A gene providing high level of late blight resistance was localized on chromosome VII. This gene is the *Rpi-mch1*, originating from *S. michoacanum* (Bitter.) Rydb., which is a natural hybrid of *S. pinnatisectum* Dunal and *S. bulbocastanum*. (Śliwka et al., 2012a).

The potato chromosome IX at the distal end of the long arm harbors two resistant genes against late blight including *Rpi-mochi* and *Rpi-dlc1*. The *Rpi-mochi* locus is close to a QTL *Ph-3* near TG591A marker in *Lycopersicon pimpinellifolium*.

On potato chromosome XI at the *R3* gene cluster most likely also the newly identified *Rpi-Smira1* late blight resistance gene is located on a sister chromatid (Rietman et al. 2012). In the same genomic region with the *R3* cluster also further R genes, the R6 and R7 were mapped (El-Kharbotly et al. 1996).



Fig. 4. Potato and tomato map for disease resistance. Position of late blight R genes underlined with solid letters and for QTL with dotted bars at the right of each chromosome (Park et al.,2009).

2.6.3. Utilization of *R* gene resources in breeding programs

Different homologs of some *R*-genes were found in different *Solanum* species and considered as an enriched source of resistance which would be applicable in breeding program to *P. infestans*. However, limited progresses have been achieved in the utilization of *R* genes of wild *Solanum* germplasm in molecular breeding (Park et al., 2009). But newly it got accelerated and many research works on potato breeding goes to exploit this approach to introduce new late blight resistant cultivars armed with R genes derived from wild *Solanum* species (Vleeshouwers et al., 2011b).

By an effectoromics approach Rietman et al., (2012) unraveled the genetic basis of late blights resistance of 'Sarpo Mira'. This Hungarian bred cultivar is one of the few that have been reported to retain resistance in the field for several years and is a candidate for delivering durable late blight resistance (Kim et al., 2012). They found that the resistance is based on the combination of four pyramided qualitative R genes and a quantitative Rgene that was associated with field resistance. The qualitative R genes include R3a, R3b, R4, and a newly identified R-gene, Rpi-Smira1. The quantitative resistance was determined to be conferred by a novel gene, *Rpi-Smira2*. This later was only detected under field conditions and was associated with responses to the RXLR effector AvrSmira2.

In order to bring the effective potentials of *R* genes, QTLs and other genes involved in resistance response into commercial cultivars through crossing, some manipulations on ploidy level of wild *Solanum* species with due regard to endosperm balance number (EBN) must be done (Bradshaw, 2008). Ploidy level in potato clones of wild species can be enhanced by polyploidization sexually or somatically (Bradeen and Kole, 2011). Unilateral or bilateral sexual polyploidization of wild *Solanum* species and production of haploid genotypes (2n=2x=24) derived from common potato (2n=2x=48) have been successfully used for hybridization of cultivated and wild tuber-bearing *Solanum* species (Muthoni et al., 2014).

Pyramiding individual *Rpi* genes in new cultivars seems to be a promising way to develop late blight resistant cultivars. This could be most effectively achieved by marker assisted selection where inheritance of the individual *R* genes is simply followed by DNA markers. Especially the late blight resistance genes from *S. bulbocastanum* are promising, since during the more than thirty years of their application in breeding programs it was noticed that potato clones harboring the *Rpi-blb2* gene remained resistant to all known races of *P. infestans* and no or hardly any spore-bearing lesion could be observed (Flier et al., 2003).

2.7. Field resistance

Quantitative resistance, the so-called field resistance is assumed to be controlled by more than one single genetic locus and is influenced by environmental conditions (Collins et al., 1999). Quantitative resistance genes often segregate as quantitative trait loci (QTL) in mapping populations of potato (Vleeshouwers et al., 2011b). In this type of resistance the progress of lesion development is slow that substantially decelerates late blight development on the plants. It is characterized with general suppression but not elimination of symptom development, thus it could be considered as rate-reducing or, in some terms, partial resistance, and was effectively used in resistance breeding programs (Song et al., 2003). Unlike to potato varieties containing *S. demissum* derived R genes no

obvious necrotic lesions which are characteristic of the classical hypersensitive response can be found in this type of resistance.

By the end of the 1950s, most potato breeders had switched to use germplasm with partial/quantitative resistance (Hawkes, 1979). However, they were not successful in combining durable resistance against *P. infestans* with early maturing foliage. Resistance was lost when short days induced early maturity (Colon, 1994). Thus, it was suggested that late blight resistance and foliage maturity type are either controlled by closely linked genes or the loss of resistance during foliage maturation is due to the physiological processes of ageing (Colon et al., 1995). The hypothesis of the role of physiological changes during foliage maturation is supported by the influence of photoperiod on resistance to late blight. In most studies it was found that in quantitative late blight resistance there is an epistatic interaction among the QTLs located on different chromosomes (Ewing et al., 2000). Two epistatic QTLs were detected by Visker et al. (2003) for foliage resistance against *P. infestans* on chromosomes III and V and another QTL for foliage maturity also on chromosome V. The foliage resistance and the foliage maturity QTLs on chromosome V were located in indistinguishable position and it was suggested that this is a single gene with pleiotropic effect on both traits. (Visker et al., 2003). Two linkage groups also on potato chromosome V associated with late blight resistance were identified in a tetraploid population, and interestingly one of them was not associated with late maturity. (Bradshaw et al., 2004). By QTL mapping of a full-sib backcross population of potato a region on chromosome IV was also identified that conferred both foliage and tuber resistance (Bradshaw et al., 2006). Park et al. (2005a) identified in a S. microdontum derived clone a QTL also on chromosome IV that was associated both with tuber and foliage late blight resistance.

Three QTLs were identified on chromosomes III, V, and XI in a population derived from a cross between two hybrid *S. phureja* x *S. stenotomum* clones, and accounted for 23, 17 and 10% of the total phenotypic variation to late blight resistance (Costanzo et al., 2005). Late blight resistance associated QTLs were identified in the tuber bearing wild potato species *S. paucissectum* Ochoa. on chromosomes X, XI and XII. However, partial reproductive barriers may exist between this wild species and cultivated potato, the few seeds obtained after crossing indicate it is a potential donor for late blight resistance in potato (Villamon et al., 2005).

Danan et al. (2011) constructed a meta-QTL map of potato by analyzing data of twentyone QTL maps and eight reference maps. By this approach they succeeded to reduce by six-fold the number of late blight resistance QTLs to 24 meta-QTLs, and by five-fold the maturity QTLs to eight meta-QTLs. Late blight resistance meta-QTLs were localized on each chromosome, while maturity meta-QTLs only on six chromosomes (Danan et al., 2011).

It can be concluded that late blight resistance QTLs are spread throughout the potato genome and only a part of them indicate pleiotropic control with maturity. Late blight resistance QTLs are independent from major *R*-genes, and contain genes involved in physiological pathways.

QTLs contribute to the general resistance against late blight and their introgression into cultivated tetraploid potato cultivars could be realized through marker-assisted selection using the same genotypes for donors in which those QTLs were detected.

2.8. Marker assisted selection in potato resistance breeding

There are two reasons for genetic variation among cultivars of a polyploid plant species like cultivated potato. The first factor is heterozygosity which refers to the presence of more than one allele at a locus, and the other one is allele dosage that means combination of alleles over the four homologous chromosomes.

To achieve high precision in selection of genotypes which harbor the desired R genes and QTLs, use of allele-specific primers and closely linked molecular markers is inevitable. Gebhardt et al. (2006) indicated that MAS could be efficiently used in resistance breeding programs. In addition to accuracy, efficiency of molecular markers could increased when multiplex PCR reactions are elaborated which allow the detection of more than one specific sequence/marker in a single reaction (Gebhardt et al., 2006).

Some of the molecular markers like restriction fragment length polymorphism (RFLP) or amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) are usually used for gene-mapping in potato. RFLP and AFLP markers are a reliable molecular technique but they are expensive, the technique is time consuming and laborious. So they are not convenient for marker assisted selection. However, among multilocus markering technologies simple sequence repeat (SSR) technique is an exception. This technique although is long and expensive, but it is frequently used, because of the co-dominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing marker size variations (Maroof et al., 1994). The SSR markers are highly reproducible and can be used in different laboratories with consistent results.

Other molecular techniques including sequence-characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) are simple to use. Sequence-characterized amplified region markers make use of polymorphisms in the primer sites resulting in an absence or presence of an amplified band, whereas CAPS markers make use of a restriction site polymorphism after PCR amplification. Moreover, with the advancement in molecular biology of potato, applying of molecular markers linked to resistance genes in genotype selection has been demonstrated(Gebhardt et al., 2006).

Recently mapping analysis of a *S. demissum* derived *R* gene, the so called *R8* in a tetraploid potato clone was done by using of SSR, CAPS and SCAR markers and showed that it is located on the distal end of the long arm of chromosome IX. Also, a co-segregating CAPS marker was developed, which will be useful for marker assisted selection (Jo et al., 2011).

Many different molecular marker types like RFLP, SSR, AFLP and CAPS also have been used for mapping quantitative trait loci in potato. The most valuable diagnostic DNA-based markers are those derived from polymorphisms in the genes causal for a trait of interest, as such markers are in complete linkage disequilibrium with the quantitative trait alleles (Pajerowska-Mukhtar et al., 2009).

Recently, loci of many R genes and QTLs were identified, but most of these genes still remain to be cloned and functionally characterized.

2.8.1. Intron Targeting Markers

The relatively conserved nature of the gene structures in *Solanaceous* plants makes it possible to use intron sequences as molecular markers. This high degree of conservation may be due to the fact that *Solanaceae* genomes have undergone relatively few genomic rearrangements and duplications and therefore have similar gene content and order (Mueller et al., 2005). One effective strategy for exploiting this information and to generating gene-specific co-dominant markers is a method called Intron Targeting (IT). This method was first applied by Choi et al. (2004) to construct a linkage map of the legume *Medicago truncatula* Gaertn. (Choi et al., 2004).

The basic principle of IT relies in the fact that intron sequences are generally less conserved than exons, and they display polymorphism due to length and/or nucleotide variation in their alleles. Expressed sequenced tag (EST)-specific primers and NGS-derived flaking exon primers allow the amplification of genomic DNA across intron regions producing the PCR products that exhibit size or presence/absence polymorphisms (Poczai et al., 2010; Ahmadvand et al., 2014). The basic assumption for this strategy is that introns contain more DNA polymorphisms than exons. Introns, as non-coding regions evolve much faster, than the coding regions (exons). Therefore, intron-targeting strategy of primer design is expected to yield higher polymorphism frequency and therefore has more efficiency than other EST–PCR-based conventional strategies. It is simple to use due to being agarose-based and produces co-dominant markers for potato research and breeding, as well as for genetic diversity analysis in the genus *Solanum* (Poczai et al., 2010).

2.9. Sequencing of transcriptome

Transcriptomic studies are often limited by the number of genes that can be surveyed simultaneously. From the 1990s to early 2000s, many analytical methods were developed for high-throughput profiling of the gene space including differential display, serial

analysis of gene expression (SAGE) microarray, cDNA- amplified fragment length polymorphism (AFLP) and massively parallel signature sequencing (MPSS). Among these tools, hybridization-based microarrays became the dominate platform and has been routinely used to analyze transcriptional changes in many species (Wang et al., 2012). Although serial analysis of gene expression (SAGE) has good capability, but the level of expression was lower than for microarrays.

Another method of transcript profiling is Digital gene expression (DGE) or Digital tag profiling which is qualitatively similar to SAGE analysis however, single transcripts induced identified a 3' end tag by this method. Although DGE method is a vast increasing in throughput by single read of cDNA which accounted as an advantage but it suffers a high difference in generating transcripts library of a population if multiple restriction enzymes are used to generate 3' end tags (Wang et al., 2012). Another disadvantage of both methods in application is producing a large number of small tags which lead to additional cost of DNA sequencing (Nobuta et al., 2010).

Such experiments will be entirely feasible with next-generation technologies, especially with the benefit of low input amount of each type of biomolecule required for a suitable library and the high sensitivity afforded by the sequencing method (Mardis, 2008b).

2.9.1. Next generation sequencing

Many analytical methods were developed for high-throughput sequencing among them a new molecular genetics approach, called next generation sequencing (NGS) is an effective and multipurpose technique. The high throughput, short read NGS systems have been successfully used in several studies for quantitative and qualitative transcriptome analysis in animal, plant and microbial model systems (Cloonan et al., 2008; Marguerat et al., 2008; Mortazavi et al., 2008; Nagalakshmi et al., 2008; Shendure and Ji, 2008) This technology is an ultra-low cost per base of sequencing and has an overwhelmingly high data output. High speed and throughput analyzing methods enable the real-time profiling of whole genome transcripts which may take several years with Sanger technique. The development and application of NGS technologies have greatly facilitated the ability to provide genome sequences of more plant species. Furthermore re-sequencing of entire plant genomes or transcriptomes is possible by this technology (Varshney et al., 2009). Rather than sequencing individual genomes, it is possible to sequence hundreds or even thousands of related genomes to sample genetic diversity within and between germplasm pools by NGS. However, a high cost for generating the sequence, short read lengths and generating huge amount of output data which need to develop appropriate analyzing software and more efficient computer algorithms are of its limitation (Ansorge,2009)

The NGS technology can have significant implications for crop genetics, genomics, and crop breeding (Varshney et al., 2009). In the latter feature one more point to put forward is breeding for developing plant against biotic and abiotic stresses. The valuable capability of this technique made a wide range of opportunities for the discovery of stress-related genes and pathways that can serve as the foundation for crop improvement during breeding program (Ma et al., 2012). For example, NGS technology can be used for high-throughput transcriptome profiling to investigate genome-wide changes in transcripts in response to stresses (Molina et al., 2011). The DNA-protein interactions which play a key role under the stress condition that underlie this type of regulation of gene expression are frequently determined by chromatin immunoprecipitation (ChIP). More recently, NGS technologies have replaced configure of former ChIP-chip in microarray with so-called ChIP-sequencing, which entails conventional ChIP followed by direct sequencing. Capability of NGS technique in sequencing of Chip-derived DNA bound by a transcription factor of interest is paving the way for whole genome transcriptomics (Wang et al., 2012). In ChIP-seq method, a data of library of the released immunoprecipitated fragments which is made by ligation with an adaptor could be analyzed by bioinformatics software followed by sequencing. This capability provide identification of genome in protein binding sites with exquisite specificity (Mardis, 2008a).

Although NGS technology is still in the early stage of its application, it has proved to be a robust tool for the genome-wide identification of genetic variation. NGS, combined with GWAS (genome-wide association studies), has been used to identify potential molecular markers, such as single nucleotide polymorphisms, insertions and deletions, and copy

number variations, which are associated with growth and development and/or stress responses (Ma et al., 2012).

There are four commercially available NGS technologies: 454 Life Sciences (acquired by Roche), Solexa (acquired by Illumina), ABI SOLID (acquired from Agencourt Biosciences), and Helicos Biosciences. Although all have their specific features, generally they can be grouped into two classes based on the lengths of the sequence reads produced. Solexa, ABI SOLID, and Helicos all produce very short reads in very large quantities, while the 454 platform can produce a more moderate amount of sequence, but with much longer read lengths (Rounsley et al., 2009; Willenbrock et al., 2009).

2.9.2. Gene quantification by NGS technique

Previous studies on gene expression based on high throughput of the transcriptome relied on microarrays and serial analysis of gene expression (SAGE) (Varshney et al., 2009). Analyzing of transcriptional changes in many species has been routinely used by hybridization-based microarrays as a dominate platform (Wang et al., 2012). Recently, however, a digital gene expression as a promising and new platform for assessing the copy number of transcripts by next-generation sequencing has been introduced, thereby providing a digital record of the numerical frequency of a sequence in a sample (Willenbrock et al., 2009). Unlike microarrays, NGS technologies are not limited to sequences of previously known genes because they generate tags without having any knowledge of gene annotation, however they require extensive sequencing and a reference genome to determine gene identity (Varshney et al., 2009).

A unique feature of high-throughput transcriptome sequencing coming by different techniques is the versatility of the data, which can simultaneously be analyzed to provide insight into the structure of genomic loci, sequence variation, exploring of single nucleotide polymorphisms (SNP) present at loci and level of gene expression (Morozova and Marra, 2008).

Quantification of transcripts is achieved by counting the density of the reads that are mapped to the exon regions of a specific gene. The calculation is usually corrected and normalized for transcript length (Wang et al., 2012). Normalization process on transcript reads over gene length and total number of mapped reads could be reflected as reads per kilobase of exon per million mapped reads (RPKM) which is a common output for mRNA expression values (Mortazavi et al., 2008; Fahlgren et al., 2009).

In order to examine transcripts of RNA during gene expression, the small fragments in exon-exon junction should be incorporated in RPKM estimation (Wang et al., 2012). This ability of NGS in which shorter reads of transcripts could be used in RPKM estimation make it more precise and high resolution in its application (Varshney et al., 2009).

2.10. Quantitative analysis by real-time PCR

Characterization of expression pattern of induced genes under various altered condition due to stresses and artificial treatments would be an important goal in order to determine the gene behavior and their role in these situations. Many different methods including RT-PCR, northern blot, southern blot and western blot were used for expression analysis of genes involved in resistance to P. infestans. RT-PCR has been more implemented recently due to its high sensitivity for quantification of rare transcripts and small changes in gene expression. Moreover this technique is easy to perform, provides the necessary accuracy and produces reliable as well as rapid quantification results (Pfaffl, 2001). Concerning to RT-PCR, two types of fluorescence methods by using of different reagents including SYBR green and Tag Man have been used for monitoring copy numbers of target genes (Applied Biosystems, life technologies, USA). There are pros and cons to each of the chemistries which is used for quantitative-PCR (qPCR) (Bookout and Mangelsdorf, 2003). Although Tag Man has been considered to be more sensitive, but SYBR Green may have a slight edge in sensitivity at low abundance of transcripts (more than >10 copies) because the reporter dye binds to any double-stranded DNA present in the sample and it is not necessary for PCR cycles to be beyond the range of detection cycle as it is in the Tag Man (Wittwer et al., 1997).

2.10.1. Expressional changes of resistance genes against late blight of potato

P. infestans is a hemibiotrophic pathogen which could parasite the host plant through biotrophic and necrotrophic phase. The pathogen is in biotrophic phase at around 24th hour post inoculation (hpi) in compatible interaction. It progresses into the host cells and turns to highly destructive in the necrotrophic phase at around 46 hpi (Vleeshouwers et al., 2000c).

Quantitation of isolated proteinase K inhibitor in potato was measured between resistant and susceptible cultivars by using of western blot technique and results showed a 19-fold increase of inhibitory activity in resistant cultivar at 24 hours after inoculation. Moreover the activity of the gene in extracts at 48 hours after inoculation was lower than the activity after 24 hours but still remained at a higher level (9-fold) than in control healthy plants and in the susceptible cultivar (Feldman et al., 2000).

Expression pattern of P69 protease genes which is a kazal-like extracellular serine protease inhibitor was studied during infection of tomato by *P. infestans* by Northern blot and semi RT-PCR analyses. Semi-quantitative RT-PCR amplifications using primers specific for P69A, P69B, and P69D showed that P69B was the only gene which was upregulated during interaction with *P. infestans* and the highest level of expression occurred 2 and 3 days after inoculation (Tian et al., 2004).

Northern blot analyses of RNA from potato leaves were performed on control and infected plants for one of the aspartic proteinases (StAsp) which has an antimicrobial activity. The assay was done on two cultivars, Bintje (susceptible cultivar) and Pampeana (resistant cultivar) to *P. infestans*. Expression analysis revealed accumulation of StAsp mRNA post inoculation in both cultivars. In cv. Pampeana the StAps mRNA level increases at 8, 12 and 24 hpi, while in cv. Bintje the StAps mRNA increases at 8 and 12 hpi with *P. infestans* but then decreased at 24 hpi. The signal intensity of StAsp mRNA levels estimated by densitometry in different treatments and control leaves at 8, 12 and 24 hpi, showed 3.54, 1.6, 1.4 and 3.27 higher fold change in cv. Pampeana than in cv. Bintje (Guevara et al., 2005).

Cysteine protease (cyp) gene is tightly up-regulated in leaves of both *R*-gene mediated and quantitatively high field resistant potato cultivars at 15 hour post inoculation with *P*. *infestans* (Avrova et al., 1999).

Analyses of apoplastic hydrophobic protein (AHP) type in potato between two different potato cultivars showing resistance and susceptibility to *P. infestans* reveal constitutive differences of AHPs level in these two cultivars, which is in correlation with potato defense response to the pathogen. Different kinds of protease inhibitors including Kunitz type, aspartic and cysteine protease inhibitors were expressed more in resistant cultivar compared to the susceptible (Fernández et al., 2012).

The activation of different metabolites of defense response including *PR-1*, *PR-5*, *PAL-1* and HMG-2 against *P. infestans* was shown to be under influence of potato cultivar. These genes were much strongly up-regulated in Kennebec (a moderately resistant cultivar) which carries the *R1* resistance gene than in the highly susceptible Russet Burbank cultivar (Wang et al., 2008). Two members of *PR-1* including *PR-1b1* and *PR-1b2* were isolated from a potato clone of the species *S. phureja* with horizontal resistance to *P. infestans*. Maximal induction of both *PR-1* members was observed on the 2nd day in the resistant parent, while in the susceptible it was on the 4th day after infection. Although sequence alignments of these two members showed some difference but they had a similar expression pattern in both the resistant and the susceptible clones in Northern blot and RT-PCR analyses (Evers et al., 2006).

Existence of PR-proteins in some non-specific resistances to *P. infestans* suggests that these genes may be expressed constitutively in different *Solanum* species and *S. tuberosum* cultivars. It was found that there is a significant positive correlation between PRs mRNA levels and resistance levels of *Solanum* species and cultivars of *S. tuberosum* to *P. infestans* (Vleeshouwers et al., 2000a). Some transcripts derived fragment (TDF) involved in potato-*P. infestans* interaction were analyzed by using of quantitative RT-PCR in two cultivars Sarpo Mira (resistant) and Bintje (susceptible). It was shown that different TDFs including patatine like protein3, Cytochrome p450, peroxidase, chitinase B, ascorbate oxydase, transcriptional factors including heat shock and WRKY were

significantly induced more higher in resistant cultivar during the early stage of infection (Orłowska et al., 2011).

2.10.2. Quantitative analysis of *Rpi* genes in potato

Many studies were done on expression pattern of R genes in compatible and incompatible interaction between potato plants and *P. infestans*. The results of some researches showed that the expression of some R genes can be influenced by host genetic background and environmental factors (Wang et al., 2001; Cao et al., 2007). In order to evaluate the expression pattern of *R3a* and their homologs and I2 gene analogues (*I2GAs*), semiquantitative RT-PCR was performed on mRNA isolated from *P. infestans* and mockinoculated leaves of resistant potato clone SH at different time points including 0, 8, 16, 24, 32, 48, and 72 hours post-inoculation. Results showed that all genes were expressed at constitutive level in all treatments (Huang et al., 2005). Relative quantity of a highly resistant gene "*Rpi-phu1*" to late blight was measured by RT-PCR. Expression profile of this gene showed a constitutive low level in a tetraploid breeding line, however transcription of *Rpi-phu1* was under influence of developmental stage and genotypes of potato. So that expression level of this gene was enhanced in diploid line and young age of a tetraploid line after challenging with pathogen (Śliwka et al., 2012b).

Ros et al. (2004) detected two to four fold changes induction of three resistance genes including R1, Rx1 and the Cf-9 gene cluster in two different time points including 16 and 72 hpi of potato cultivars Indira and Bettina with *P. infestans*. No significant changes in gene induction were found in both cultivars at the early stage of the infection process. Expression of the three resistance genes occurred only at 72 h post-infection in both 2-and 4-week-old plants (Ros et al., 2004).

Transcript level of the RB gene was increased significantly after infection of the wild species *S. bulbocastanum* and different lines of the tetraploid transgenic potato cultivar "Katahdin" with the late blight pathogen. The level of transcription in the wild species was much higher than in the transgenic lines. Level of resistance in transgenic lines was correlated with amount of RB transcript (Kramer et al., 2009).

3. MATERIALS AND METHODS

3.1. Plant material and pathogen isolates

3.1.1 Plant material

The potato cultivar White Lady that possesses high tolerance to *Phytophthora infestans* and extreme resistance to PVX and to PVY has been used in the whole analysis. The late blight susceptible breeding line S440 was used in crossings with White Lady to generate an F_1 population (White Lady x S440) for assessing the efficiency of NGS derived intron-targeting markers. A differential set of Mastenbroek R lines was used for assessing phenotypical reactions of potato to *P. infestans* (Table1).

Differential R-line	Cultivar/clone ¹	R-gene content	Cross combination/line ¹	Country of origin ²	Potato species Origin of R gene
r	Craig royal	-	line	United Kingdom	-
R 1	Craigs snow white	<i>R1</i>	CRAIGS DEFIANCE	United Kingdom	S.demissum
R2	1512(16)	<i>R2</i>	Unknown	-	S.demissum
R3	Pentland Ace	<i>R3</i>	CRAIGS DEFIANCE	United Kingdom	S.demissum
R4	1563c(14)	<i>R4</i>	CRAIGS DEFIANCE	United Kingdom	S.demissum
R5	3053-18³	R1, R2, R3b	Line	-	S.demissum
R6	XD2-21³	R1, R2, R3a	Line	-	S.demissum
R 7	$218ef(7)^{3}$	R3, R4	Line	-	S.demissum
R 8	2424a(5)	R3a, R4, R8	Line	-	S.demissum
R 9	$2573(2)^3$	R1, R2, R3, R9	Line	-	S.demissum
R10	3681ad (1)	R3b, R10	Line	-	S.demissum
R11	5008ab(6)	R3b, R10	Line	-	S.demissum
R1, R2,	2070(54)	R1, R2, R3, R4	Line	-	S.demissum
R3. R4					

Table 1. Specification of the used differential set of Mastenbroek R lines

1,2: Data retrieved from http://www.plantbreeding.wur.nl/potatopedigree, (Pieterse et al., 1992)

3: Presence of additional R genes in these clones, besides their R genes contents

The applicability of some intron-targeting markers developed from NGS transcriptome dataset for identification of R genes derived *S. demissum* was analyzed. Plant materials for this analysis include F_1 population of White Lady X S440 and 11 potato cultivars with known resistance or susceptibility to *P. infestans* (Table 2). Analyses of the reaction of potato all genotypes were done on healthy plants grown in a vector-free greenhouse.

Cultivar	Country of origin ¹	Reaction to late blight	Cross combination ²	Pedigree
White Lady	Hungary	R	KE.40 x 1-71.17/6 N+B	-
Victoria	England	R	AGRIA x ROP J 861	-
Luca XL	Hungary	R	79.60 x CHIEFTAIN	-
Agria	Germany	S	QUARTA x SEMLO	-
Rosita	Germany	R	Unknown	-
Vénusz Gold Gold	Hungary	R	Unknown	-
Desiree	The Netherlands	S	URGENTA x DEPESCHE	-
Sante	The Netherlands	R	Y 66-13-636 x AM 66-42	CPC 1673-20
Russet Burbank	USA	S	BURBANK mutant	Rough purple chili
Démon	Hungary	S	88.635 x KURODA	
Cara	Irland	R	ULSTER GLADE x A 25/19	-

Table 2. Potato cultivars used for IT marker evaluation

1,2: Data retrieved from http://www.plantbreeding.wur.nl/potatopedigree

3.1.2. Pathogen isolates

Aggressive isolates of *P. infestans* (collected by István Wolf) with different avirulence genes (*avr*) were maintained in the Potato Research Centre (Table 3.). Also a pathogenic isolate having *Avr* genes and considered as race 0 was used in the experiments.

Late blight isolate	Type of avirulence gene	Races of pathogen	Region	
Race0	Avr	Avr1,2,3,4,10,11	Keszthely	
H12/10	avr	avr1,3,4,7,10,11	Sarmellek	
10/2010	avr	avr1,2,3,4,6,7,10,11	Sarmellek	

Table 3. Pathogenicity of the applied *P. infestans* isolates

3.1.3. Preparation of inoculums of P. infestans

Culture of the H12/10 isolate was grown for one week on tuber slices of the susceptible potato cv. Hópehely, and then it was suspended and sieved through a cheesecloth. The purified suspension was incubated at 25°C for 1 hour to induce sporangium production. Suspension was maintained at 4°C for 2 hours and at room temperature for 20 minutes to induce zoospore formation. The sporangium concentration was determined with a hemocytometer microscope slide and adjusted to 1.5×10^4 spore/ml in a 50 ml total volume. Artificial inoculation of potato clones and cultivars was done in greenhouse and in the laboratory as described below.

3.1.4. Inoculations in greenhouse

Healthy tubers of cultivar White Lady, S440 and of the Mastenbroek R lines were planted into pots and kept in a greenhouse with max 25°C and min 15°C temperature. Four weeks after planting, the plants were sprayed with the suspension of the pathogen. All plants in the pots were sprayed from the abaxial surface. For maintaining the required humidity for infection, plants were covered by net cloth and sprayed every day.

Potato leaflets were sampled from plants at 7th day after inoculation. Water soaked dark grayish lesion appeared on the foliage of plants were assessed in two grade scale: 0—lack of symptoms or non-sporulating lesions, 1—presence of sporulating lesions (Lebecka and Sobkowiak, 2013). Scale 0 and 1were assigned as resistant and susceptible, respectively.

3.1.5. Detached leaf assay

Preparation of the inoculum was the same as described in point 3.1.3. Fully expanded leaves of plants in the beginning of the flowering stage were used and terminal leaflets were detached for inoculation. After rinsing the leaflets with sterile distilled water, 50 μ l of sporangia suspension was dropped to the abaxial surface of the leaves. Leaflets treated with sterile distilled water were used as control. All leaves were incubated in humid plastic chambers in a culture room with 16/8 light/dark period at 21°C. The detached leaves were examined six days after inoculation. The detached leaves were examined six days after inoculation.

3.2. Detection of *R* genes in White Lady by specific primers

Existence of *R*-genes in White Lady was tested with specific published primers (Table 4). Genomic DNA of White Lady, S440, Mastenbroek R line and 24 F₁ genotypes of the WL x S440 cross were isolated using the Walbot & Warren protocol (Walbot and Warren, 1988). PCR was performed in an Eppendorf Mastercycler ep384 (Eppendorf, Germany) thermal cycler. PCR reactions were carried out in a total volume of 12 μ l, comprising the following reagents: 1.5 μ l of the template DNA (100 ng/ μ l), 1.2 μ l of dNTP (0,2 mM), 0.1 μ l Dream Taq Polymerase (5 U/ μ l) , 1.5 μ l 10 x Buffer Dream Taq Green Buffer (Fermentas, Lithuania), 1.2 μ l (100pmol/ μ l) of each of primer. PCR profiles were basically the same as in the references for each gene, although some minor optimization was done to get the appropriate amplicons.

		Tm	Target	Product	Dofononoog
Name	Sequence	(°C)	gene	length (kb)	Kelerences
76-2SF2 76-2SR	CACTCGTGACATATCCTCACTA CAACCCTGGCATGCCACG	50	R1	1.4	(Ballvora et al., 2002)
R2F R2R	ATGGCTGATGCCTTTCTATCATTTGC TCACAACATATAATTCCGCTTC	55	R2	2.5	(Kim et al., 2012)
SHa-F SHa-R	ATCGTTGTCATGCTATGAGATTGTT CTTCAAGGTAGTGGGCAGTATGCTT	56	R3a	0.982	(Huang et al., 2005)
R3bF4 R3bR5	GTCGATGAATGCTATGTTTCTCGAGA ACCAGTTTCTTGCAATTCCAGATTG	55	R3b	0.378	(Rietman, 2011)
184-81F 184-81R	RRAGATTCAGCCATKGARATTAAGAAA ACTCGATTCTCAACCCGAAAG	55	R8	0.500	(Jo et al., 2011)

Table 4. Characteristics of the *R*-gene specific primers

3.3. Bulked analysis of transcriptomes captured in multiple time points by next generation sequencing

In order to get a better understanding about the genetic background of pathogen induced resistance response in White Lady a so called RNA-Sequencing (hereafter RNA-Seq) was carried out by next generation sequencing (NGS).

Since preparative parts, like infection of plants, RNA-extraction and related experiments of this research, as well as the RNA-sequencing and some analysis of data was published by Ahmadvand (2013), here (point 3.3) we just briefly summarize the methodology to be able to understand our analyses and results which are based on the transcriptome dataset generated by this RNA-sequencing.

Next generation sequencing of bulked transcriptomes captured in multiple time points was performed to harvest as much as possible molecular information about biotic stress response in tetraploid potato. Two important potato viruses, PVX and PVY, as well as the worldwide possibly most dangerous pathogen of potato, the P. infestans were inoculated in the same way as described in point 3.1.4. on White Lady to trigger the resistance response. Post inoculation mRNA from three replications, for the viruses at eleven and for P. infestans at eight time points (Table 5.) was isolated with RNAzol (MRC Inc., USA) and mixed into a single bulk so that each individual sample should be represented with the same amount of mRNA. For control in the same time points and also in three replications mock inoculated (for the viruses with healthy tobacco leaf sap, and for P. infestans with sterile distilled water) samples were also collected and the purified mRNA was mixed into a single bulk. These two bulks as treated and control were then used for RNA-sequencing with a Life Tech SOLID RNA Sequencing Kit (Life Technologies, USA) and on a 5500 XL SOLiD (Life Technologies) sequencer. Low-quality and broken sequences were removed. After contig assembling and normalization the fold change and the number of reads per thousand bases per million mapped reads (RPKM) (Mortazavi et al. 2008) was analyzed by CLC Genomics Workbench 4.8 (64 bit) software. The ratio of RPKM-treated/RPKM-control value was applied for fold change with the threshold of \geq + 2 and \leq -2, in treated and control samples and was considered for up- and downregulated genes, respectively. Contigs were mapped to the potato genome sequence: Solanum tuberosum L. group Phureja clone DM1-3 516 R44 (hereafter potato-DM) (Xu et al., 2011) and were annotated.





Parallel with the treated, the process of mock inoculated sampling was done in the same way

Fig. 5. Experimental design of the bulked analysis of transcriptomes captured in multiple time points.

Table 5. Post inoculation time points when treated and mock inoculated samples were taken

Pathogen	1	Minute	9		Hour						Day			Week		
ΡVΧ	5	10	30	1	2	4	8	12	-	-	-	2	-	-	1	2
ΡVΥ	5	10	30	1	2	4	8	12	-	-	-	2	-	-	1	2
P. infestans	-	-	-	1	-	4	-	-	16	24	30	2	3	6	-	-

It should be emphasized that while important data were expected from this RNA-Seq analysis our main interest was to generate a cumulated dataset for the isolation and subsequent functional analysis of genes with major role in biotic stress response.

3.4. Phylogenetic analysis of P. infestans resistance gene homologues

Based on the functional annotation, the TC database was screened for *P. infestans* resistance gene homologs. Selected homologs were identified in the SOL Genomics Network (SGN - <u>http://solgenomics.net/</u>) database, were extracted and blasted in NCBI (National Center for Biotechnology Information, USA). According to arbitrarily chosen thresholds sequences shorter than nine-hundred nucleotides, as well as those with lower than 80% sequence similarity to any of the *P. infestans* resistance genes were excluded from the analysis. After this pre-analysis selected sequences were used for phylogenetic analyses as described in the followings.

3.4.1. Sequence alignment

Multiple sequence assemblages were aligned with MUSCLE (Edgar, 2004) as implemented in the Geneious v.4.8.5 (<u>http://www.geneious.com/</u>) program using default settings. Final datasets were concatenated and formatted to different extension files (FASTA, NEXUS) using the export options of Geneious.

3.4.2. Applied phylogenetic analyzing approaches

3.4.2.1. Parsimony analysis

Phylogenetic analysis with parsimony was performed as an optimality criterion using the program Nona (Goloboff et al., 2008) within a winclada (Nixon, 2002) shell. Five separate analyses (using processor time as a seed to randomize the order of the terminals) were also performed with the following settings: hold 3,000 (holding defined number of trees), 100 replications (search performed with multiple tree-bisection-reconnection algorithm mult*max*), hold/20 (keeping twenty starting trees for each replication). In addition a larger analysis by holding up to 30,000 trees (hold 30,000) but keeping only

two starting trees for each replication (hold/2) was performed too. Jackknife (Farris et al., 1996) support values were calculated using 1,000 replications, with 10 search replications (multi*10) and with one starting tree per replication (hold/1).

3.4.2.2. Maximum Likelihood Analysis

Phylogeny was also inferred using the maximum likelihood (ML) approach implemented in RAxML 7.2.6. (Stamatakis, 2006). All runs were performed with the graphical user interface raxmlGUI 0.93 (Farris et al., 1996). Thorough bootstrap searches (1,000 replicates) were performed under the default general time reversible model of nucleotide substitution with rate heterogeneity following a discrete gamma distribution (GTR+ Γ). RAxML implements only the GTR model and is therefore applied in our analysis. Throughout this paper, 70–84% bootstrap support is considered moderate and 85–100% as strong support. Trees from all analyses were summarized as majority-rule consensus trees and edited with TreeGraph2 (Stöver and Müller, 2010).

3.4.3. Selection test of homologues P. infestans resistance genes in TC database

Pairwise alignment of amino acids sequences of the homologues related to different R genes showed that they have undergone more or less multiple variation thought their entire domain. To investigate about the type of diversifying existed in different R gene homologues a selection test was done according to the Kimura model in Mega 5.2 and statistical support were provided for each estimation at probability level of 95% and 99% (Tamura et al., 2011).

The strength of selection was measured for homologues of each gene separately. Amino acid of TC sequences were subjected to the HyPhy program in the Mega 5.2. and numbers of diversed codons in the alignments of TC were measured.

Test of selection on R gene homologues in the TC dataset and their correspond known Rpi genes was done by using the program to compare the relative abundance of synonymous and nonsynonymous substitutions codons between the gene sequences. For a pair of sequences, this is done by estimating the number of synonymous substitutions

per synonymous site (dS) and the number of nonsynonymous substitutions per nonsynonymous site (dN). Null hypothesis (H0: dN = dS) and level of significance in the case of null hypothesis rejection was calculated by using of a Z-test in the program. The level of significance at 5% probability in which the null hypothesis was rejected (alternative hypothesis) and calculation of $d_N - d_S$ determine the type of selection according to the b or c definition as below:

H₀: $d_{\rm N} = d_{\rm S}$

- HA: (a) $d_N \neq d_S$ (test of neutrality).
- (b) $d_{\rm N} > d_{\rm S}$ (positive selection).
- (c) $d_N < d_S$ (purifying selection).

3.4.4. NBS-LRR motifs in *R* **gene homologs alignments**

Protein alignments of the *R*-gene homologues were analyzed for the nucleotide binding site (NBS) and leucine rich repeat (LRR) domains. NBS domains were identified with the InterProScan 4 (<u>http://www.ebi.ac.uk/Tools/pfa/iprscan/</u>) and with the Motif-Scan (<u>http://myhits.isb-sib.ch/cgi-bin/motif_scan</u>) program. LRR domains were identified with the LRR finder program (<u>http://www.lrrfinder.com/lrrfinder.php</u>) and those LRR alignments with significant hits were selected. Protein alignments of TC homologs were screened for the NBS and LRR domains of related R genes. Multiple alignment of these regions of the homologs was done by the Mega 5.2. software.

3.5. Developing of transcriptome-based primers for the identification of *P. infestans* resistance genes and homologs

The biotic stress induced transcriptome (TC) database of White Lady was used for developing of primers from the sequences of *P. infestans* resistance genes and gene homologues. Especially, for the R1 gene there are many homologues in the TC database which may contribute to the resistance against *P. infestans*.

Three different markering techniques, the intron-targeting, the CAPS (cleaved amplified polymorphic sequence) and the SCAR (sequence characterized amplified region) were applied for primer development. Transcript sequences were extracted with the Tablet

software (Milne et al., 2010) and intron regions of them were determined by the intron finder in the SOL Genomics Network. For those genes where introns identified primers were designed on the adjacent exon regions of the intron using the SIM4 program (Florea et al., 1998). NGS derived SCAR primers were designed for less similar, unique regions of the analyzed sequences. This was true especially for the exon region of R1 homologs in the TC dataset for which some changes in the site of forward primer were found for the published primer. CAPS primers were designed on the highly similar exon regions of the R1 homologs. Primer sequences and their characteristics are listed in Table 6.

3.5.1. PCR procedures for the detection of *P. infestans* resistance genes and homologues

Genomic DNA of White Lady, S440, Mastenbroek R1 lines and 24 F_1 population of WL x S440 was extracted using the Walbot & Warren's protocol (Walbot and Warren, 1988). PCR reactions were carried out in a total volume of 12 µl, comprising the followings: 1.5 µl of the template DNA (100 ng/µl), 1.2 µl of dNTP (0.2mM), 0.1 µl Dream Taq Polymerase (5 U/µl), 1.5 µl 10 x Dream Taq Green Buffer (Fermentas, Lithuania) and 1.2 µl (100pmol/µl) of each of primer (Table 6).

For each primer pairs the appropriate annealing temperature was determined by using of gradient PCR on an Eppendorf Mastercycler ep384 (Eppendorf, Germany) thermal cycler (Table 6). All PCR products were separated on 1.5 % agarose gel post stained with ethidium bromide, visualized and analyzed on a Gene Genius (Syngene, UK) gel documentation system.

For the CAPS markers PCR products were digested with restriction endonuclease enzyme *Rsa*I (10 U/ μ l). The 30 μ l reaction mixture (including 18 μ l nuclease-free water, 1-2 μ l *Rsa*I and 10 μ l PCR products) was incubated at 37°C for 14 hours. The enzyme inactivation was done by incubating the mixture at 80°C for 20 minutes. Electrophoresis, staining and gel documentation was performed as described above.

In all experiments electrophoretic pattern was evaluated for the existence/absence of the expected band.

3.5.2. Cloning and sequencing of the amplified fragments

The amplified fragments with the expected size were cloned with a pGEM-T Easy kit (Promega,USA) and plasmids were transformed to JM109 Z-competent cells. Transformed bacteria were cultured in liquid LBA medium for 1.5 hours and 100 µl was spread on solid LBA on which 20µl X-gal (50mg/ml) and 100 µl IPTG (100 mM) was added. White colonies were selected and evaluated by colony PCR for the presence of the expected size band. In colony PCR the M13 plasmid specific primers were used. Colonies with the expected fragments were selected, their plasmid was extracted with the Gene JET Plasmid Miniprep Kit (Thermo Scientific) and prepared for sequencing process with ABI system analyzer 3/10 (ABI PRISM 310 Genetic Analyser, user's manual). Sequences were analyzed in NCBI using the BLASTn function.

Name	Sequence	Tm (°C)	Marker type	enzyme	Target gene (TC)
R1L506-2-F	TCAACTTCATCAACTCGCACTT				
R1L506-2-R	CTCAGCAACATATCTACTGTATCACAA	56	IT	-	RI
R1L380-1-F	TCAAAGCAAAGATTCAGGAAAA				
R1L380-1-R	TCATTCATCCTCGGAGTCCT	55	IT	-	RI
R1L333-1-F	CCAGAACACAAGGAACAAATAGAA				
R1L333-1-R	GCTAGCCTCAATTAAAGCATGA	58	IT	-	RI
RP3a35-1-F	TGAAAATGCTTCACTCCACA				
RP3a35-1-R	TTGTTCTTTCCGTTTTTCAGTG	58	IT	-	RP3a
Rp3a80-1-F	TTGGAAATTGATAGTGTAGAGAGTGAA				
Rp3a80-1-R	CCTCTCTCAGCTGACCATCAT	58	IT	-	RP3a
Rp3a94-1-F	GGAAGAAGAGCTTCCCTCCT				
Rp3a94-1-R	GGCAGTGCTGATTCAGAAAG	56	IT	-	RP3a
R1A4-1F	CCAATACTTTGCCGATCGTCC				
R1A4-1R	TATATCTGGCAGCTGATCTACGC	57	NGS-caps	Rsal	RI
R1B23-1F	TAGCCTTCGCAATGAGTACA				
R1B23-1R	GCATCTTCACTTTCTGCGCT	57	NGS-caps	Rsal	RI
R1B23-3F	AAGCTGCTCCCCTCTCCTAA				
R1B23-3R	GCCATCCACGCAAAACCAAT	57	SCAR	-	RI
R1C3-2F	AAATGCGCTACTGTTCACGC				
R1C3-2R	CAGTTCCACGGTACAAGGCT	62	SCAR	-	RI
R1A4-3F	AGCAATGGCAAGTTCCCTCA		0015		
R1A4-3R	GGCTGACTTGACAACCGACT	58	SCAR	-	RI

Table 6. Characteristics of the transcriptome-based IT primers

3.6. Quantitative analysis of HR-mediated late blight resistance genes

In order to check the expression level of some genes which may play a role in resistance response to *P. infestans*, biotic stresses induced genes were selected from the

transcriptome dataset and analyzed by qPCR. The criteria for selection were the function and RPKM value of the genes, as well as the type of gene homology in the dataset and sequence homology of these transcripts in NCBI and in SOL Genomics Network. Quantitative analysis of the genes was performed by real-time PCR (RT-PCR) by comparing the expression of the genes between the treated to and mock inoculated control plants. White Lady plants, grown from pathogen-free explants were inoculated with the H12/10 and 10/2010 isolates. The inoculation was done according to the method mentioned in section 3.1.4. Samples for mRNA isolation were collected at eight time points i.e. at 1, 4, 17, 24, 35, 48 and 65 hours post inoculation (hpi) then they were frozen in liquid nitrogen immediately. Samples from mock inoculated plants were collected at the same time points. Isolation of mRNA was done with RNAzol (MRC Inc., USA) according to the recommendations of the producer. The transcribed sequences of selected genes were used for designing specific RT-primer pairs by the primer express software 3 (Life Technologies, USA). The quantitative evaluation was done with a StepOne Real-Time PCR Systems (Life Technologies, USA) machine. Gene expression in this system is analyzed on the basis of monitoring of thermal cycling with a fluorescent chemical reagent, the so called SYBR green binding dye, which is incorporated into the newly amplified DNA fragments (Shepherd et al., 2009). The changes in fluorescence during PCR-reaction was measured by the system and after running the experiment, the output data was analyzed by the StepOne software v2.3 (Life Technologies, USA).

3.6. 1. Preparation of RNA for qPCR

For qPCR analysis mRNA was isolated as above, and was either kept at -80°C until reverse transcription and synthesis of complementary DNA (cDNA) or was immediately used for these processes.

3.6. 2. cDNA synthesis by reverse transcription reaction

In order to quantify the gene expressional changes induced by *P. infestans*, a two-step reaction was used. That involves two separate reactions including the reverse transcription of a poly(A)+ RNA into cDNA and the PCR procedure. The reaction was carried out with High-Capacity cDNA Reverse Transcription Kit with RNAse inhibitor

kit, according to the protocol of the manufacturer (life Technologies, USA). This firststrand cDNA synthesis reaction was primed with random primers, oligo(dT), or genespecific primers (GSPs), but to give an equal representation of all targets in real-time PCR applications and to avoid the 3' bias of oligo (dT) primers as it is advised, random primers were utilized to sythesize cDNA from mRNA by using the Multiscribe Reverse reverse transcriptase in the tests (Life Technologies).

Procedure of reverse transcription was carried out as follows:

1. The reagents of reverse transcription mixture were prepared according to the manual of the 2X RT master mix (High-Capacity cDNA Reverse Transcription Kit) and adjusted the final volume to 10 μ l. For prevention of any kinds of RNase contamination of the mastermix, 1 μ l RNase inhibitor was added to the mixture.

2. 10 μ l of the purified mRNA was added to the mastermix, thus the total volume became 20 μ l. The final concentration of mRNA was 1 ng/ μ l.

3. Pipetting up and down and briefly centrifuge to spin down the content and to remove any bubbles from the reaction solution.

4. Loading the tubes on the thermo-cycler with the following program:

Temp	Time	Step1	Step2	Step3
Temperature	25°C	37°C	85 [°] C	4°C
Time	10min	120min	5min	œ

Table 6. Program for reverse transcription of mRNA

The cDNA was either directly used for qPCR experiment, or it was store at -20° C.

3.6.3. Designing the RT-PCR primer pairs

Primer pairs for RT-PCR were designed using of Primer Express software version 3.0. (Life Technologies, USA). After uploading the file of gene sequences saved from transcriptome dataset to the software with the finding primer option, candidate primers and probes were outputted by the program, including some information about the primers, probes and amplicons. Those primers pairs were selected that had a low penalty score and low amplicon length.

3.6.4. QPCR reaction

The quantitative real-time PCR analyses of the genes were performed using the StepOne Real-Time PCR Systems (Life Technologies, USA). For detection of the products the power SYBR green PCR master mix (life Technologies, USA) was used. The master mix contains reagents and enzyme including the fluorescence SYBR green as a reporter dye, highly purified AmpliTaq Gold DNA polymerase, and a proprietary version of ROX dye as passive dye. Besides, RT-primer pairs of the target gene and first-strand cDNA of the transcript as template were added to the PCR master mix according to setup section of the experiment.

The process of qPCR experiment with the real-time machine was carried out in 3 steps:

1. Setup the experiment:

The quantitation type of experiments were set up in the 3-color, 48-well system with comparative CT ($\Delta\Delta$ CT) method. The reagent SYBR green dye was used for fluorescence signaling and cDNA as template. Two targets including a housekeeping gene (ß-tubulin) and the gene of interest were quantified in the range of samples with three replications for each sample. Endogenous control was the housekeeping gene and the reference sample was untreated (control) sample. The qPCR reaction mix contained the followings:

- 0.45μ L of each primer (0.5 μ M final concentration)
- 2.1 μ L of cDNA (final concentration = 1 ng/ μ l)
- $10 \,\mu\text{L}$ of power SYBR green master mix.
- 7.45 μL of molecular biology water (AccuGENE, Belgium)

2. Run the experiment:

The PCR reaction mixture was loaded on a MicroAmp Fast Optical 48-Well reaction plate (life Technologies, USA), then the plate was sealed with a

Microamp Fast Optical 48-Well adhesive film (Life Technologies, USA) and centrifuged at 400.

For obataining the optimal results, the standard ramp speed (two-hour time) was selected for running the system. The cycling program was as follows:

95°C for 10 min in holding stage, 40 cycles of 95°C for 30s, annealing at the temperature for each primer pairs for 60 s, 95°C for 15 s in cycling stage and 95°C for 15 s, 60°C for 1min and +3 °C in step and hold to 95°C for 15 s during melting curve stage.

3. Data analysis:

The comparative Ct method (or Δ Ct) was used to assess relative changes in mRNA levels between two or more samples in RT-PCR. The StepOne software v2.3 measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. With calculation of the Δ Ct in treatments and control, the software determines the relative quantity of the target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample (Applied Biosystems, life Technologies, USA).

3.6.5. Gel electrophoresis, cloning and sequencing of qPCR amplified fragments

In order to checking the qPCR amplified fragments whether they are at the expected size or not, they were separated on 2% agarose gel at 200 mA direct current, then stained with ethidium bromide and analyzed on a GeneGenius (Syngene, UK) gel documentation system. Cloning and sequencing was performed as described in point 3.5.2.

Alignment and analysis of the amplified qPCR sequences was done with the Mega 5.2 software (Tamura et al., 2011).

3.7. Analysis of the effect of *P. infestans* inoculation on the protein profile of White Lady leaves

The protein profile of treated (*P. infestans* inoculated) and mock inoculated White Lady leaves were analyzed by the SDS-PAGE method. Total extracted proteins were loaded and run on polyacrylamide gel. After de-staining with Methanole-Acetic acid 7%, gels were dried in a gel-drier and the protein profile was scanned.

The protocol of Griga et al. was applied (Griga et al., 2007) with some modification as follows:

- Homogenizing of leaf tissue at a ratio of 100 mg weight to 1 ml extraction buffer with 100 Mm Tris-HCL (PH 6.8) containing SDS 2% and 20% sucrose which was added during mixing
- Sample mixtures were transferred to eppendorf tubes and put on a water bath with 70°C for 5 minutes
- Centrifugation of homogenized samples at 14000 rpm for 15 minutes
- Transferring the 1 ml supernatants in a 1.5 Eppendorf tube and incubating at 20°C for overnight
- Applying of the Acrylamide-Bisacrylamide gel on a vertical cassette and keep it until solidification
- Loading the 60 µl samples + 30 µl dye (BPB+ Mercaptoethanol) per well
- Running the gel with constant current 27 mA until the tracking dye reached the bottom of the gel
- Staining the gel in 0.25% Coomassie Brilliant Blue solution for overnight

The Acryl amide-Bisacrylamide gel was composed of 7.5 ml Tris buffer 0.5 M, 300 μ l SDS 10%, 12ml Acrylamaide-bis, 15ml TEMED, 150 μ l Aps10% and 10ml H2O. The concentration of protein samples in infected and mock inoculated plant was measured by Nanodrop at A280 nm. The final concentration was determined and adjusted to 1 μ g/ μ l, and then electrophoresis was done with equal volume for each sample

4. Results

4.1. The *R* gene content of White Lady according to inoculation tests

Phenotypic reaction of Mastenbroek differential R lines by isolates of *P. infestans* showed a combination of virulence genes in the genetic composition of isolates H12/10, H10/2010 and WL1 (Table 7). Applying four different isolates with different avirulence gene content in inoculation tests indicated that at least 8 genes, *R1*, *R2*, *R3*, *R4*, *R6*, *R7*, *R10* and *R11* may be present in the genetic background of White Lady (Table 7). The Mastenbroek differential set of R lines was used to assure the avirulence gene content of the isolates. Phenotypic reaction of all R lines was as expected. Frequency of avr*R5*, avr*R8* was low and avr*R9* was not at all present in any isolate of *P. infestans* collected from different locations in Hungary. This may be due to the fact that these avirulence genes are rare in Hungary.

 Table 7. Reaction of the Mastenbroek R lines and White Lady (WL) to different P.

 infestans isolates

Isolate	Avirulence gene	•	Mastenbroek differential R line								WL			
		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R1,2,3,4	
H12/10	avr1,3,4,7,10,11	+		+	+			+			+	+	-	-
Race 0	AVR1,2,3,4,10,11	-	-	-	-						-	-	-	-
H10/2010	avr1,2,3,4,6,7,10,11	+	+	+	+		+	+			+	+	+	-
WL1	avr1,2,3,4,5,6,7,10,11	+	+	+	+	+	+	+			+	+	+	+

+: compatible interaction

-: incompatible interaction

*: the results of reaction test on R line and White Lady with this isolate was provided by Istvan Wolf and included in the table for comparison

4.2. Identification of resistance genes to P. infestans

4.2.1. Detection of *P. infestans* major resistance genes by molecular markers

Among the *R* genes, that derive from *S. demissum R1, R2, R3*a and *R3b* have already been cloned (Ballvora et al. 2002, Lokossou et al 2009, Huang et al. 2005, Li et al. 2011) and their presence in White Lady was investigated here with published gene-specific primer pairs.

While the expected products were obtained for the *R2*, *R3a* and *R3b* genes, the *R1* gene could not be amplified (Fig. 6, 7). The PCR products of each gene were cloned and two clones from each were sequenced. Sequences of each gene were assembled with the Codon Code Aligner program and a contig was constructed for each gene and analyzed in the NCBI database.

With the R2 specific primers a 2,500 bp contig could be created that showed 97% identity with the R2 gene in the NCBI. With the R3a and R3b primers the sequence similarity of the two 1,000 bp and 380 bp size contigs were 98% and 99% in NCBI, respectively (Table 8).

However, inoculation experiments clearly indicated the presence of the R1 gene in White Lady, this gene could not be amplified by the published (Ballvora et al. 2002) 76-2S specific primers. As shown in Figure 2 with this primer pair the gene could be amplified in the differential R1 line. Hence, it is suggested that sequence differences between the primers and one or both priming sites could be the reason that no products were obtained with this primer pair.

The primer which is used for R8 gene is for mapping of the genes since this gene wasn't cloned yet. PCR experiment with this anchor marker failed to amplify the fragment containing this gene in the White Lady, however it is not clear that this gene is absent in this cultivar.

Fragment	Primers	Matched homologs in TC	Contig length	Similarity
R2	R2F,R	R2	2500 bp	97%
R3a	SHa-F,R	R3a	1000 bp	98%
R3b	R3bF4R5	R3b	380 bp	99%

	Table 8. Similarity	v of <i>R</i> -genes	with White	Lady contigs	of the same lo	ci
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Fig. 6. Results of fragment amplifications with the *R1* specific primer pair 76-2S (left) and with the *R2* specific primer pair R2 (right)

M: 100 bp molecular ladder; WL: White Lady; S440: breeding line; r: Mastenbroek line with recessive allele; BR1, 2: Mastenbroek R-line with dominant allele. The arrow indicates the expected and cloned band.


Fig. 7. Results of fragment amplifications with the *R3a* specific primer pair SHa (left) and *R3b* specific primer pair R3b (right)

M: 100 bp molecular ladder; WL: White Lady; S440: breeding line; r: Mastenbroek line with recessive allele; BR1, 2, 3: Mastenbroek R-line with dominant allele. The arrow indicates the expected and cloned band.

4.2.2. Results of transcriptome (TC) analysis

Since main data of this TC analysis have already been published by Ahmadvand (2013), here in point 4.3 we just cite some of those data which are necessary for understanding our results of further analyses of that huge TC dataset and of consecutive experiments based on the TC dataset. Published results of Ahmadvand (2013) are indicated with a *. For transcriptome analysis a so called RNA-sequencing (RNA-Seq) strategy was used that belongs to the next generation sequencing (NGS) technologies. The purpose of this RNA-Seq analysis was to generate a huge dataset about biotic stress induced gene expressional changes in tetraploid potato. RNA-sequencing of transcriptomes captured at multiple time points of the treated (inoculated with PVX, PVY and *P. infestans*) sample resulted in 12,060,751* and of the control in 9,861,651* reads. Reads were assembled into contigs and were mapped to the doubled haploid potato-DM reference genome. By this, out of the 39,031 known protein-coding genes of the doubled haploid potato-DM reference genome 38,062* and 37,926* expressed genes could be identified in the treated and control samples, respectively. In total, only 358 genes of the reference genome were either not present or were not expressed in the treated as well as in the control sample. Almost 60% of these (211 out of 358) were genes of unknown function.

4.2.3. Copy number and gene regulation

Mapped reads for each transcript were normalized and expression of them was defined by the RPKM value (Mortazavi et al., 2008) (Fig. 8). By comparing the fold change of the treated to control transcripts 8,464* up-regulated as well as 4,671* down-regulated genes were identified.



Fig. 8. Number of reads of the mapped genes in the treated sample. Y-axis: number of reads; X-axis: number of mapped genes

As illustrated in Figure 5, while the copy number in the 51-100 read spectrum was almost the same in the control (18,9%) and treated (19) samples, a decreasing of contribution of lower copy number reads and a corresponding increasing contribution of higher copy number reads could be observed in the treated sample (Fig. 9).



Fig. 9. Distribution of the copy numbers of the reads in the control and treated samples.

For approximately one third of both the up-regulated and down-regulated genes of mapped sequences annotation was not possible, since these sequences matched proteins of unknown function. There were 747* up-regulated genes in the treated sample which were detected in the control, indicating that those genes were activated by the stress response. On the other hand, 611 genes were found to be inactivated in the treated sample, indicating that the pathogen attack had induced strong genome-wide expressional modification. According to their annotations, 747 resistance genes and analogues were identified: of these, 385 were NBS and/or LRR domain containing genes, 142 were late blight resistance genes and homologs, and the remainder was resistance response genes and analogs.

4.2.4. Heat map analysis

Out of the 747 annotated resistance genes, 11 were expressed only in the control sample, while 13 were found to be expressed only in the treated sample. These later genes expressed just in the treated sample included 3 NBS type, 1 LRR type, 3 late blight resistance and 6 other resistance genes. The highest read number 232,365 was observed for a conserved gene of unknown function in the treated sample.

A scale of spectra for fold change of transcripts was used to construct a heat-map to visualise the rate of up-regulation and down regulation of some genes. Fold changes were determined with the Log₂ RPKM values in a range between -2 to 2 (Fig. 10).



Spectra of fold change (log₂ RPKM)



Fig. 10. Heat-map of some of the most up-, and down-regulated genes. Log₂ of the RPKM values was used as a scale to reveal color intensity. Red color indicated higher RPKM values compared to the green with lower values. The heat map was created by the Java tree view software (Saldanha, 2004).

4.2.5. Identification of resistance genes to P. infestans in the transcriptome dataset

Based on the functional annotation a total of 142 *P. infestans* resistance genes and homologs were expressed in the treated sample. In the White Lady genome the presence of the *R1*, *R2*, *R3a* and *R3b* genes was proved either by sequence amplification or phenotypic reaction test. In the transcriptome dataset only the *R1*, *R2*, and *R3a* genes could be identified by annotation. Since, as described above the presence of *R3b* could be revealed in White Lady, it is suggested that possibly due to sequence similarity with the *R3a* gene or with other homologs, this gene could be either miss-annotated, or reads of it are present in the unmapped bulk of reads. For the remaining 139 sequences it is concluded that these are *Rpi* homologs or other non-isolated *R* genes.

4.2.6. Selection test of different *R* gene homologs

Selection test with amino acid alignment of the resistance genes R1, R2, R3a, R3b, Rpiblb1, Rpi-blb2 and Rpi-vnt1 of the TC database reveal different types of diversification for each gene homolog. As a model for the test, synonymous/non synonymous substitutions were evaluated by the Kimura method (Kimura, 1980) with the MEGA5 software for each gene separately and positively selected sequences of homologs were determined (Appendix 1). The probability level for all R gene homologs in the TC was significant. Results of selection showed that R1 homologs positively diversified in the selection test with probability level higher than 95% (Table 9). Analysis of sequences revealed seven diversified homologs in this gene homologs with significant level. Not only gene sequences of R2 in the TC showed the highest convergence with the related gene in NCBI, but also show the highest level of synonymous amino acid between them. Although the calculated means of values of dN > dS for R1 homologs was more than other *R*-gene homologs, but only one, the *Rpi-blb2* homolog in the TC showed positive diversity to the accession no. DQ122125 and most of the Rpi-blb2 homologs showed purifying with synonymous substitution in the test in the test (Table 9). Among different R genes identified in the TC, and have a main role in resistance to late blight disease R2 and *Rpi-blb2* gene homologs were more convergent to their cloned resistance genes and R2 homologs were accounted as purifying with the synonymous substitution of amino acid.

R-gene	No. of diversified homologs ^a	Positive selection of paired sequences				
it gone		Probability ^b	Value (dN-dS)			
R1	7	0.014 , 0.000 , 0.001 , 0.011 0.015, 0.048 , 0.002	2.213, 3.376, 3.134, 2.336, 2.193, 1.683, 3.015			
R2	1	0.023	2.020			
R3a	2	0.015, 0.022	2.187, 2.045			
R3b	2	0.030, 0.018	1.90, 2.128			
Rpi-blb1	2	0.026, 0.010	1.964, 2.353			
Rpi-blb2	1	0.002	2.893			
Rpi-vnt1	1	0.010	1.477			

Table 9. Evolutionary analysis of codon-based test of positive selection on sequences of *Rpi* gene homologs in TC

a: Z-test of selection for measuring number of positively diversified homologs on the basis of synonymous/non synonymous substitutions per site

b: Probability level of diversified ones with hypothesis rejection at 5% level

4.2.7. Phylogenetic analyses of the *Rpi* homologs

To study the relationship of the Rpi homologs of White Lady phylogenetic trees were constructed from the nucleotide alignment.

Pre-analysis revealed massive size differences and a high degree of sequence difference among the homologs. Therefore arbitrary thresholds were chosen and sequences shorter than 900 nucleotides, as well as those with less than 80% similarity to their homologous Rpi gene were excluded from the analysis. Using this approach, 82 sequences were included in the final phylogenetic analysis with 21 being *P. infestans* resistance genes. The *I2* gene of tomato was included also, which is similar to the *R3* genes, and used the *Bs2* gene of pepper as an out-group.

Phylogenetic analysis was performed using maximum parsimony and maximum likelihood (Appendix 2 and 3). Both approaches revealed large phylogenetic distance

among the analyzed sequences. While with maximum likelihood analysis members of the R1 gene group grouped together, they separated in the parsimony analysis. Excepting this, the results of the two analyses were almost identical.

The main clades were formed by known *Rpi* genes and matching homologs. The gene *R*2 (including also *Rpi-abpt, Rpi-hjt, Rpi-snk* and *Rpi-blb3* genes) represented a separate clade, and separated well from the groups formed by *R1* and *Rpi-blb2*. *Rpi-blb1* (including *Rpi-sto* and *Rpi-bt1*) and *Rpi-vnt* genes formed separate clades, while *R3a* with *R3b* was part of a larger clade with 12 homologs. In the maximum likelihood analysis 12 homologs didn't grouped with any *Rpi* gene.

Out of the 82 homologs in the treated sample, 17 were up-regulated, while 15 were down-regulated. Interestingly, the 8 adjacent homologs of the *R2* block of *Rpi* genes and 5 homologs in the *Rpi-blb2* sub-clade showed significant expressional changes, while in the *R1* sub-clade pathogen inoculation did not induce expressional changes, except in one homolog. Sequence analysis of the *Rpi* homologs indicated that except five, all of them belong to the NB-LRR family of resistance genes (data not shown).

4.2.8. NBS-LRR alignments of *R*-gene homologs of the TC dataset

It was hypothesized that the NBS and LRR regions of the *R* gene homologs may contain conserved sequence motifs. To test the hypothesis the nucleic acid sequence of all homologs with an NBS and/or LRR motif were translated to amino acid sequence with the Expasy program and the NBS and LRR regions of them were selected and aligned with the MEGA5.2 program. These two regions of the *P. infestans* resistance genes (*R* genes and *Rpi* genes) were also used in the analysis. According to the clustering in the phylogenetic analysis amino acid sequence of the homologs of the *R1*, *R2*, *R3a*, *R3b*, *Rpi-bt1*, *Rpi-blb2* and *Rpi-vnt1* genes were aligned in separate groups. Both the NBS and the LRR alignments showed similarity between the appropriate *R* gene and the homologs of it, but to different extent. Except short identical sequences or single amino acids in given positions, hardly any homogeneous regions could be detected which are characteristic for groups of the homologs. In contrast to this, in those clusters which contained more than

one R gene, extensive sequence similarity could be observed in both regions among the R genes. Sequence similarity is especially high among the R genes of the R2 cluster (Appendix 4). Although, many LRR regions were determined which exhibit more changes than the NBS region of the same homolog, but the magnitude of changes in residues depends on the type of diversity of the Rpi-gene homologs

4. 3. Development of intron-targeting primers for the detection of the R1 gene

Different published and analyzed homologs of *R1* including *R1A4*, *R1B23*, *R1C3*, *R1-B16* and *R1-Tken* (Kuang et al., 2005) could be identified in the TC dataset. A study on homologs of three haplotypes of *R1* including *-A*, *B* and *C* (Kuang et al., 2005) showed that some of these including *R1A4*, *R1B23*, *R1C3* are located in the fast evolving regions of *R1* which exhibit high level of sequence similarity.

Based on the TC data and on genebank (NCBI) data of the *R1* gene, three intron-targeting primers were designed on the intron flanking exon regions. Among them, the R1L333 primer pair amplifyed a 380 bp sequence in White Lady. This band was polymorphic in an F_1 population of 24 genotypes derived from a cross of White Lady and the susceptible breeding line S440. This band was amplified in White Lady, in resistant cultivars and in resistant F_1 genotypes (Figure 11 and 12), was extracted from the gel, cloned and sequenced.



Fig. 11. The R1L333 IT marker of the *R1* gene in White Lady (lane 1), in S440 (lane 2), in R1 (lane 3), in resistant (lane 4-7) and in susceptible (lane 8-10) F₁ genotypes. Lane M is 100 bp molecular ladder.



Fig. 12. Amplified band in PCR by using of IT primer in different potato cultivars. Lane 1 - White Lady (resistant), lane 2 - Venus gold (resistant), lane 3 - Desiree (susceptible), lane 4 - Cara (resistant), lane 5 Luca (resistant), lane 6 - Russet Burbank (susceptible), lane 7 - Victoria (resistant), lane 8 - Demon (susceptible), lane 9 - Sante (resistant), lane 10 - Agria (susceptible), lane 11 - Rosita (resistant). Lane M is 100 bp molecular ladder.

The 380 bp amplified band obtained with the R1L333 IT-primer was extracted from several resistant cultivars and two resistant F_1 genotypes of the White Lady x S440 cross, and was sequenced and blast analysis was performed in NCBI an in SGN databases. We found that this fragment has high degree of similarity (98%) with the *R1* gene originating from *S. demissum*, although this sequence is located in an intron region in some haplotypic homologs of this gene including R1A4 R1C3, R1B23 which homologs were also identified in our TC database and according to Kuang et al. (2005) these homologs are located in the proximal region of the gene. Other homologs of the *R1* gene like R1B16 and R1-Tken have not this intron sequence (Table 10). Sequence analysis of the homologs of *R1* with the vector NTI software (Lu and Moriyama, 2004) showed that the amplified fragment of White Lady is in the first intron region of the main open reading frame (ORF) on the forward strand of each homolog.

Fragment	Sequence coverage (%)	E-value	Identity (%)		
R1-A4	95	4e-111	89%		
R1-C3	95	4e-100	87%		
R1-B23	69%	4e-86	91%		
R1-B16	-	-	-		
R1-Tken	-	-	-		

 Table 10. Results of pairwise alignment of the R1L333 marker of White Lady and published R1 homologs

4.3.1. Analysis of IT-amplified fragment in gene databases

In order to study the similarity of the R1L333 IT marker in both the NCBI and SGN databases, sequences of the amplified fragments of each genotype were blasted both in these databases (Table 11). All amplified fragments showed high similarity with the R1 gene or with R1 homolog. Interestingly, all sequences showed an 87-96% similarity with the same sequence in SGN. Sequence similarity of the same amplified fragments was in the range between 90-98% in NCBI where different accessions of the R1 gene derived from *S. tuberosum* clones were in data output.

Genotype	SGN BLAST				NCBI BLAST					
(cultivar [*] , line ^{**})	Seq ID	Function	Coverage E-Value	E-Value	Identity	Accession no	Function	Coverage	E-Value	Identity
			(%)		(%)	Accession no.		(%)		(%)
White Lady	PGSC0003DMG400003380	late blight resistance protein homolog R	e 95.41	1.8e-49	89.42	AY547666.1	Solanum tuberosum clone 1327 R1 (R1) gene, partial cds	95%%	2e-152	98%
Venus gold	PGSC0003DMG400003380	late blight resistance protein homolog R	e 82.51	1.7e-46	88.52	AY547666.1	Solanum tuberosum clone 1145 R1 (R1) gene, partial cds	91%	2e-103	91%
Cara	PGSC0003DMG400003380	late blight resistance protein homolog R	e 84.08	3.5e-47	87.19	XM_006363186.1	<i>Solanum tuberosum</i> putative late blight resistance protein homolog <i>R1</i> A-4 (LOC102594522), mRNA	90	2e-102	92
Sante	PGSC0003DMG400003380	late blight resistance protein homolog R	e 72.42	1.1-45	87.87	AY547596.1	<i>Solanum tuberosum</i> clone 1215 <i>R1</i> -like (R1) gene, partial sequence	90	8e-102	90
Luca	PGSC0003DMG400003380	late blight resistance protein homolog R	e 100	7.7e-24	96.53	XM_006363186.1	Solanum tuberosum putative late blight resistance protein homolog R1A-4 (LOC102594522), mRNA	97%	2e-59	97%
85	PGSC0003DMG400003380	late blight resistance protein homolog R	e 84.31	3.5e-47	87.19	AY547583.1	<i>Solanum tuberosum</i> clone 1245 <i>R1</i> -like (<i>R1</i>) gene, partial sequence	92	4e-101	90
467	PGSC0003DMG400003380	late blight resistance protein homolog R	e 84.22	6.2e-49	88.36	AY547583.1	<i>Solanum tuberosum</i> clone 1245 <i>R1</i> -like (<i>R1</i>) gene, partial sequence	92	3e-107	91

Table11. Blasting results of the R1L333 IT marker in SGN and NCBI

*: resistant potato cultivars against late blight pathogen **: resistant F₁ genotypes of WL X S440

4.4. Quantitative analysis

4.4.1. Protein analysis of *P. infestans* inoculated potato leaves

Total protein was extracted from White Lady leaves at different time points after inoculation with *P. infestans*. The samples were separated in a polyacrylamide gel (Figure 9). Differences in the banding pattern of the first two samples which were taken one (lanes 1 and 2), four (lanes 3 and 4) and seventeen (lanes 5 and 6) hours after inoculation indicated a strong band in the 50-60 kDa interval and 80-150 kDa in the inoculated compared to the mock inoculated sample (Fig. 13), although there was a difference between lane 13 and 14 at sixty five hours after inoculation. Obvious differences in banding patterns during the first time points after inoculation imply that more proteins were expressed in this period in the treated plants which may be involved in resistance to the pathogen.



Fig. 13. Protein profile of mock inoculated (lanes: 1, 3, 5, 7, 9, 11 and 13) and *P. infestans* inoculated (lanes: 2, 4, 6, 8, 10, 12 and 14) samples

Post inoculation sampling time points were: 1 hpi (lanes 1 + 2), 4 hpi (lanes 3 + 4), 17 hpi (lanes 5 + 6), 24 hpi (lanes 7 + 8), 31 hpi (lanes 9 + 10), 48 hpi (lanes 11 + 12), 65 hpi (lanes 13 + 14).

4.4.2. Gene expression analysis of biotic stress response genes by qPCR

To characterize the *P. infestans* inoculation triggered expression of biotic stress response genes during a successful resistance response, genes of several resistance gene families were selected from the transcriptome dataset and were analyzed. To this end, leaves of White Lady plants were inoculated with the H12/10 and 10/2010 isolates and for expression tests samples were collected in the following time points: just before infection (for control), then 1, 4, 17, 24, 35, 48 and 65 hours post inoculation (hpi). RNA was extracted and quantitation of the transcripts for each time point was done by qPCR. Genes for analysis were chosen from the NGS generated transcriptome dataset. From among the more than 38,000 transcriptomes of the TC dataset biotic stress response genes were chosen for quantitation according to their RPKM (reads per kilobase per million mapped reads) value, which indicates the pathogen inoculation induced changes in the copy number of a transcriptome (Table 12). Sequence similarity and homology was also considered in selection, especially in such cases as for example the respiratory burst oxidase family (*Rboh*) which has different homologs, distinguished as A, B and C. These homologs were identified in the TC dataset as inoculation induced genes, hence they were used in the analysis.

Different types of protease inhibitor genes were identified in the transcriptome dataset. Among them four up-regulated homologs of different types including aspartic protease inhibitor, cysteine protease inhibitor, Kunitz-type inhibitor and serine protease inhibitor were evaluated quantitatively.

It should be noted that reason of the present expressional analysis was also to determine if the observed up-regulation of the selected genes of the TC dataset was really caused by the *P. infestans* inoculation, or possibly by the other two pathogens.

As indicated in Table 12, in total four different protease inhibitors, four genes belonging to the reactive oxygen species (ROS), two pathogenesis related protein (PR) genes, one immune receptor gene, one *P. infestans* resistance gene and four NBS-LRR type *P.*

85

infestans resistance gene homologs were selected and analyzed by qPCR. The stably expressed beta-tubulin gene was applied as housekeeping gene in the analysis.

Among different homologs of pathogenesis related proteins (PR-protein) one upregulated homolog, the PR1 (PGSC0003DMG400037874) was expressed only in the treated sample. Since PR10 proteins are also involved in the resistance response, the only PR10 protein of the TC dataset, the PGSC0003DMG402001494 was also used in the expressional analysis, although, it showed no significant expressional changes in the transcriptome analysis (Table 12).

In selection of the *P. infestans* resistance gene homologs results of the phylogenetic analysis were also considered. Among the 82 homologs used in the phylogenetic analysis 17 were up-regulated, while 15 were down-regulated, and expression of the remaining homologs doesn't changed over the RPKM criteria. The most pronounced expressional changes were found among the homologs of the *Rpi-blb2* and *R2* gene clusters. The fewest changes were observed in the *R1* sub-clade where among the nine homologs, except one the pathogen inoculation didn't induced expressional changes.

According to the phylogenetic trees, besides eight *Rpi*-genes 26 homologs clustered together with the *R2* gene. Among them, 11 homologs showed significant expressional changes with five up-regulated and six down-regulated genes.

Among the eight homologs of the *Rpi-blb2* sub-clade four were up-regulated and one was down-regulated.

After screening of originally 22 homologs of *Rpi-bt1* found in the White Lady TC dataset, 14 were selected and used for phylogenetic analysis. Except two, 12 homologs grouped to the same clade together with the *Rpi-bt1*, *Rpi-sto1* and *Rpi-pta1* genes. In this clade the PGSC0003DMG401007609 homolog showed the highest fold change (Fig. 14).



Fig.14. Partial dendrogram of the maximum parsimony tree showing the clusters of the *R1*, *R2* (right) and *Rpi-bt1* and *Rpi-blb2* (left) genes. (The complete dendrogram can be found in Appendix 3.)

	Gene family	Gene function	Transcript*	SGN ID**	RPKM- Control	RPKM- Treated
1		Aspartic protease inhibitor (AspI)	3	PGSC0003DMG400009513	1.48	10.42 ^{up}
2	Protease inhibitors	Cysteine protease inhibitor (CyspI)	5	PGSC0003DMG400010134	0.66	9.46 ^{up}
3		Kunitz-type protease inhibitor (KtpI)	6	PGSC0003DMG400015267	3.89	6.00
4		Serine protease inhibitor (SerpI)	7	PGSC0003DMG400026953	3.12	6.99 ^{up}
5	Development in the state of the	RbohC	1	PGSC0003DMG400014168	5.82	11.10
6	homologs	RbohA	1	PGSC0003DMG400012316	2.54	3.43
7		RbohB	1	PGSC0003DMG400024754	1.66	3.12
8	Antioxidant	Glutathione peroxidase (Glutp)	4	PGSC0003DMG402004978	2.99	18.01 ^{up}
9	PR-proteins	PR1	9	PGSC0003DMG400037874	0	2.43 ^{up}
10		PR10	1	PGSC0003DMG402001494	2.93	2.96
11	Immune receptor	PPR1	16	PGSC0003DMG400020554	3.26	18.52 ^{up}
12	<i>R</i> -genes & homologs from	R1 homolog	9	PGSC0003DMG400025545	1.54	2.23
13	S. demissum	R2 homolog	26	PGSC0003DMG401011522	2.42	8.30 ^{up}
14		R3a gene	12	PGSC0003DMG402030235	2.14	5.10 ^{up}
15	Rpi-homologs from	Rpi-btl homolog	11	PGSC0003DMG401007609	1.22	6.69 ^{up}
16	S. bulbocastanum	Rpi-blb2 homolog	9	PGSC0003DMG400023253	2.97	8.02 ^{up}

Table 12. Characteristics of genes identified in the transcriptome dataset and analyzed by qPCR

*: Number of transcripts in the TC dataset for that type of gene

**: Potato gene identification numbers of the SOL Genomics Network (SGN) database according to which transcripts of White Lady were annotated

^{up}: Up-regulated genes according to the >+2 RPKM value criterion

Based on the selection criteria, 16 homologs of 6 gene family groups were selected for RT-PCR studies. As a criterion for resistance against *P. infestans, PR1* gene which proved to have expressional changes in resistant potato cultivar treated with the late blight during the first time point (Orłowska et al., 2011), was selected and evaluated with other gene homologs in these experiments.

4.4.2.1. Expression analysis of protease inhibitor genes

Among the protease inhibitors three different types, the aspartic-, the cysteine and the serine protease inhibitors with different numbers of homologs were expressed in White Lady. No expression could be detected for the Kunitz-type protease inhibitor. Since the level of expression of the selected aspartic- and cysteine protease inhibitor gene are almost similar they are shown in the same figure (Fig. 15). Nevertheless, there is a strong difference in expressional peak of the two genes. The aspartic protease inhibitor is as its maximum at the 1st hour after inoculation with a 7 times increase, thus the inoculation triggers an immediate up-regulated of this gene. Then, its expression decreases rapidly. The cysteine protease inhibitor was expressed at low level and had the maximal up-regulation with 12.25 times fold change at 31 hours after inoculation, and it then decreased again in the following hours post inoculation (Fig. 15).



Fig. 15. Expression profile of the Kunitz-type protease inhibitor (*Ktpi*), of the asparatic- (*Aspi*) and cysteine (*Cyspi*) protease inhibitor genes in seven different post inoculation (hpi) time points

RQ: Relative quantitation

The serine protease inhibitor showed a gradual increase of expression and reached its maximum at 17 hours post inoculation with more than 180 times copy number increase (Fig. 16). Comparison between relative quantitation of different protease inhibitors at the peak point presented significantly higher expression levels for the serine protease inhibitor with a 26.76 and 15.13 times more than for the aspartic protease inhibitor and cysteine protease inhibitor, respectively.



Fig. 16. Expression profile of the serine protease inhibitor gene (*Serpi*) in in seven different post inoculation (hpi) time points RQ: Relative quantitation

4.4.2.2. Expression analysis of reactive oxygen species

All respiratory burst oxidase homologs were significantly induced after inoculation. Respiratory burst oxidase homologs (*Rboh*) are assigned as the main sources of reactive oxygen species and play a critical role in the signal transduction pathway and programmed cell death in the physiological process during HR (Thoma et al., 2003; Mur et al., 2008b).



Fig. 17. Expression profile of respiratory burst oxidase inhibitors type A (*RbohA*) and C (*RbohC*) and of the glutathione peroxidase gene (*GlutP*) in seven different post inoculation (hpi) time points

RQ:Relative quantitation

Expression levels of *Rboh* type *A* and *C* increased only at four hours post inoculation with a maximum 10 fold change in *RbohA* (Fig. 17), while for *RbohB*, the gene was rapidly activated and reached its maximum with ~180 fold change at four hpi, that is 24 and 17.9 times more expressional change than for *RbohA* and *RbohC* at 4 hpi (Fig. 18).

The analyzed glutathione peroxidase homolog was not significantly induced, it implies that this gene may not be induced in the early period of the succesful defense response to *P. infestans*.



Fig. 18. Expression profile of respiratory burst oxidase inhibitors type B (*RbohB*) in seven different post inoculation (hpi) time points

RQ: Relative quantitation

4.4.2.3. Expression analysis of PR proteins and immune receptor genes

Both pathogenesis-related proteins in this study have different activation ranges. For *PR1* the expression maximum was at 4 hpi and it then decreased rapidly, while *PR10* activation peaked at 31 hpi when the *PR1* was almost inactive. (Fig. 19)

The only plant immune receptor homolog which showed a high fold change of RPKM value in the transcriptome dataset expressed maximum at 4 hpi (Fig. 19).



Fig. 19. Expression profile of pathogenesis related proteins (PR1 and PR10) and of the immune receptor gene (PPR1) in seven different post inoculation (hpi) time points

RQ: Relative quantitation

4.4.2.4. Expression analysis of NB-LRR genes

The *R1* and *R2* homologs as well as the *R3a* gene showed a low constituent expression during the examined time period, but at 65 hpi the *R2* homolog got a very strong up-regulation (169 fold change) and the *R1* homolog a much less however significant (15.4 fold change) up-regulation, while the *R3a* gene expression showed no change at all (Fig. 20).



Fig.20. Expression profile of *R* gene (*R1*, *R2 and R3a*) derived from *S. demissum* in seven different post inoculation (hpi) time points

RQ: Relative quantitation

Among the *Rpi*-gene homologs which show similarity with *S. bulbocastanum* derived *Rpi* genes the *Rpi-bt1* homolog was activated by the inoculation and had an expression maximum (~21 fold change) at 4 hpi and it then was inactivated rapidly. The *Rpi-blb2* homolog showed a very low expression level during the examined period, which is somewhat surprising since for this gene much higher up-regulation was registered in the TC dataset. If this up-regulation is due to the infection of the other pathogens, it needs further examinations. (Fig. 21).



Fig. 21. Expression profile of *Rpi* genes (*Rpi-bt1* and *Rpi-blb2*) derived form *S*. *bulbocastanum* in seven different post inoculation (hpi) time points

RQ: Relative quantitation

4.4.3. Gel electrophoresis and sequence analysis of qPCR amplified fragments

Electrophoretic pattern of qPCR amplified fragments of all analyzed genes showed the expected size which is shown in Appendix 5. Each band was cloned and sequenced. Sequencing of the amplified genes proved that the true genes have been analyzed by qPCR (Fig. 22).

*Lane 1 in all electrophoretic patterns is beta-tubulin (β -tub) as a housekeeping gene. The arrangement of the profile band in each pattern is as below:

Protease inhibitors: aspartic protease inhibitor (AspI), cysteine protease inhibitor (*CyspI*) and serine protease inhibitor (*SerpI*); Rboh-s and Glutathion peroxidase genes: *Rboh A*, *B*, *C*, glutathione peroxidise (*GlutpI*); PRproteins and immune receptor gene: *PR1*, *PR10* and *PPR1*; Rpi-genes: *Rpibt1* homolog, *Rpi-blb2* homolog, *R1*, *R2* homologs and *R3a* gene



Fig. 22. Gel electrophoresis pattern of the genes analyzed by qPCR

5. DISCUSSION

5.1. Rpi-genes, importance and struggle for resistance against P. infestans

It is strongly believed that stacking of multiple *R*-genes in plants delays the onset of the pathogen invasion and potentially provide enhancement of durability against it.

High rate of recombination and sexual diversity of the pathogen stimulate evolution in potato to overcome and defeat the new races of the pathogen genetically. Variation in resistance gene resources to late blight may take place either by allele changes or segmental gene duplications during recombination. In general clustering of functional genes for qualitative and quantitative resistance to various pathogens suggests their evolution from common ancestors by local gene duplication followed by functional diversification (Friedman and Baker, 2007). There are at least 21 cloned major *P. infestans* resistance genes in potato and related *Solanum* species, while there is a supposedly large reservoir of up-to-now undetected genes that may also be utilized in cultivar improvement against this pathogen. Two important factors including pathogen effectors and gene nature during evolution have driven the evolution of gene variation. Race specific resistance genes generally have many homologs in the same *Solanum* species or different orthologs in other *Solanaceae* which may play a role in late blight resistance.

Phenotypic studies using also the Mastenbroek R lines revealed the existence of some *S*. *demissum* originating *R* genes in the genetic background of the Hungarian potato cultivar White Lady. This cultivar has high tolerance to *P. infestans* races presently widespread in Hungary, but gene compositions involved in resistance to late blight haven't been identified yet. Newly, multifunctional high throughput sequencing techniques called next generation sequencing offer novel, rapid ways for genome-wide characterization and profiling among others of mRNAs, small RNAs, transcription factor regions, chromatin structure and DNA methylation patterns, in any organism or in metagenomics studies (Ansorge, 2009). This technique is expected to create condition for identification of R genes and further accelerates their cloning (Vleeshouwers et al., 2011b). Implementation of next generation sequencing on biotic stress induced mRNA samples of White Lady

revealed the existence of a number of gene homologs of different race-specific -genes and broad spectrum *P. infestans* resistance genes in this cultivar. From a total of 142 *P. infestans* resistance gene homologs identified in this cultivar, more than 60 belong to race-specific *R*–genes, while the others were homologs of broad spectrum resistance genes (*Rpi*-genes) of *S. bulbocastanum* and other *Solanum* species. Out of these genes, *R1, R2, R3a* and *R3b* which have already been cloned (Ballvora et al., 2002; Huang et al., 2005; Lokossou et al., 2009; Li et al., 2011) were detected also by specific primers in the cultivar White Lady in this study.

5.2. Phylogenetic relationship of *P. infestans* resistance genes and gene homologs identified in the TC dataset

In the present analysis, 142 annotated late blight resistance gene homologs for 21 of the above *P. infestans* resistance genes (*R* genes and *Rpi* genes) were found, and except for five homologs, all of them proved to be of the NB-LRR type and could be identified among the potato-DM NB-LRR list of Jupe et al. (2012).

Recently, broad spectrum *P. infestans* resistance genes (Rpi) were identified and cloned of the diploid wild potato species *S. bulbocastanum* Dunal. These isolated genes are the *Rpi-blb1* (Van Der Vossen et al., 2003) also known as *RB* (Song et al., 2003), the *Rpi-blb2* (Vossen et al., 2005), the *Rpi-blb3* (Lokossou et al., 2009) and the *Rpi-bt1* (Oosumi et al., 2009). An *Rpi-*gene which possibly derives from *S. bulbocastanum* is the *Rpi-abpt*, (Lokossou et al., 2009) that was isolated from a complex quadruple hybrid of *S. acaule*, *S. bulbocastanum*, *S. tuberosum* Group *Phureja* and *S. tuberosum* (Park et al., 2005b). Further late blight resistance genes were cloned from *S. stoloniferum* (Vleeshouwers et al., 2008), from *S. edinense* Berthault, *S. hjertingii* Hawkes, *S. schenckii* Bitt., (Champouret, 2010), and from *S. venturii* Hawkes & Hjert. (Foster et al., 2009). From each of the four later species several *Rpi*-genes were cloned. Late blight resistance genes from *S. okadae* Hawkes & Hjert., and *S. mochiquense* Ochoa (Jones et al., 2013) were isolated and patented. While in White Lady only *S. demissum* derived *R* genes could be detected, interestingly, many *Rpi* gene homologs of different *Solanum* species, such as S. *bulbocastanum*, *S. hjertingii, S. schenkii, S. stoloniferum*, and *S. venturii* were found in

the TC dataset. It is suggested that there was a common ancestor of potato which contain some resistance gene homologs for any kinds of biotic stresses including *P. infestans*. During speciation, many of these genes have undergone diversification which led to emergence of new variants of the genes in a number of different species and their derivative cultivars.

In our phylogenetic analyses based on nucleotide sequences large distances were observed, which could mainly be attributed to large differences appearing in the sequences. Interestingly, we observed both homologous and non-homologous blocks corresponding to different regions of the P. infestans resistance genes. Most of the nonhomologues regions were unalignable forming autoapomorphic changes being characteristic to separate clades of the phylogenetic tree (Appendix 1, 2). Clades formed from the 82 homologues and the 21 Rpi genes corresponded to those found in the potato-DM NB-LRR tree of Jupe et al. (2012), which was based on analysis of the amino-acid chain of NB-LRR motifs of 438 genes were analyzed (Jupe et al., 2012). In the present phylogenetic analysis 50 late blight resistance gene homologs did not show expressional changes. These annotated late blight resistance genes were not affected by the inoculations, indicating that they have other roles in the genome. Nevertheless, the function of these 50 annotated genes is not clarified. It can be hypothesized that these homologs are also involved in the evolution of biotic stress response genes. The evolution of disease resistance genes is dynamic involving divergent selection and birth-and-death processes (Michelmore and Meyers, 1998).

Resistance genes are frequently clustered in the genome, and show significant similarities in sequence homology and structural motifs resulting from sequence exchanges among paralogs which generate novel chimeras (Kuang et al., 2005). To clarify the role of the 17 up-and of the 15 down-regulated genes in late blight resistance needs further investigation.

Gao et al. (2013) studied the interaction of potato tubers and *P. infestans* on the nontransformed late blight susceptible cultivar Russet Burbank, as well as on a transformed late blight resistant Russet Burbank line carrying the *RB* (*Rpi-blb1*) late blight resistance gene (Gao et al., 2013). They identified ~30,000 potato genes by 36 RNA-Seq reactions of samples taken at different time points (0, 24 and 48 hours post inoculation) and found expressional changes in 2,531 genes during this period of time. They concluded that time and treatment (pathogen treatment vs. and mock inoculation) have larger influences than the genotype on overall transcriptome differences. The *RB* gene confers resistance to all known *P. infestans* strains, while the White Lady cultivar carries strain specific late blight resistance genes and was challenged with an isolate containing six different avirulence genes. In our study leaves and three different pathogens were used, the post inoculation sampling time was much longer, and more sampling times were included. It is proposed that any or all of these factors could explain the observed changes affecting a much higher number of genes.

Structure based prediction of the function of genes lacking functional information is challenging, since homologous proteins often have distinct and sometimes multiple functions (Alberts, 2002). It would be very important to determine the role of the genes with unknown functions in the pathogen-potato interaction in order to utilize key resistance response genes in marker assisted potato breeding.

5.3. Intron targeting marker development for the detection of the R1 gene

The presence of the *R1* gene in White Lady was identified by phenotypic tests and highly similar transcripts of this gene were found in the TC dataset. Among the different *R1* primers the intron primers which were developed in this analysis resulted only the expected marker. Interestingly, it was found that this intron sequence of the *R1* homologs has significantly higher nucleotide similarity than the flanking exon sequences which are usually highly conserved sequences. The relatively high nucleotide identities for the intron sequences were not caused by the frequent sequence exchanges, and homogenized intron sequences among the *R1* homologues at the proximal sub-cluster suggest that they are type I resistance genes. So they haven't diverse region in this homologues genes as it is for coding region (Kuang et al., 2005).

IT-primers were designed for the flaking exon regions of the homologous sequences of the R1 gene. One IT-primer showed polymorphic bands between White Lady and the late blight susceptible S440 breeding line. Sequence analysis of the amplified intron fragment

in the NCBI database showed that it has highest similarity with the R1 gene of S. *demissum*.

Pairwise alignment of the amplified intron region of White Lady with different homologues showed that it covers the first intron of the A4, B23 and C3 R1 homologs, but doesn't gives any product in B16 that is another R1 homolog (Fig. 23). Although the region of this marker is an intron but this region was embedded in two exon regions with around 2600 bp length (Fig 23).



Fig.23. The intron region detected with the R1l333 IT primer pair in the *R1* homologs

A4: R1-A4 homolog, B23: R1-B23 homolog, C3: R1-C3 homolog, B16: R1-B16 homolog Inverted red colored V shape shows the intron region of the A4, B23 and C3 R1 homologs. First and second blue arrows are exon regions of the main ORF of the R1 homologs A4, B23 and C3.

5.4. P. infestans inoculation induced expressional changes as revealed by qPCR

The highly sensitive real-time PCR enables the quantification of gene expression changes. The results of quantification by this method are invaluable in the evaluation of the role of genes at the early stage of biotic stress response. Our study of the selected genes which are known to be involved in biotic stress responses revealed that Aspi, Serpi, Rboh A, B, C, PR1 and PPR1 are expressed more significantly at the biotrophic phase of infection with *P. infestans*. The first two time points, the 1 and 4 hours post inoculation showed up-regulation in most of these cases. These genes are involved in suppression of the pathogen by encoding of antimicrobial products, inhibitor proteins or controlling host cell death at the site of infection. It is clearly perceived that pathogens at biotrophic phase require living host plant cells for their survival and suppress the defense responses (Heath, 2000; Vleeshouwers et al., 2000c). A comparison between observed detached leaves and whole Sarpo Mira plants after inoculation with P. infestans showed a significant expression of some important genes including *PR1*, *Chitinase A*, *Chitinase B* and patatin-like. They were expressed more in leaves of whole plants, than in detached leaves at 1 hpi (Orłowska et al., 2012). It is strongly believed that very early expression has a great impact on resistance to the pathogen which is in relation to systemic acquired resistance (SAR) characterized by coordinated activation of a specific set of genes in both local and distal tissues (Durrant and Dong, 2004). Disruption of the SAR phenomena by leaf detachment deprives it of early induced response genes and leads to susceptibility (Orłowska et al., 2012). Among five transcript derived fragments (TDFs) which significantly up-regulated in the resistant potato cultivar Sarpo Mira compared to susceptible Bintje at 1 hour after inoculation with P. infestans, four genes showed different expression profiles throughout the whole infection process between both cultivars, hence they are considered to be involved in the resistance response to the pathogen (Orłowska et al., 2011).

5.4.1. Expression pattern of non-specific resistance genes to P. infestans

During the infection process, the *Phytophthora* produces enzymes that target host cells. In return plants encode secreted proteins that inhibit enzymatic activities of the pathogen. SDS-PAGE banding patterns of total leaf protein obtained at different time points after inoculation showed in some cases stronger bands in the treated samples. In a research study Fernandez et al. (2012) performed a densitometric analysis of SDS-PAGE bands of apoplastic hydrophobic proteins that were extracted from tubers of resistant and susceptible potato cultivars 24 hours after inoculation with *P. infestans*. The results showed higher amount of aspartic-, serine- and cysteine protease inhibitors in resistant samples compared to the control (mock-inoculated) and susceptible tubers (Fernández et al., 2012). Nevertheless, for the Kunitz-type protease inhibitors they didn't found expressional changes. Among different protease inhibitors in this study, Kunitz-type protease inhibitor with Id homolog PGSC0003DMG400010129 didn't express significantly in the treated White Lady over control. In a study, Kunitz PIs gene homologs were analyzed quantitatively in a 24 hpi treated resistant potato to control (sterile water). Among different homologues, only Kunitz type PIs didn't show any expressional changes (Fernández et al., 2012).

More abundant protease inhibitors which have been linked to plant defense response against pathogens are the trypsin and chemotrypsin inhibitors. Increased levels of these compounds correlated with the plants resistance to the pathogen (Kim et al., 2009) They belong to the serine protease type that inhibits the enzyme activity of the pathogen. Many different types of these molecules including *BTI-1*, *BTI-2*, *PT-1* have been found to play a role in resistance against pathogens in potato (Tian et al., 2004; Kim et al., 2005; Fernández et al., 2012). Sequence identity of one type of serine protease inhibitor in the test which was expressed more than other protease inhibitors was done by using of potato genomics resources database. The function of this gene was determined as '*BTI-2* trypsin inhibitor'.

Highest level of expression for all *Rboh* genes occurred at the 4th hour post inoculation. ROS signaling in the plant cell has a dynamic and rapid nature. This event is a consequence of contrasting processes between ROS production and scavenging. To reach the pinnacle of balance between scavenging and production rate, rapid alteration of ROS levels occur in plant cells (Mittler et al., 2011). Therefore it is possible that in a resistant potato plant like White Lady upon the pathogen attack, a leap in value of ROS followed by activation of NADPH oxidase *Rboh* genes are triggered by different stimuli.

The expressional changes of Rboh homologs showed a high fold changes of RbohB compared to other homologs in the test. Co-infiltration of *Nicotiana benthamiana* by Agrobacterium-containing *StCDPK5VK* gene originated from potato lead to activate four homologues of *Strboh* including A, B, C and D. By measuring the relative chemiluminescence intensity of *Strboh* homologs induced in transgenic plants highest intensity was measured for *StrbohB* (Kobayashi et al., 2012).

The results of this study as well as other similar studies indicate, *PR1* is an antimicrobial compound which is expressed mostly at the first time points after inoculation with the pathogen (Orłowska et al., 2011; Orłowska et al., 2012). This protein has an inhibitory effect on zoospore germination and mycelial growth (Niderman et al., 1995). At the early stage after inoculation, the sprayed sporangia and germinating zoospores of the fungi are present on the surface of the leaves and penetration process of infection and creation of haustoria develop. Production of *PR1* with its antimicrobial activity in the early stage of infection which is the biotrophic phase of pathogen would be a good criterion for resistance against *P. infestans*. Therefore White Lady with around 10 and 23 relative quantitation values at 1 and 4 hours after inoculation respectively assigned as a resistant cultivar to the pathogen. Another antifungal pathogenesis-related protein is *PR10*. Only one homologue of this gene was found in the TC dataset of White Lady. Unlike previous studies which indicates that *PR10* had no effect on *P. infestans* (Constabel et al., 1993), in our study proved that it has a role in resistance against this pathogen, however PR10 was expressed later than *PR1*.

Some plant resistance proteins have been found that could make physical interaction with pathogen effectors. These interactions agree with the model of effector-triggered immunity following direct recognition of effectors like *ATR1* by plant immune receptors *PPR1* (Postma et al., 2012). Sixteen homologues of plant immune receptors *PPR1* were identified in our TC dataset, and interestingly 10 of them were up-regulated and the most up-regulated was analyzed by qPCR. Nevertheless, our results indicated much less up-regulation of this PPR1 gene, indicating, that possibly either or both of the other two pathogens (PVX and PVY) may have triggered stronger up-regulation of this gene.

5.4.2. qPCR analysis of race-specific and broad spectrum resistance genes to *P*. *infestans*

Some homologs of race-specific (R) and broad spectrum (Rpi) resistance genes were found in the TC dataset that were significantly up-regulated after the inoculations. In the examined time period only the R1 and R2 homologs showed up-regulation but only at 65 hpi, while for the R3a gene no expressional change was observed. Interestingly the R2homolog had the highest expression level among the analyzed resistance gene homologs. Our results of the R1 homolog are in accordance with the results of Ross et al. (2004) who found that changes in expression of the R1 gene occurred only at the third day post inoculation. Our results on the R3a gene are somewhat astonishing, since in the TC dataset significant up-regulation was registered for this gene. Nevertheless, Huang et al. (2005) found that the R3a gene is a constitutively expressed gene which is in accordance with our qPCR results. The observed up-regulation in the TC analysis for this gene may be considered as an experimental mistake or a mistake of the assembling of the reads, where small homologous reads from different genomic regions amplified the signal of the R3a gene.

QPCR of the *Rpi-bt1* gene homolog showed a rapid and extended expression in the first half of the examined period. This gene is constitutively expressed in the wild *S*. *bulbocastum* in which it was identified, but introgression into different genetic backgrounds may affect both basal expression as well as the transcription activation after infection with late blight pathogen (Kramer et al., 2009).

In general, quantitative analysis of NGS-identified genes involved in resistance by qPCR contributes to our understanding about their role in resistance. The qPCR analysis revealed up-regulation of 11 genes from the analyzed 16 genes, indicating their role in the resistance response to *P. infestans* and determining the homologues of the target gene for resistance in biotic and abiotic stresses. In this study, some non-specific resistance gene homologues including *Serp1* with *BT1-2* trypsin inhibitor function and *RbohB* have higher expression value than other analyzed genes. On the other hand, some genes belong to NB-LRR including *R1* gene homolog with a high expression level and *Rbt1* with a high and extended level of expression, were identified which can be used in functional analysis studies. Since White Lady is a valuable source of different resistances and is used in our breeding programs, evaluation of the role of the above genes contribute to our efforts in developing biomarkers, which could be applied in resistance breeding to *P. infestans*.

LIST OF NEW FINDINGS

1) Identification of the *R1*, *R2*, *R3a* and *R3b* race-specific *P*. *infestans* resistance genes in cultivar White Lady by infection tests and/or by specific primers.

2) By analysis of the transcriptome dataset new data on pathogen inoculation induced genome-wide expressional changes have been obtained, which created new opportunities to design further genomics researches in the field of biotic stress response of potato. (These researches were partially realized in the present study.)

3) By phylogenetic analysis of *P. infestans* resistance gene homologs it was revealed that not only *S. demissum* derived *R*-gene homologs are present in White Lady, but also homologs of broad spectrum resistance (Rpi) genes of such species which are not present in the genetic background of this cultivar. This indicates the common ancestral origin of *P. infestans* resistance genes in potato and sheds light on their evolution.

4) Development of an intron targeting marker for the R1 gene based on sequence analysis of the transcriptome dataset of White Lady. The usefulness of this marker in the analysis of R1 homologs was proven. Since the R1 sequence in the NCBI for that region that was analyzed in the present study is somewhat different, it is suggested that the White Lady either has an allelic version of that R1 sequence or a functional homolog of this gene.

5) By quantitation analysis of biotic stress response genes eleven genes could be identified which were up-regulated by the *P. infestans* inoculation. The expression of these genes was characterized in seven different time points during the early period of the successful resistance response.

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PUBLICATION LIST

Referred articles related to thesis:

1. Hajianfar, R., Polgár, Z, Wolf, I., Takács, A., Cernák, I, and Taller, J.Complexity of late blight resistance in potato and its potential in cultivar improvement. Accepted for publication in Acta Phytopathologica Vol. 49 No. 2, 2014.

2. Ahmadvand R., Poczai, P., **Hajianfar, R**., Kolics B., Gorji A.M., Polgár Z. and Taller J. (2014). Next generation sequencing based development of intron-targeting markers in tetraploid potato and their transferability to other Solanum species. Gene 540, 117-21

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2. Hajianfar, R, Ahmadvand, R., Polgár,Z., Wolf, I, and Taller,j. (2013). *R1* late blight (*Phytophthora infestans*) resistance gene homolog in the cultivar White Lady. A Pannon Növény-Biotechnológiai Egyesület konferenciája PhD hallgatók számára, 2013. Május 15

3. Hajianfar, R, Ahmadvand, R., Mousapour Gorji, A, Wolf, I., Cernák, I., Taller,J, and Polgár,Z. (2013). Next generation sequencing based analysis of genes for resistance to *Phytophthora infestans* in cultivar White Lady. Abstract book of the EAPR - EUCARPIA Congress "The challanges of improving both quality and resistance to biotic and abiotic stresses in potato", June 30 - July 04. 2013, Hévíz, Hungary pp:26

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Appendixes

Appendix 1. Codon-based Test of Positive Selection for analysis between sequences of each clade of *R*-gene homologs of the TC data set of White Lady

Title: fasta file Description: R1 homologs of TC Analysis Analysis ----- Z-test of Selection Scope ----- In Sequence Pairs Test Hypothesis (HA: alternative) --- Positive selection (HA: dN > dS) Estimate Variance Variance Estimation Method ----- Bootstrap method No. of Bootstrap Replications ------ 500 Substitution Model Substitutions Type ------ Syn-Nonsynonymous Model/Method ------ Kumar method (Kimura 2-para) Data Subset to Use Gaps/Missing Data Treatment ----- Pairwise deletion No. of Sites : 1127 Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level [yellow backgroud]) Stat (blue) : Statistic used to compute the probability (blue).

[1] #PGSC0003DMG400028339
[2] #PGSC0003DMG400003380
[3] #PGSC0003DMG400018441
[4] #PGSC0003DMG400018442
[5] #PGSC0003DMG400025547
[6] #PGSC0003DMG400025545
[7] #PGSC0003DMG400023062
[8] #PGSC0003DMG400013506
[10] #R1_gene(AF447489.1)

	1	2	3	4	5	6	7	8	9	10
[1]	[2	2.569][3	3.329][2.944][1.227][3.086][[1.862]	[2.283]	[1.353]	[2.213]
[2]	0.006	[2	2.209][1	1.974][1.756][2.225][1.484]	[0.972]	[2.013]	[0.868]
[3]	0.001	0.015	[1	.650][1	1.591][3.128][2.651]	2.953][1.821]	[3.376]
[4]	0.002	0.025	0.051	[1	.831][1.851][2	2.147][2.346][1.738][3.134]
[5]	0.111	0.041	0.057	0.035	[0.752][1.522][2.280][2.616][2.336]
[6]	0.001	0.014	0.001	0.033	0.227	[]	1.802][-	-0.484][0.532][1.160]
[7]	0.033	0.070	0.005	0.017	0.065	0.037	[1.882][2	2.729][2.193]
[8]	0.012	0.167	0.002	0.010	0.012	1.000	0.031	[]	2.598][1.683]
[9]	0.089	0.023	0.036	0.042	0.005	0.298	0.004	0.005]	3.015]

 $[10] \quad 0.014 \quad 0.194 \quad 0.000 \quad 0.001 \quad 0.011 \quad 0.124 \quad 0.015 \quad 0.048 \quad 0.002$

Title: fasta file
Description: R2 homologs of TC
Analysis
Analysis Z-test of Selection
Scope In Sequence Pairs
Test Hypothesis (HA: alternative) Positive selection (HA: $dN > dS$)
Estimate Variance
Variance Estimation Method Bootstrap method
No. of Bootstrap Replications 500
Substitution Model
Substitutions Type Syn-Nonsynonymous
Model/Method Kumar method (Kimura 2-para)
Data Subset to Use
Gaps/Missing Data Treatment Pairwise deletion
No. of Sites : 754
Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level
[yellow backgroud])
Stat (blue) : Statistic used to compute the probability (blue).

[1] #PGSC0003DMG400006531
[2] #PGSC0003DMG400006533
[3] #PGSC0003DMG400011517
[4] #PGSC0003DMG400011518
[5] #PGSC0003DMG400011520
[6] #PGSC0003DMG400011521
[7] #PGSC0003DMG401011522
[8] #PGSC0003DMG400011523
[9] #PGSC0003DMG400011524
[10] #PGSC0003DMG400011525
[11] #PGSC0003DMG400011527
[12] #PGSC0003DMG400011528
[13] #PGSC0003DMG400011529
[14] #PGSC0003DMG400011920
[15] #PGSC0003DMG400032572

[16] #PGSC0003DMG400032576
[17] #PGSC0003DMG402032547
[18] #PGSC0003DMG400032578
[19] #PGSC0003DMG400032581
[20] #PGSC0003DMG400032584
[21] #PGSC0003DMG400024231
[22] #PGSC0003DMG400024234
[23] #PGSC0003DMG400023288
[24] #PGSC0003DMG400023288
[25] #PGSC0003DMG401010614
[26] #PGSC0003DMG400023273
[27] #R2_gene_(FJ536325)
[28] #Rpi_protein_gene_ABPT(FJ536324.1)
[29] #Rpi-blb3_(FJ536326.1)

*: Only homolog no. 23 has significant diversified codons in the analysis of positive selection

Title: fasta file
Description: R3 homologs of TC
Analysis
Analysis Z-test of Selection
Scope In Sequence Pairs
Test Hypothesis (HA: alternative) Positive selection (HA: $dN > dS$)
Estimate Variance
Variance Estimation Method Bootstrap method
No. of Bootstrap Replications 500
Substitution Model
Substitutions Type Syn-Nonsynonymous
Model/Method Kumar method (Kimura 2-para)
Data Subset to Use
Gaps/Missing Data Treatment Pairwise deletion
No. of Sites : 1055
Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level
[yellow backgroud])

Stat (blue) : Statistic used to compute the probability (blue).

[1] #PGSC0003DMG400007344 [2] #PGSC0003DMG400014047 [3] #PGSC0003DMG400018570 [4] #PGSC0003DMG400018574 [5] #PGSC0003DMG402018576 [6] #PGSC0003DMG403019668 [7] #PGSC0003DMG400018694 [8] #PGSC0003DMG401030236 [9] #PGSC0003DMG400030238 [10] #PGSC0003DMG400030239 [11] #R3a_gene|AY845382| [12] #R3b_gene|JF900492| [13] #AF118127.1_L.esculentum_-I2_gene 3 10 13 1 4 5 6 8 11 12 [1.299] [2.784] [1] [2.260] [2.440] [2.187] [1.905] 0.169 [2] [-0.501] [0.155] [-0.995] [-1.304] [3] 0.002 [2.182] [-0.248] [1.597] -0.090] [0.512] [1.653] 0.039 [4] 1.000 0.280 [0.056] [1.457] [-0.751] [1.243] [0.619] 1.425] [0.822] [0.845] [1.553] [5] [6] [7] [8] -21.000 [1.271] 0.054 1.000 1.000 [0.145] -2.364 [-2.656] [-2.160 [-1.138] [-2.285] [-1.193] 0.003 0.266 0.490 0.074 0.443 -0.186 -0.954] [0.257] [0.142] -0.239 [0.296] [.486] 0.103 0.070 1 000 1 000 0.051 1 000 [1.659] 0.1721 [1.826] [2045] 2 128 1 2.1351

0.050

0.432

0.035

0.022

0.018

0.017

1.000

1.000

0.399

0.444

1.000

0.384

0.013

0.098

0.008

0.015

0.030 0.003

[9] [10]

[11]

[12] [13]

1.000

1.000

0.438

1.000

 $1.000 \\ 1.000$

0.016

1.000

0.056

1.000

0.305

0.050

0.108 1.000

0.268 1.000

0.078 1.000

0.206 1.000

 $\begin{array}{ccc} 0.200 & 1.000 \\ 0.062 & 1.000 \end{array}$

[0.262]

[0.417]

1.000

1.000

-0.562]

[1.121]

[-1.521]

0.339

1.000

0.167

[-0.633]

1.000

1.000

 $1.000 \\ 1.000$

1.000

0.132

0.397

0.294 0.069

[0.543]

[-0.671] [-0.010]

[-1.150]

1.000

[1.496]

[0.969]

[-0.431]

[-0.025]

-0.066]

Title: fasta file
Description: <i>Rpi-blb1</i> homologs of TC
Analysis
Analysis Z-test of Selection
Scope In Sequence Pairs
Test Hypothesis (HA: alternative) Positive selection (HA: $dN > dS$)
Estimate Variance
Variance Estimation Method Bootstrap method
No. of Bootstrap Replications 500
Substitution Model
Substitutions Type Syn-Nonsynonymous
Model/Method Kumar method (Kimura 2-para)
Data Subset to Use
Gaps/Missing Data Treatment Pairwise deletion
No. of Sites : 875
Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level
[yellow backgroud])

Stat (blue) : Statistic used to compute the probability (blue).

[1] #PGSC0003DMG400009324

[2] #PGSC0003DMG400019804

[3] #PGSC0003DMG400030855

[4] #PGSC0003DMG400017060

[5] #PGSC0003DMG400017062

[6] #PGSC0003DMG400018050

[7] #PGSC0003DMG402009305

[8] #PGSC0003DMG400007608

[9] #PGSC0003DMG401007609

[10] #PGSC0003DMG400029588

[11] #PGSC0003DMG400029590

[12] #S._bulbocastanum_Rpi-bt1(FJ188415)

[13] #Rpi-pta1(EU884422)

[14] #S._bulbocastanum_RPI_gene(AY426259)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
[1]	[-	1.016][0.304][1.418][2.370]	[0.113]	[-0.888]	[0.697][1.030]][0.064][-0.050][1.964]][0.938]	[0.789]
[2]	1.000	[-().399][-	0.286][0.013][-2.324]	[-0.426]	[-1.600][-1.249][-0.349	9][-1.604][0.420][-0.818][-0.886]
[3]	0.381	1.000	[().057][1	1.242][-	0.546][1.189][-0.799]	[-0.534]	[-0.168][-0.720][0.438]	[-0.003]	[-0.237]
[4]	0.079	1.000	0.477	[0	.559][().013][0.649][-	0.483][-0.012][-0.646	[-2.178]	[2.353]	[1.531][1.505]
[5]	0.010	0.495	0.108	0.289	[().441][-(0.111][0.192][0.943][·	-0.612]	[-1.152][1.500][0.926][0.867]
[6]	0.455	1.000	1.000	0.495	0.330	[0	.660][-]	1.776][-	2.389][-	-1.287][-2.021][-0.521][-0.822][·	-0.704]
[7]	1.000	1.000	0.118	0.259	1.000	0.255	[1	.165][().501][-().735][·	0.328][-	0.265][-	0.173][-(0.173]
[8]	0.244	1.000	1.000	1.000	0.424	1.000	0.123	[-0	0.027][-1	1.168][-	2.387][0.033][-().525][-0).590]
[9]	0.153	1.000	1.000	1.000	0.174	1.000	0.309	1.000	[-1.	.168][-2	2.710][0	.248][-0	.328][-0.	.382]
[10]	0.475	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	[0	.000][-0	.157][-1	.093][-1	.093]
[11]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	[-1	.787][-2.	298][-2.	459]
[12]	0.026	0.338	0.331	0.010	0.068	1.000	1.000	0.487	0.402	1.000	1.000	[0.3	90][0.5	46]
[13]	0.175	1.000	1.000	0.064	0.178	1.000	1.000	1.000	1.000	1.000	1.000	0.348	[0.8	15]

[14] 0.216 1.000 1.000 0.067 0.194 1.000 1.000 1.000 1.000 1.000 1.000 0.293 0.208 Title: fasta file Description: Rpi-blb2 homologs of TC Analysis Analysis ----- Z-test of Selection Scope ----- In Sequence Pairs Test Hypothesis (HA: alternative) --- Positive selection (HA: dN > dS) Estimate Variance Variance Estimation Method ----- Bootstrap method No. of Bootstrap Replications ------ 500 Substitution Model Substitutions Type ------ Syn-Nonsynonymous Model/Method ------ Kumar method (Kimura 2-para) Data Subset to Use Gaps/Missing Data Treatment ------ Pairwise deletion No. of Sites : 1023 Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level [yellow backgroud]) Stat (blue) : Statistic used to compute the probability (blue).

#PGSC0003DMG400023253
 #PGSC0003DMG400020736
 #PGSC0003DMG400025511
 #PGSC0003DMG400020749
 #PGSC0003DMG400010895
 #PGSC0003DMG400020741
 #PGSC0003DMG400021987
 #PGSC0003DMG400020732
 #Rpi-blb2_(DQ122125)

[1	2	3	4	5	6	7	8	9
[1]	[0]).896][().408][-	0.916][-0.080]	[-0.407]	[0.525][-0.903	3][0.307]
[2]	0.186	[3	.006][().417][4.861][2.117][2.318]	1.775][2.893]
[3]	0.342	0.002	[-().659][2	2.288][-	0.664][0.199]	[0.468]][-0.151]
[4]	1.000	0.339	1.000	[0]).492][-(0.168][-	1.066][-1.065	[-1.162]
[5]	1.000	0.000	0.012	0.312	[-().128][1.859][1.556]	[1.353]
[6]	1.000	0.018	1.000	1.000	1.000	[1	.056][-	1.551][-1.210]
[7]	0.300	0.011	0.421	1.000	0.033	0.146	[-().751][·	-0.494]
[8]	1.000	0.039	0.320	1.000	0.061	1.000	1.000	[-	0.372]
[9]	0.380	0.002	1.000	1.000	0.089	1.000	1.000	1.000	

Title: fasta file Description: Rpi-vnt1.1 homologs of TC Analysis Analysis ----- Z-test of Selection Scope ----- In Sequence Pairs Test Hypothesis (HA: alternative) --- Positive selection (HA: dN > dS) Estimate Variance Variance Estimation Method ----- Bootstrap method No. of Bootstrap Replications ----- 500 Substitution Model Substitutions Type ------ Syn-Nonsynonymous Model/Method ------ Kumar method (Kimura 2-para) Data Subset to Use Gaps/Missing Data Treatment ----- Pairwise deletion No. of Sites : 812 Prob (black) : Probability computed (must be <0.05 for hypothesis rejection at 5% level [yellow backgroud]) Stat (blue) : Statistic used to compute the probability (blue).

#PGSC0003DMG400024363
 #PGSC0003DMG401020585
 #PGSC0003DMG400020584
 #PGSC0003DMG400020587
 #PGSC0003DMG403020585
 #Rpi-vnt1.1(FJ423044)
 #Rpi-vnt1.2(Fj423045)
 #Rpi-vnt1.3_(FJ423046)
 #Rpi-vnt1_like_protein(oka_745-1)

2 4 5 9 1 3 6 7 8 [1] [0.528][1.793][1.645][1.494][0.814][0.899][0.899][0.905] 0.299 [1.845][0.601][-0.378][0.304][0.542][0.542][0.892] [2] [3] 0.038 0.034 [1.877][1.084][0.045][0.544][0.544][1.252] [4] 0.051 0.275 0.032 [1.308][2.169][2.350][2.350][1.477] [5] 0.069 1.000 0.140 0.097 [1.561][1.566][1.566][0.786] [6] 0.209 0.381 0.482 0.016 0.061 [-0.536][-0.536][0.315] 0.294 [7] 0.185 0.294 0.010 0.060 1.000 [0.000][0.540] 0.294 0.294 [8] 0.185 0.010 0.060 1.000 1.000 [0.540] [9] 0.184 0.187 0.106 0.071 0.217 0.377 0.295 0.295



Appendix 2: Most parsimonious tree found by the phylogenetic analysis of the late blight resistance gene homologues of the potato cultivar White Lady^{*}



Appendix 3: Maximum likelihood (ML) majority rule consensus tree of the late blight resistance gene homologues of the potato cultivar White Lady^{*}

*: Late blight resistance genes are abbreviated as in NCBI and are written in bold-italics.

Appendix 4: NBS-LRR region alignments of *R*-gene homologues of White Lady

ENLRKKLLNO ENLRKKLLNO ENLRKKLLNO ZTLKGKLIKO ZTLKGKLIKO DELIGKLKGO ETLVHKLIRO DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGESP-TI DAIGESP-TI 150 	I I STKGQDVISI STKGQDVISI STKGQDVISI SKLDLISI SSRLDVIAI SSRLDVIAI SSRLDVIAI SSRLDISI 90 I I P0 I I RRELHANELA HRELHDNELA SDKLPENELA CREKQADELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA LSKETDELA	I HGMPGLGKT I HGMPGLGKT I HGMPGLGKT I HGMPGLGKT I VGMAGLGKT VVGMPGLGKT I VGMAGLGKT I VGMA	. TLANSLYSDRS TLANRLYSDRS TLANRLYSDRS TLANKLFLDQI TLAYRLYYDKS TLANKLYSDES TLANKLYSDES TLANKLYSDES 110 . RRYLILVDDVW RRYLILVDDVW RRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDVW KRYLILVDDW KRYLILVDDW	II.VFSQFDICAQQ VVSQFDICAQQ VVSQFDICAQQ VVSQFDICAQQ VVSHFDIRAQQ VVSHFDIRAQQ VVSHFDIRAQQ VVSHFDIRAQQ VVSKFDIRAKQ I20 II ENSVWDDLRG VETSVWDDLRG	I I CCVSQVYSYK CCVSQVYSYK CCVSQVYSYK CCVSQVYSK CCVSQVYSK CCVSQYSK CCVSQYSK CCVSQAYSR CCVSQAYSR 130 I I CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNSSR CFPDNNSS CFPDNNSSR CFPDNNSSR	 DLIL DLLL DLLL DLLL GLLLSL SVLL I40 IILTTR IILTTR IILTTR IILTTR IILTTR IILTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR ILLTTR
ENLRKKLLNG ENLRKKLLNG ENLRKKLLNG ZTLKGKLIKG EKLRDQLIKG DELIGKLKG ETLVHKLIRG DAIGEGS-VI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI DAIGEDSDQI SVKKDTV-IS DAIGESP-TI 150 	STKGQDVIS1 STKGQDVIS1 STKGQDVIS1 SSKLDLIS1 SSKLDLIS1 SSRLDVIS1 SSRLDVIS1 SSRLDVIS1 SSRLDVIS1 SSRLDVIS1 SSRLDDIS1 SSRLDDIS1 SSRLDDIS1 SSRLPENELS SCKLPENELS SCKLPENELS SCKLPENELS SCKLPENELS LLSKETDELF LTKLSTDVLS 160 	IHGMPGLGKT IHGMPGLGKT IHGMPGLGKT IVGMAGLGKT IVGMAGLGKT IVGMAGLGKT IVGMPGAGKT 100 . ADMLRKTLLPI ADKLRKTLLPI ADKLRKTLLPI ADKLRKTLLPI ADKLRKTLLPI ADKLRKTLLPI ADKLRKTLLPI ADKLRKTLRI ADKLRKTLFG ADLLRKTLFG ADLLRKTLFG ADLLRKTLWI IT70 . SSWKLLEKKVI	TLANSLYSDRS TLANRLYSDRS TLANRLYSDRS TLANRLFLDQI TLAYRLYYDRS TLANRLFLDQI TLAYRLYYDRS TLANRLYSDS TLANRLYSDS TLANRLYSDS TUAN	VFSQFDICAQQ VVSQFDICAQQ VVSQFDICAQQ VVSQFDICAQQ VVSFFDIRAQQ VVSFFDIRAQQ VVSFFDIRAQQ VVSFFDIRAQQ VVSFFDIRAQQ VVSFFDIRAQQ I20 ETTACDDLMPQ IETSVWDDLRGQ IETSVWDDLRGQ IETRAWDDLKLQQ IETRAWDDLKLQQ IETRAWDDLKQQ IETRAWDDLKQQ IETRAWDDLKQQ IETRAWDDLKQQ IANGURIAKMQQ	CCVSQVYSYK CCVSQVYSYK CCVSQVYSYK CCVSQVYSK CCVSQVYTRK CCVSQVYTRK CCVSQEYTRK CCVSQAYSRR 130 II CFPDVNNRSR CFPDVNNRSR CFPDVNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNRSR CFPDNNSSN CFPDNNSSN CFPDNNSSN CFPDNNSSN CFPDNNSSN CFPDNNSSN CFPDNNSSN	DLIL DLLL DLLL DLLL GLLLSL SVLL I40 IILTTR IILTTR IILTTR IILTTR IILTTR IILTTR IILTTR IILTTR IILTTR IILTTQ 210 AGILSE
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GVKKDTV-IS DAISENF-EC DITDERA-KI VSIDETSI DAIGESP-TI 150 KYASVHSDPI	SDKLPENELA CREKQADELA LRRETENELA LLSKETDELF LTKLSTDVLA 160 LHLRMFDEVE LHLRMFDEDE	ADKLRKLLFG ADLLRKTLFSI ADKLRKLLMRI KDILSRILRSI ADQLRKTLLWI 170 	2RYLILIDDVM KRYLILVDDVM KRYLLLIDDVM KRYLILLDDVM KRYLILVDDIM 180 . FGEESCSPLL	ETTACDDLMP(ETSVWDDLRG: ETRAWDDLKL IDHKAWDDLKC IEASVWDDLRC(190 INVGLRIAKMC(CFYEANNGSR SFRDSNNGSR CFPEDNNRSR CFPDDNTGSR CFHDSNNASR 200 GQLPLSIVLV	LILTTR IILTTR IILTTR ILLTTR IILTTQ 210 AGILSE
DAISENF-EC DITDERA-KI VSIDETSI DAIGESP-TI 150 KYASVHSDPI KFASVHSDPI	CREKQADELA LRRETENELA LLSKETDELF LTKLSTDVLA 160 LHLRMFDEVE LHLRMFDEDE	ADLLRKTLFSI ADKLRKLLMRI KDILSRILRSI ADQLRKTLLWI 170 	KRYLILVDDVW KRYLLLIDDVW KRYLILLDDVW KRYLILVDDIW 180 . FGEESCSPLLK	ETSVWDDLRG ETRAWDDLKL DHKAWDDLKC EASVWDDLRC 190 NVGLRIAKMC	SFRDSNNGSR CFPEDNNRSR CFPDDNTGSR CFHDSNNASR 200 3QLPLSIVLV	IILTTR IILTTR ILLTTR IILTTQ 210 AGILSE
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DAIGESP-T 150 KYASVHSDPI KFASVHSDPI	160 LHLRMFDEVE LHLRMFDEDE	ADQLRKTLLWI 170 . ESWKLLEKKVI	180 . FGEESCSPLLE	EASVWDDLRC(190 NVGLRIAKMC(200 200 	11LTTQ 210 AGILSE
150 KYASVHSDP1 KFASVHSDP1	160 LHLRMFDEVE LHLRMFDEDE	170 . SWKLLEKKVI	180 . FGEESCSPLLE	190 NVGLRIAKMC	200 <u>30LPLSIVLV</u>	210 A <u>GILS</u> E
 KYASVHSDP1 KFASVHSDP1	 LHLRMFDEVE LHLRMFDEDE	. ESWKLLEKKVI ESWKLLEKKVI	. FGEESCSPLLE	 NVGLRIAKMC	 <mark>GQLPLSIVLV</mark>	 AGILSE
KYASVHS D PI KFASVHS D PI	LHLRMFDEVE LHLRMFDEDE	ESWKLLEKKVI ESWKLLEKKVI	FGEESCSPLLK	NVGLRIAKMC	GQLPLSIVLV	AGILSE
KFASVHSDP1	LHLRMFDEDE	ESWKT.LEKKVI	CROCCODIT			
			GEQSCSPLLE	DVGLRIAKMC	GQLPLSIVLV	AGILSE
YHAKLVS D PI	HFLRKFTLEE	SWILLKNKVI	FNKKSCPAVLE	DVGQKIAQKC	GGLPLSVVLV	AGILET
MYTKIRSDP1	LLLRMFNSDE	ESWELLRKKVI	FGEESCSPLL1	'EIGQQIANKC	GQLPLSVVLV	AGILAE
SHA K HDSDPI	HKLRFLNSDE	SWMLLNKKVI	ENNESGPLILE	DVSQEIVRKC	GGLPISIILV	AGILTR
DYVKSVNKPI	HHLSLLTYEE	ESWELLKMKVI	FGNGSCSPLLE	KVGQEIVRKC	GGLRLSIVLV	AGILSK
220	230	240	250	260	270	280
•••• ••••		.	.			
ECWEQVANNI	L <mark>GSYIHND</mark> S <mark>F</mark>	RAIVDKSYHVI	LPCHLKSCFLY	FGAFLEDRVI	DISRLIRLWI	SEAFIK
ECWEQVANNI	L <mark>GTHIHND</mark> S F	RAIVDQSYHVI	LP CHLKSCFLY	FGAFLEDRVI	DISRLIRLWI	SESFIK
RCWEQVAIN	LGPHIQAKS <mark>E</mark>	EDIINLSYQD1	LPFHLKPCFLY	FGVFSEDEEI	KVSKI TWLWT	AEGLV-
ECWEQLANNI	L <mark>GPHIHKD</mark> SF	RTVIEQSYQII	LP YRLRPCFLY	FGALLEDSVI	S VPK LTQLWI	S EGFVK
	L <mark>GTNIQDQME</mark>	EGTLDLSYQN1	LPP YLK P CFLY	LGVFPEDGEI	QVS K LTWLWI	AEGFIK
HCWEQMATNI				CORT PETET	NVSKLTWMWI	GEGFVN
HCWEQMATNI ECWSEVAKHI	L <mark>GINMLSAL</mark> N	N DIIE QS Y QHI	LPYHLKSCFLY	FGIFLEFREI		
	SCWEQVANNI SCWEQVANNI SCWEQVANNI RCWEQVAINI SCWEQLANNI HCWEQMATNI	ECWEQVANNLGSYIHNDS ECWEQVANNLGTHIHNDS RCWEQVAINLGTHIQAKS ECWEQLANNLGPHIQAKS HCWEQMATNLGTNIQDQM	ECWEQVAINLGSYIHNDSRAIVDKSYHV SCWEQVAINLGTHIHNDSRAIVDQSYHV RCWEQVAINLGTHIHNDSRAIVDQSYHV ECWEQLANNLGPHIQAKSEDIINLSYQD ECWEQLANNLGPHIHKDSRTVIEQSYQI	2CWEQVANNLGSYIHNDSRAIVDKSYHVLPCHLKSCFLY 2CWEQVANNLGTHIHNDSRAIVDQSYHVLPCHLKSCFLY 2CWEQVAINLGTHIHNDSRAIVDQSYHVLPCHLKSCFLY 2CWEQLANNLGPHIQAKSEDIINLSYQDLPFHLKPCFLY 2CWEQLANNLGTHIQDMEGTLDLSYQNLPYLKPCFLY 2CWEQMATHLGTNIQDMEGTLDLSYQNLPYLKPCFLY	ZCWEQVANNLGSYIHNDSRAIVDKSYHVLPCHLKSCFLYFGAFLEDRVI ZCWEQVANNLGTHIHNDSRAIVDQSYHVLPCHLKSCFLYFGAFLEDRVI ZCWEQVANNLGTHIHNDSRAIVDQSYHVLPCHLKSCFLYFGAFLEDRVI RCWEQVAINLGPHIQAKSEDIINLSYQDLPFHLKPCFLYFGVFSEDEEI ZCWEQLANNLGPHIHKDSRTVIEQSYQILPYRLRPCFLYFGALLEDSVI HCWEQMATNLGTNIQDQMEGTLDLSYQNLPPYLKPCFLYLGVFPEDGEI ECWSEVAKHLGINMLSALNDIIEQSYQHLPYHLKSCFLYFGTFLEFKEI	SCWEQVANNLGSYIHNDSRAIVDKSYHVLPCHLKSCFLYFGAFLEDRVIDISRLIRLWI SCWEQVANNLGTHIHNDSRAIVDQSYHVLPCHLKSCFLYFGAFLEDRVIDISRLIRLWI SCWEQVANNLGTHIHNDSRAIVDQSYHVLPCHLKSCFLYFGAFLEDRVIDISRLIRLWI RCWEQVAINLGPHIQAKSEDIINLSYQDLPFHLKPCFLYFGVFSEDEEIKVSKITWLWT. SCWEQLANNLGPHIHKDSRTVIEQSYQILPYRLPCFLYFGALLEDSVISVPKLTQLWI HCWEQMATNLGTNIQDQMEGTLDLSYQNLPPYLKPCFLYLGVFPEDGEIQVSKLTWLWI. ECWSEVAKHLGINMLSALNDIIEQSYQHLPYHLKSCFLYFGTFLEFKEINVSKLTWMWI

Protein sequence alignment of the NBS region of *R1* homologs

	• • • •
R1-AF447489	SSEG
400003380	SCEG
400025545	
400013506	
402004578	SCE-
400028339	PHTG
400018442	DLEG
400023062	

Protein sequence alignment of the LRR region of *R1* homologs

	10	20	30	40	50	60	70
R1 gene-AF447489	 LKVLDLEHRVFIDFI	 PT <mark>ELVYLK</mark> Y	 FSAHIEQNSII	 PSSISNLWNL <mark>I</mark>	 Tlil k spiy	 ALRCTLLLPS	 IVW <mark>D</mark> MV
400003380 400025545							
400013506				<i>I</i> 1	NLSYODLPFH.	LKPCFLYFG	VFS
402004578				TVI	ZQSYQILPYR	LRPCFLYFG	A LL
400028339				TL1	DLSYQNLPPY	LKPCFLYLG	VF P
400018441 400023062	LKVLNLES-TVINSF	PTVLVYLRY	FAAQTDQDSI	TSLIANLWNL	TLILKP	-TKGKLKLPVI	" IMKMV
	80	90	100	110	120	130	140
							••••
R1 gene-AF447489	KLRHLYIPDFSTRIE	AALLENSAK	LYNLETLSTL	YFSRVEDAELI	ILRKTPNL	RKLICEVECLE	Y PPQ Y
400003380	SALK	KALLENSPK	LDDLETLSNS	YFTRVEDAELI	ILRKTPNL	RNLTCKIECLE	(YPHQY
400025545	SALK	KALLENSPK	LDDLETLSNS	YFTRVEDAELI	<i>ILT</i>	CEVESLE	<u>YPHQ</u> Y
400013506	EDEEIKVSKITWLWT	AEGLVKTHK	EKLSEDIAEN	LKNLIGRNL	MVSKKSSNG	KTKTCRIHDLI	LEFCK
402004578	ED SVISVP K LTQLWI	SEGFVKSCE	GKRLEDIAEG	LENLIGRNL	MGTKRSSRG	KIKACHIHDLI	HDFCK
400028339	EDGEIQVSKLTWLWI	AEGFIKPHT	GKTLEEIAEN	LENLVGRNL	MIDKRSSDG	RIKTCHIHDLV	THEVCR
400018441	RLRHLCIDNTYFTLN	GEEGL	LEKLEVLSTPO	CFSCAKDVELI	VQKTPNL	RELRCSFVGFF	RQECL-
400023062			L R 1	THKNKKSLI	MDHKS	<i>HKGM</i> Ç	20
	150						
R1 gene-AF447489	HVLNFPIR						
400003380	HALNFPIR						
400025545	HVLNFPIR						
400013506	<i>KKAKVENFLQ</i>						
402004578	<u>ERA</u>						
400028339	KKAKLEN						
400018441							
400023062							

Protein sequence alignment of the NBS region of R2 homologs

	10	20	30	40	50	60
70						
			1			
R2-FJ536326	LODVVOKLLAOLLKA	EPRRTVLSI	HGMGGLGKTT	LARKLYNSSA	ILNSFPT <mark>RA</mark> WI	CVSOEYNTMDLLRNI
Bb1b3-FJ536325		EPRRSVIST	YGMGGT.GKTT	T.ARNT.YKNPN	TASSFPTRTWT	CVSOEYNTMDLLRNT
Bniabnt-E.T536324	FODVVOTELAOLLK	EPRRSVIST	VCMCCL.CKTT	T.ARKT.VTSPD	TINSFRTRAWT	CVSOEVNTMDLL.PNT
R_{p_1}			VCMCCI CKTT	TADAT VACOD		CUCOEVNEMDLIDNT
Rpinjt1.1-G0565971		EPRRSVLSI	IGMGGLGKIT	LARNLINSPD.	LINSPPIRAWI	CVSQEINIMDLLRNI
Rpinjt1.2-G0563972	FQDVVQTFLAQLLKA	EPRRSVLSI	YGMGGLGKIT	LARKLYTSPD	LLINSERTRAWI	CVSQEYNIMDLLRNI
Rpihjt1.3-GU563973	LQDVVQKLLAQLLK	EPRRSVLSI	YGMGGLGKTT	LARNLYNSPD	ILNSFPTRAWI	CVSQEYNTMDLLRNI
Rpiedn1.1-GU563963	LQDVVQKLLAELLK	EPRRSVLSI	YGMGGLGK TT	LARNLYKNPN:	IASSFPTRTWI	CV SQ EY NT MD LL R NI
Rpisnk1.1-GU563975	FQDVVQTLLAQLLKA	EPRRSVLSI	YGMGGLGK TT	LARKLYTSPD	ILNSFPT <mark>RA</mark> WI	CVSQEYNTMDLLRTI
Rpisnk1.2-GU563976 400024234	LQDVVQTLLAELLKA	EPRRSVLSI	YGMGGLGKTT	LARNLYNS	-LKSFPTRTWI	CVSQEYNTMDLLRNI
400011527	FODVVHTLLSELLK	EPRRRVLSV	YGMGGLGK TT	LARKLYTSPN	IASSFLTRAWI	CVSOEYNTMDLLKTI
400011525	LODVVOKLLAELLKA	EPRRSVISI	HGMGGLGKTT	LARNLYNSSN	IVSSFPTRAWI	CVSOEYSTMDLLKTI
400011524						MDT.T.KTT
400011522	FODVVEST.LAELLK	EPRRNXHT.H	TRYCRXARPY		TLSSLASOHAT.	GVVFLKSTTPWTSLG
400011521		FDCPSVTST	VCMCCT CKTT		TUNCEHTDAWT	CUSOEVNTUDITENT
400011020		TEP CRSVISI	VCMCCI CKUU	TADEL VECUD		CUCOEVNENDLIDNI
400011920	TODIVIECTIARTIQU	EPRRSVLSI		LARKLIISHD.	LUNSEPTRAWI IVCCEDED	CVSQEINIMDLERNI
400011517		EPRRIVUSI	HGMGGLGKIT	LARNLINSPN.		CVSQEINIMDLEKTI
400011520	FQDVVQTLLAELLK	EPRRSIVSI	HGMGGLGKTT	LARILYNSPN.	LVSSFPTRAWI	CVSQEYNIMDLLKII
400011528	LQDVVEKLLDELLR	EARPSVISI	CGMGGLGKTT	LARNLYINPN.	LVNSFHMRAWI	CAPEEYNTVDVLKNI
400032547	-QDIVETLLAELLKI	P <mark>EPRRSVISI</mark>	YGMGGLGK TT	LARNLYISPN	IASSFPT RA WI	CVSQEYNTMDLLWNI
400011523	-QDVVERLLSELLKA	EPCRSVISI	YGMGGLGK TT	LARNLYISPD:	IVNSF <mark>H</mark> T RA WI	CVSQEYNTVDLLRYI
400032581						
400032582				Y NSPN	IVNSFPT <mark>RA</mark> WI	CVSQEYNTMDLLRNI
400023273	LQDVVQKLLAELLKA	EPRRSVLSI	YGMGGLGKTT	LARKLYTSPD	ILNSFPT <mark>RA</mark> WI	CVSQEYNTMDLLRNI
400032578	FQDVVQTLLTELLK	PEPHRRVISI	YGMGGLGKTT	LARNLYISPN	TASSFPT <mark>R</mark> AWI	CVSQEYNTMDLLWNI
400023288			GGLGKTT	LARKLYTSPD	ILNSFPT <mark>RA</mark> WI	CVSOEYNTMDLLKNI
400024231						
400032572						
400032576				TAPNTVECON	TVCCEDTDAMT	
401010614			VCMCCI CKTT	T A DAT VACOC	TATEOTRANT	CVSQLININDILKII
401010014			VCKCCLCKIN	LARNLINSPG	LAIFUIKAWI	CVSQQISILKSI
400006535	FQDVVQI LLAQLLKE	EPRRAVISI	VCMCCI CKUU	LARREIISPI.	LASSIFIKAWI	CVSQDINIMDLLKII
400006551	<mark>E</mark> LLK	EPRRSVLSI	IGMGGLGKIT	LARKLI INPN.	LASSIPICAWI	CVSQLINIMDLLSII
	0.0	00	100	110	100	120
	80	90	100	110	120	130
140						
R2-FJ536326	IKSVQGRTKETLDL1	LERMTEGDLE	IYLRDLLKER	KYLVMVDDVW(2KEAWDSLKRA	FPDSKNGSRVIITTR
Rb1b3-FJ536325	IKSIQGRTKETLDL1	LERMTEGDLE	IYLRDLLKER	<u>KYLVVVDD</u> VW(2 REAWE SL KRA	FP D GKNGSRVIITTR
Rpiabpt-FJ536324	IKSIQGRTKETLDL1	LERMTEGDLE	IYLRDLLKER	<u>KYLVVVDDVW</u>	Q REAWE SL KR S	FPDGKNGSRVIITTR
Rpihjt1.1-GU563971	IKSIQGRTKETLDL1	LERMTEGDLE	IYLRDLLKER	<u>KYLVVVDD</u> VW(Q REAWE SL KRA	FP DGK NGSRVIITTR
Rpihjt1.2-GU563972	IKSIQGRTKETLDLI	LERMTEGDLE	IYLRDLLKER	KYLVVVDDVW(Q REAWE SL KR S	FPDGKNGSRVIITTR
Rpihjt1.3-GU563973	IKSIQGRTKETLDL1	LERMTEGDLE	I YLRDLLKER	KYLVVVDDVW(Q REAWE SL KRA	FPDGKNGSRVIITTR
Rpiedn1.1-GU563963	IKSIQGRTKETLDL1	LERMTEGDLE	IYLRDLLKER	KYLVVVDDVW(Q REAWE SL KRA	FPDGKNGSRVIITTR
Rpisnk1.1-GU563975	IKSIQGCAKETLDLI	L <mark>EKMAEIDLE</mark>	NHLRDLLKEC	KYLVVVDDVW(2 REAWESLKRA	FPDGKNGSRVIITTR
Rpisnk1.2-GU563976	IKSIOGRTKETLDLI	LERMTEGDLE	TYLRDLLKER	KYLVVVDDVW	- O REAWE SL KRA	FPDGKNGSRVIITTR
400024234						
400011527	TKSTOGCAKETLDL		NHT.RKT.T.T.R	KYT.VVVDDVW	OREAWEST.KRA	FPDSKNGSRVTTTTR
400011525	TKSTOCPTKCTIDE			KYT WWW	DEAMEGT KDA	FDFCKMCCDVTTTTT
400011523	TRETOCCARETION				OPP AMEGI KPA	FDDEWCCDVTTTT
400011524	TOWLOW			KILVVIDDVW,	ZKEAWESLKKA	FFDRRWGSRV1111R
400011522	ISNLSKVAPRKLIC	RGQKEIKFT	FV1YEKN	ANTLLSLMMY	SREKHGKVKEH	SWIARMEA-ELLPXR
400011521	IKSIQGCTKETLDLI	ERMIERDLE	1 Y LRDLLKKR	KYLVVVDDLW(ZREAWESLERA	PDSKNGSRVIITTR
400011920	IKSVQGRTTETLDL1	LERMTEGDLE	IYLRDLLKER	<u>KYLMVVDD</u> VW(2 REAWESLKRA	FP DGKNGSRVII TTR
400011517	IKSIQGCAKETLDL1	LEKMAETDLE	NHLRDLLEGC	<u>KYLVVVDD</u> VW(2 KEAWE SL KRA	FP DR NNGS R VIITTR
400011520	IKSIQGCTKETLDL1	L <mark>EKMAETDLE</mark>	NHLRDLLKGR	<u>KYLVVVDDVW</u>	KREAWKSLKRV	FPDNKNGSRVIITTR
400011528	IKSIQSCSKEILDW1	LERMTERDLA	IYLRALLKKR	KYLVVVDDLW	HREAWESLKKA	FRDGKKGSRVIITTR
400032547	IKSIQGCTKETLDL1	LEKMTERDLE	IYLRDLLKEP	KYLVVVDDLW	HREAWESLKRA	FPDSKNGSRVVITTR
400011523	IKSIQGCTKETLDL1	LERMTERDLE	IYLRDLLKKR	KYLVVVDDLW(Q REAWE SL KRA	FPDSKNGSRVIITTR
400032581						<i>TT</i> H
400032582	IKSIQGCTKETLDSI	LERMTERDLE	IYLCDLLKEH	KYLVVVDDVW(QREAWESLKRA	FP DGKNGSRVIITTR
400023273	IKCIOGLTKETLDI.		IYLRDLLKER	KYLVVVDDVW	KEAWESLKRA	FPDSKNDSRVIITTR
400032578						
400023288	IKSIOGCTKETIIII	EKMIERDIE	IYLRDT.T.KEP	<u>KYX(:(-;(-;R#1</u>	MARSMGKENEH	FOIARTAAET.XTTTR
	IKSIQGCTKETLDLI	LEKMTERDLE LETMTECDI E	IYLRDLLKEP	KYXCGGREI	MARSMGKENEH	FQIARTAAELXITTR
400024231	IKSIQGCTKETLDLI IKSIQGRTKETLELI	LEKMTERDLE LETMTEGDLE	IYLRDLLKEP FHLRDLLKER	KYXCGGRF1 KYLVVVDDVW(MARSMGKENEH QREAWESLKRA OREAWESLKRA	FQIARTAAELXITTR FPDGKNGSRVIITTR FPDGKNGSPVIITTR
400024231	IKSIQGCTKETLDLI IKSIQGRTKETLELI	LEKMTERDLE LETMTEGDLE GVLA	IYLRDLLKEP FHLRDLLKER AYMLDLS	KYXCGGRFI KYLVVVDDVW(ISAVW(MARSMGKENEH QREAWESLKRA QREAWESLKRA	FQIARTAAELXITTR FPDGKNGSRVIITTR FPDGKNGSRVIITTR

400032576	INSIQGCTKETLDLV	EKMAETNLEN	HLRKLLTERK	YLVVV <mark>DD</mark> VWQI	REAWESLKRA	FPDSKNGSRVIITTR
401010614	IKSIQ <mark>GYHEKM</mark> LKLL	KEMTETDLE T	HLRNLLKERK	YLVVV <mark>DD</mark> VW <mark>H</mark> I	REAWESLKRA	LPDNNNGSRVILTTR
400006533	IKSIQGCTNETLNLL	ERMTEGDLEI	YLRDLLKERK	YLVVVDDVWQI	NEAWESLKRA	FPDSKNGSRVIITTR
400006531	IKSIQGRTKVTLDLL	ESMPEGDLEI	YLRDLLTERK	<u>YLVVVDDVRQ</u>	KEVWERLKRA	FPDSKNGSRVIITTP
	150	160	170	180	190	200
210						
					I I	
R2-F.1536326	KODVA - ERADDIGEV	HKT.RFT.SOFF	SWDT.FRKKT.T.		MENT.A K	DMVEKCRGLPLATVV
Rb1b3-E1536325	KEDVA - ERADDRGEV	HKT.BET.SOFE	SWDT.FRRKT.T.			DMVEKCRCI.PI.ATVV
Prichot-FIE26224						DMUERCECT DI ATUN
P_{r}	KECKA ERADDROFV				MEGIAN	DMVERCEGIF LATVV
Rpinjt1.1-G0563971	KEGVA-ERADDRGFV	IKLKI LSQLL	SWDLF RRKLL		MESLAR	DMVERCEGLPLAIVV
Rpinjt1.2-G0563972	KEDVA-ERADDRGEV	HKLRFLSQEE	SWDLFRRKLL.	DVRAMVPE	MESLAK	DMVEKCRGLPLAIVV
Rpihjt1.3-GU563973	KEGVA-ERADDRGFV	HKLRFLSQEE	SWDLFRRKLL.	DVRAMVPE	MESLAR	DMVEKCRGLPLAIVV
Rpiedn1.1-GU563963	KEDVA-ERADDRGFV	HKLRFLSQEE	SWDLFRRKLL	DVRAMVPE	MESLAK	DMVEKCRGLPLAIVV
Rpisnk1.1-GU563975	KEDVA-ERVDNRGFV	HKLRFLSQEE	SWDLFHRKLL	DVRAMVPE	MESLAK	DMVEKCRGLPLAIVV
Rpisnk1.2-GU563976	KEGVA-ERADDRGFV	HKLRFLSQEE	SWDLFRRKLL	DVRAMVPE	MESLAK	DMVEKCRGLPLAIVV
400024234						
400011527	KEDVA-ERADDRGFV	HKLRFLTOGE	SWDLFCRKLL	DVRAMVPE	MESLAR	DMVEKCRGLPLAIVV
400011525	KHDVA-ERADNRGFV	HELRFLSREE	SWDLFCRKOL	DVRAMVPE	MVRIAR	DMVEKCRGLPLAIVV
400011524	KXXMX-ERADNKGEV	VRT.RFT.SOFF	SWDT.FCRKT.T.		T F RT.4K	MVDKCGGLPLATVV
400011522		UPT PET SOFE	SWDI FCPKI V			DMUDKCCCL PLATIV
400011522	KEDVA ERADORGEV		CHDI FCDKI I		MEDIAN	DMUDKCCCI DI ATUU
400011521	KEDVA-ERADSRGFV	HALKE LSQLL	SWDLF CRKLL	DVQAMISI		DMVDRCGGLPLAIVV
400011920	KEDVA-ERADNRGF1	HKLRFLSQEE	SWDLFCRKLL.	DVRAMV		
400011517	NQDVA-ERANNRGFV	HKLRFLKQEE	SWDL			
400011520	QGXCRXERADDKGFV	HKLRFLSQEE	SWDLFCRKLL	DVRAMVPA	MESLAK	DMVGKCRGLPLAIVV
400011528	RVDIA-KTAD-EGFV	HNLRFLSQ DE	SWDLFCRKQL	HVQAMVPK	MERLAR	DMVEKCGGLPLSIVV
400032547	KEDVA-ERADNKGFV	YKLRFLSQEE	SWDLFCRKLL	DVQAMVSA	MERLAK	DMVDRCGGLPLAIVV
400011523	KEDVA-ERADSRGFV	HKLRFLSQEE	SWDLFCRKLL	DVQAMTST	MERLAK	DMVDKCGGLPLAIVV
400032581	KEDVA-ERADDRGFV	HKLRFLSHEE	SWDPFCRKLL	DVRAMTSA	MERLAN	DMVDKCRGLPLAIVV
400032582	KEDVA-ERADNRGEV	HKTRFT.SOFE	NWDT.FSRKT.T.			DMVEKCRGLPLATVV
400023273	KEDVA - ERADDRCEV	HKT.PFT.S				
400023273		VDI DET COFF				
400032378	KEDVA-ERADINGEV	IKIKI DEL CORR	SWDLF CKKLL			ENVDRCGGLFLAVVV
400023288	REDVA-ERADDRGFV	HRLRFLSQEE	SWDLF RRKLL	DVRSIVPE	MESLAR	DMVERCRGLPLAIVV
100021231				M (((((((((((((((((((MKSLAK	DMVEKCRGLPLAIVV
400024251	REDVA-ERADDRGEG	HELRFLSQEE	SWDLF CRKLL	DVGAMVPE	1120201	
400032572		HELRFLSQEE				
400032572 400032576	KEDVG-SKSRRQRFC	HELRFLSQEE P-TSFLSQ <mark>E</mark> E	SWDLFCRKLL SWDLFCRKLL	DVGAMVPE	MESLAR	DMVEKCRGLPLAIVV
400032572 400032576 401010614	KEDVA-ERADDKGFG KEDVG-SKSRRQRFC KEDVA-ERVDDKGFS	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH	DVGANVPE DVRAMVPE PENKMSSADLi	MESLAR FSPSMKRLAI	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV
400032572 400032576 401010614 400006533	KEDVA-EKADDAGFG KEDVG-SKSRRQRFC KEDVA-ERVDDKGFS KEDVA-ERADDRGFV	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL	DVRAMVPE	MESLAK FSPSMKRLAI MENLAK	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV
400032572 400032576 401010614 400006533 400006531	KEDVA-ERADDKGFS KEDVA-ERVDDKGFS KEDVA-ERADDRGFV KKDVA-ERADDRGFV	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE HELRFLTQEE	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL SWDLFRRKLL	DVGAMVPE DVRAMVPE PENKMSSADL DVRAMVPE DVQAMTFT	MESLAR FSPSMKRLAT MENLAR MENLAR	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV NMVGKCRGLPLAIVV
400032572 400032576 401010614 400006533 400006531	KEDVA-ERADDKGFG KEDVA-ERVDDKGFS KEDVA-ERADDRGFV KKDVA-ERADNRGFV	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE HELRFLTQEE	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL	DVRAMVPE DVRAMVPE PENKMSSADLI DVRAMVPE DVQAMTFT	MESLAR FSPSMKRLAT MENLAR MERVAR	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV NMVGKCRGLPLAIVV
400032572 400032576 401010614 400006533 400006531	KEDVA-ERADDKGFS KEDVA-ERADDKGFS KEDVA-ERADDKGFV KKDVA-ERADDKGFV 220	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE HELRFLTQEE 230	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL SWDLFRRKLL 240	DVRAMVPE DVRAMVPE PENKMSSADLI DVRAMVPE DVQAMTFT 250	MESLAR FSPSMKRLAT MENLAR MERVAR 260	DIVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV INMVGKCRGLPLAIVV 270
400032572 400032576 401010614 400006533 400006531	KEDVA-ERADDRGFO KEDVA-ERVDDKGFS KEDVA-ERADDRGFV KKDVA-ERADDRGFV 220	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE HELRFLTQEE 230	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL SWDLFRRKLL 240	DVRAMVPE PENKMSSADL DVRAMVPE DVQAMTFT 250	<i>MESLAR</i> FSPSMKRLAI <i>MENLAR</i> <i>MERVAR</i> 260	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV NMVGKCRGLPLAIVV 270
400032572 400032576 401010614 400006533 400006531	KEDVA-ERADDKGFS KEDVA-ERVDDKGFS KEDVA-ERADDRGFV KKDVA-ERADDRGFV 220	HELRFLSQEE P-TSFLSQEE HKLRFLNKEE HKLRFLSQEE HELRFLTQEE 230	SWDLFCRKLL SWDLFCRKLL SWDLLCKKLH SWDLFRRKLL 240	DVRAMVPE PENKMSSADL DVRAMVPE DVQAMTFT 250	<i>MESLAR</i> FSPSMKRLAT <i>MENLAR</i> <i>MERVAR</i> 260	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV NMVGKCRGLPLAIVV 270
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<pre>400032572 400032576 401010614 400006533 400006531 280 R2-FJ536326 Rblb3-FJ536325 Rpiabpt-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563972 Rpihjt1.3-GU563973 Rpisnk1.1-GU563963 Rpisnk1.1-GU563963 Rpisnk1.2-GU563976 400024234 400011527 400011525 400011524 400011521 400011521 400011521 400011527 400011521 400011520 400011520 400011528 400032547</pre>	KEDVA-EKADDAGFG KEDVA-ERADDAGFG KEDVA-ERADDAGFV KEDVA-ERADDAGFV KKDVA-ERADDAGFV Z20 LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ	HELRFLSQEE 	SWDLFCRKLL SWDLFCRKLL SWDLFCRKLL SWDLFRRKLL SWDLFCS SWDLFCRKLL SWDLFRRKLL SWDLF SWD SWDLF SWD SWDLF SWD SWD SWD SWD SWD SWD SWD SWD SWD SWD	DVRAMVPE DVRAMVPE PENKMSSADL DVRAMVPE 250 ILSLSYNDLS'	MESLAR FSPSMKRLAT MENLAR MENLAR 260 TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV 270 GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVEADNII GIFPEDQVEADNII GIFPEDQVEADNII GIFPEDQELEAENII GIFPEDQELEAENII YFS
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<pre>400032572 400032576 400032576 401010614 400006533 400006531 280 R2-FJ536326 Rblb3-FJ536325 Rpiabpt-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563973 Rpiedn1.1-GU563973 Rpisnk1.2-GU563976 400024234 400011527 400011527 400011522 400011522 400011521 400011522 400011521 400011522 400011521 400011522 400011524 400011522 400011524 400011522 400011524 400011522 400011524 400011522 400011524 400011522 400011524 400011523 400032547 400011523 400032581 400032582</pre>	KEDVA-EKADDAGFG KEDVA-ERADDAGFG KEDVA-ERADDAGFV KEDVA-ERADDAGFV KKDVA-ERADDAGFV KKDVA-ERADDAGFV Z220 LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ	HELRFLSQEE 	SWDLFCRKLL SWDLFCRKLL SWDLFCRKLL SWDLFRRKLL SWDLF SW	DVRAMVPE DVRAMVPE PENKMSSADL DVRAMVPE 250 ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSFSDLS ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS' ILSLSYNDLS'	MESLAR FSPSMKRLAT MENLAR 260 TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF TALKQCFLYF	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV 270 GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVEADNII GIFPEDQVVEADNII GIFPEDQVEEADNII GIFPEDQVEEADNII GIFPEDQVEEADNII GIFPEDQVEEADNII GIFPEDQVEEADNII GIFPEDQUEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQVEEAENII GIFPEDQEVEAENII GIFPEDQEVEAENII GIFPEDQEVEAENII GIFPEDQEVEAENII GIFPEDQEVEAENII
<pre>400032572 400032576 401010614 400006533 400006531 280 R2-FJ536326 Rblb3-FJ536325 Rpiabpt-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563972 Rpihjt1.3-GU563973 Rpiedn1.1-GU563973 Rpisnk1.1-GU563975 Rpisnk1.2-GU563976 400024234 400011527 400011527 400011524 400011521 400011521 400011521 400011521 400011521 400011521 400011521 400011521 400011524 400011523 400032547 400011523 400032581 400032582 400032582 400032582 4000323273 400032582 400032582 400032582 400023273 400032582 4000328 4000 400 400 400 400 400 400 400 400 4</pre>	KEDVA-ERADDAGFG KEDVA-ERADDAGFG KEDVA-ERADDAGFV KEDVA-ERADDAGFV KKDVA-ERADDAGFV Z220 LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ	HELRFLSQEE P-TSFLSQEE P-TSFLSQEE HELRFLNKEE HELRFLTQEE 230 KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI KVKDHLWKNI RVKDHLWENI RVKDHLWENI RVKDHLWENI RVKDHLWENI RVKDHLWENI RVKDHLWQN- KVKDHLWQN- KVKDHLWQN- KVKDHLWQN-	SWDLFCRKLL SWDLFCRKLL SWDLFCRKLL SWDLFRRKLL SWDLF S	DVRAMVPE DVRAMVPE PENKMSSADL DVRAMVPE 250 ILSLSYNDLS ILSLSYND	MESLAR FSPSMKRLAT MENLAR 260 TALKQCFLYF	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV 270 GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVEADNII GIFPEDQVEADNII GIFPEDQVEADNII GIFPEDQVEADNII GIFPEDQVEANII GIFPEDQUELAENII GIFPEDQVEAENII GIFPEDQVEAENII GIFPEDQVVEAENII GIFPEDQVVEAENII GIFPEDQVVEAENII GIFPEDQEVDAEYII GIFPEDQEVDAEYII GIFPEDQEVDAEYII GIFPEDQEVDAEYII
400032572 400032576 401010614 400006533 400006531 280 R2-FJ536326 Rblb3-FJ536325 Rpiabpt-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563973 Rpiedn1.1-GU563973 Rpisnk1.2-GU563973 Rpisnk1.2-GU563975 Rpisnk1.2-GU563976 400024234 400011527 400011525 400011524 400011521 400011521 400011521 400011520 400011521 400011520 400011523 400032547 400032581 400032582 400032578	KEDVA-EKADDAGFG KEDVA-ERADDAGFG KEDVA-ERADDAGFV KEDVA-ERADDAGFV KKDVA-ERADDAGFV KKDVA-ERADDAGFV LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLNQWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKKGLDEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLDEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEEWQ LSGLLSHKRGLEWQ LSGLLSHKRGLEWQ	HELRFLSQEE	SWDLFCRKLL SWDLFCRKLL SWDLFCRKLL SWDLFRRKLL SWDLFRRKLL SWDLFRRKLL SWDLFRRKLL SWDLFRRKLL SWDLFRRKLL SWDLFRRKLL SWDFSIEISN KEDKSIEISN KEDKSIEISN KEDKSIEISN KEDKSIEISN KEDKSIEISN KEDKSIEISN KEDKSIEISN IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY IEDDSIEISY SKEDKSIEISY	DVRAMVPE DVRAMVPE PENKMSSADL DVRAMVPE 250 ILSLSYNDLS'	MESLAR FSPSMKRLAT MENLAR 260 TALKQCFLYF	DMVEKCRGLPLAIVV EMVEKCRGLPLAIVV EMVENCRGLPLAIVV 270 GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVKADDII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVVEADII GIFPEDQVIEADII GIFPEDQVEAENII GIFPEDQVVEAENII GIFPEDQVVEAENII GIFPEDQEVDAEYII GIFPEDQEVDAEYII

400023288	LSGLLSHKKGLNOWHKVKDHLWKNIKEDKSIEISCILSLSFNDLSTALKOCFLYIGIFPEDOVIDVENII
400024231	LSGLLSHRGGVDKWOEVKDHLWKNIMKDKSIEISCILSLSYNDLSTVLKOCFLYFGIFPEDOVLKAENII
400032572	LSTVLKOCFLYFGIFPEDOEVDAEKII
400032576	LSGLLSHKKGLSEWHKVKDHLWKNIKEDKSIEISCILSLSYNDLSTALKKCFLYFGIFPEDOEVEAENII
401010614	LGGLLSYRKGVDEWOKVKTHLWOH-MKNDSVEISHILSLSYNDLSFELKOCFLYIGSFOEDHVIDAEKLM
400006533	LSGLLSHKKGLKEWQKVKDCLWKDIEEDSFHEISSILSLSYNDLSTALKKCFLYFGIFPEDQVVEADNII
400006531	LSGLLSHKKRLDEWQKVKDHLWKNNVEDEYIEISNILSLSYNDLSTALKQCFLYFGIFPEDQVVKAENII
	290
	••••••••••••••••••
R2-FJ536326	RLWMAEGFIPRGEE-
Rb1b3-FJ536325	RLWMAEGFIPRGEE-
Rpiabpt-FJ536324	RLWMAEGFIPRGEE-
Rpihjt1.1-GU563971	RLWMAEGFIPRGEE-
Rpihjt1.2-GU563972	RLWMAEGFIPRGEE-
Rpihjt1.3-GU563973	RLWMAEGFIPRGEE-
Rpiedn1.1-GU563963	RLWMAEGFIPRGEE-
Rpisnk1.1-GU563975	RLWMAEGFIPRGEE-
Rpisnk1.2-GU563976	RLWMAEGFIPRGEE-
400024234	RLWMAEGFIPRGEE-
400011527	RLWMAEGFIPRGEE-
400011525	LLWMAEGFIPNGEE-
400011524	WLWMAEGFVPTGKE-
400011522	RLWMAEGFIPRGEE-
400011521	
400011920	
400011517	
400011520	RLWMAEGFIIPRGEE
400011528	RLWMAEXF
400032547	WLWMAEGFIPTGEE-
400011523	
400032581	WLWMAEGFIPNGEE-
400032582	RLWMVEGFTPIGEE-
400023273	
400032578	
400023288	HLWMAEGFIPRGEE-
400024231	RLWMAEGFVPNGDE -
400032572	LLWMAEGFIPNGEE-
400032576	WLWMAEGFVPRGEE-
401010614	RLWLAEGFIPRIEH-
400006533	RLWMAEGFIVPRGEE
400006531	RLWMAEGFIPRGEE-

Protein sequence alignment of the LRR region of R2 homologs

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70															
	· · · · I			I				1						I	
R2-FJ536326	SNILS.	LSYND.	LSTAL	KQCF1	YFGI	PEDQ	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		ADGE	LNEL	IRRSL
Rpiblb3-FJ536326	SNILS	LSYND.	LSTAL	KQCF1	YFGII	PEDQ	VKVL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
Rpiabpt-FJ536324	SNILS.	LSYND.	LSTAL	KQCF1	YFGI	PEDQ	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		ADGE	LNEL	IRRSL
Rpihjti.1-GU563971	SNILS	LSYND.	LSTAL	KQCFL	YFGII	SEDK	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
Rpihjt1.2-GU563972	SNILS	LSYND.	LSTAL	KQCFL	YFGII	PEDQ	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		ADGE	LNEL	IRRSL
Rpihjt1.3-GU563973	SNILS	LSYND.	LSTAL	KQCFL	YFGII	SEDQ	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
Rpiedn1.1-GU563963	SNILS.	LSYND.	LSTAL	KQCF1	YFGII	PEDQ	/V <mark>K</mark> VL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>	RMED	AEGE	LNEL	IRRSL
Rpisnk1.1-GU563975	SNILS.	LSYND.	LSTAL	KQCF1	YFGI	PEDQ	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
Rpisnk1.2-GU563976	SNILS.	LSYND.	LSTAL	KQCF1	YFGI	SEDK	VVKAL	DIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
400024234	SNILS.	LSYND.	LP <mark>AA</mark> L	KQCF1	YFGII	PEDQ	/FEAL	NIIR	LWMA	EGF-	IP <mark>RGEE</mark>	RMED	AEGF	LNEL	IRRSL
400011527	SNILS.	LSYND.	LSTPL	KQCFL	YFGII	PEDQ	/L <mark>EAL</mark>	NIIR	LWMA	EGF-	IP <mark>RGEE</mark>	RMED	AEGE	LNQL	IRRSL
400011525	SYILS.	LSYND.	LSATL	KQCFL	YFGII	PEDQI	EVDAE	KIIL	LWMA	EGF-	IPNGEE	RMED	AEGE	LS <mark>E</mark> L	IRRSL
400011524	SYILS.	LGYND.	LSTAL	KQFF I	YFGI	PEDR	VVHVL	HILW	LWMA	EGF-	VP T <mark>GKE</mark>		AEGE	LNEL	IRRSL
400011522	SYILS.	LSYND.	LSTAL	KQCFL	YFGII	PEDQI	<u>CLEAF</u>	NIIR	LWMA	EGF-	IP <mark>RGEE</mark>	RMED	AEDE	LNEL	IRRSL
400011521	SYILS.	LSYND.	LSTAL	KQCF1	YFGI	PEDQ	<u>eleaf</u>	NIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEDE	LNEL	IRRSL
400011920	-NILS	LSYND.	LSTAL	KQCF1	YFGI	PEDQ	VVKAL	NIIR	LWMA	EGF-	IP <mark>RGEE</mark>		AEGE	LNEL	IRRSL
400011518	EK G	TSHLX	LSNL <mark>K</mark>	LRSIM	IFF D QI	v	-FRKM	ISLXK	LQEC	VPTS	ICVS-1	VP DA	GSLY	HLKF	LRLRG
400011517		L X	LSNS <mark>K</mark>	L <mark>R</mark> SIM	FFDPI	IICN-V	7FQ <mark>H</mark> 1		HIYV.	LYLD	I <mark>DYGK</mark> V	IP <mark>DA</mark> I	GSLY	NLKL	L R LSV
400011520	SYILS.	LSFSD.	LSAAL	KLCFL	YFGI	PEDQ	/IKT <mark>E</mark>	NIMR	LWMA	EGFI	IP <mark>RGEE</mark>		AEGE	LN <mark>E</mark> L	IRRSL

400011529	SNILSLSYNDLSTALKQCF	LYFSIFP EDK VL <mark>E</mark>	AENIIWLWMAEGF	-IP RGEERMED V	AEGFLNELIRRSL
400011528					
400032547	SYILSLSYNDLPTELKQCF	LYFGIIPEDHEVH	VDHILWLWMAEGF	-IPTGEEIMEDV	AEGFLNELIRRSL
400011523					
400032581	SYILSLSSNDLSTALKQCF	LYFGLFPEDQEVL	AEYIIWLWMAEGF	-IPNGEERMEDE	AFKWLEHFGRK
400032582	SKILSLSYNDLSTALKQCF	L YFGIF PQ D QVLG	ADSIIRLWMVEGF-	-TPIGEERMDDV	AEGXLEXL
400023273			DVWKKEAW-	<u>E</u> SL	KRAFPLQFVNCAQ
400023288	SCILSLSFNDLSTALKQCF	L YIGIFPED QVIL	VENIIHLWMAEGF	-IP RGEERMED V	AEGFLNELIRRSL
400024231	SCILSLSYNDLSTVLKQCF	LYFGIFPEDQVLK	AENIIRLWMAEGF-	-VPNGDERMEDV	AEGFLNELIRRSL
400032572	LSTVLKQCF	LYFGIFPEDQEVL	AEKIILLWMAEGF-	-IPNGEERMEDV	AEGFLNELIRRSL
400032576	SCILSLSYNDLSTALKKCF	LYFGIFPEDQEVE	AENIIWLWMAEGE	-VPRGEERMEDV	AEGFLNELIRRSL
401010614	SHILSLSYNDLSFELKQCF	LYIGSFQEDHVIL	AEKLMRLWLAEGE -		AENFLHELISRSL
400006531	SSILSLSINDLSIALKKCF	INFGIPPEDQVVE	ADNIIRLWMAEGEI AFNITTRIWMAECE	TDRCEERMEDV	ALGELNELIKKSL
400008551	SNILSLSINDLSIALKQCF	LIFGIFFEDQVVK	ALNIIKLWMALGE -	-IPRGEERMEDV	ALGILNELIKKSL
	80	90 100	110	120	130
140					
			.		
R2-FJ536326	VQVAKTFWEKVTDCRVHDL	LRDLAIQKVLEVN	FFDIYDPRSHSISS	SL CIRHGIHSEG	ERYLSSLDLSNLK
Rpiblb3-FJ536326	VQVNNTFWQRVTECRVHDL	LRDLAIQKASEVK	FFDVYDPRSHSIPS	SLCIRHGIHSEG	ERYLSSFDLSNLK
Rpiabpt-FJ536324	VQVAKTFWEKVTDCRVHDL	LRDLAIQKALEVN	FFDIYDPRSHSISS	SLCIRHGIHSEG	ERYLSSLDLSNLK
Rpihjti.1-GU563971	VQVAKTFWEKVTECRVHDL	LHDLAIEKALEVN	FFDVYDPRSHSISS	SL CIRHGIHSEG	ERYLSSLHLSNLK
Rpihjt1.2-GU563972	VQVAKTFWEKVTDCRVHDL	LRDLAIQKALEVN	FFDIYDPRSHSISS	SL CIRHGIHSE G	ERYLSSLHLSNLK
Rpihjt1.3-GU563973	VQVAKTFWEKVTECRVHDL	LHDLAIEKALEVN	FFDVYDPRSHSISS	SLCIRHGIHSEG	ERYLSSLHLSNLK
Rpiedn1.1-GU563963	VQVNNTFWQRVTECRVHDL	LRDLAIQKASEVK	FFDVYDPRSHSIPS	SL CIRHGIHSE G	ERYLSSFDLSNLK
Rpisnk1.1-GU563975	VQVAKTFWEKVTDCRVHDL	LRDLAIQKALEVN	FFDVYGPRSHSISS	SLCIRHGIHSEG	ERYLSSLDLSNLK
Rpisnk1.2-GU563976	VQVAKTFWEKVTECRVHDL	LHDLAIEKALEVN	FFDVYDPRSHSISS	SLCIRHGIHSEG	ERYLSSLHLSNLK
400024234	VQVAKTFWERVTECKVHDL	LHDLVIQKALEVN	FFDIYDPKSHSISS	SLCIKHVIHSQG	ERYP-SLDLSNLK
400011527	VQVTRTFWERVTECRVHDL	LHDLAIQKALEVN	FFDVYDPRSHSISS	SLCIRHTIHSQR	ERYLS-LDLSNLK
400011525	TORUBUENERVERVERVERVERVER		EFDIYDPRKHSISS	SLCIRHVIHGQG	ERILS-LDLSILK
400011524	VOVA CTEWERV SACRIADL	UPDISTOKASEVN	FEDITOPRESISS	TCTPHATHDOG	FKVI SI YSFI KVF
400011522	VOVAGTEWEKVILCRVHDV	VRDLSIQKASEVN	FFDIIDERNHSISI	T.CTRHATHDOG	EKILSLXSFLKVE
400011920	VOVA VTYWERVIECRVHDI.	THDIATOKALEVN	FFGTYDPRSHSTS	SLCTRHVVHSHG	ERVIS-I.DI.SNI.K
400011518	TOG				
400011517	VSK				
400011520	VQVVDTFWEKVTECRVHDL	LRDLAIQKALEVN	FFDIYDPRKNLKS	SCIRHAIHSEG	ERYLSSLDLSNSK
400011520 400011529	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL	LRDLAIQKALEVN LRDLAIQKALEVN	FFDIYDPRKNLKS FFDIYDTRYHSISS	TS CIRHAIHSE G SS CIRHAIH SQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL	LRDLAIQKALEVN LRDLAIQKALEVN	FFDIYDPRKNLKS FFDIYDTRYHSIS	SCIRHAIHSEG SCIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLAIQKALEVN LRDLAIQKALEVN LRDLAVQK	FFDIYDPRKNLKS' FFDIYDTRYHSIS	TS CIRHAIHSEG SS CIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLAIQKALEVN LRDLAIQKALEVN LRDLAVQK	FFDIYDPRKNLKS FFDIYDTRYHSIS	TS CIRHAIHSEG SS CIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLAIQKALEVN LRDLAIQKALEVN LRDLAVQK	FFDIYDPRKNLKS' FFDIYDTRYHSIS	TS CIRHAIHSEG SS CIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLAI _O KALEVN LRDLAI _O KALEVN LRDLAV _O K	FFDIYDPRKNLKS' FFDIYDTRYHSIS	SCIRHAIHSEG SSCIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLAI _O KALEVN LRDLAI _O KALEVN LRDLAV _O K	FFDIYDPRKNLKS' FFDIYDTRYHSIS	SCIRHAIHSEG SSCIRHAIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023288	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLAIQKALEVN LRDLAIQKALEVN LRDLAVQK	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS	SCIRHAIHSEG	ERYLSSLDLSNSK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023288 400023288 400024231	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV- VQVVDTFWERVTECRVHDL VQVVDTFWEKVTDCRVHDL	LRDLA I QKALEVN LRDLA I QKALEVN LRDLAVQK	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS	SCIRHAIHSEG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023288 400023273 400032572 400032572	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV- VQVVDTFWERVTECRVHDL VQVVDTFWEKVTCCRVHDL VQVANTFWEKVTECRVHDL	LRDLA I QKALEVN LRDLA I QKALEVN LRDLAVQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTM	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS LFDIYHPRKHSKS	SCIRHAIHSEG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023273 400023288 400024231 400032572 400032576 401010614	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA V QK LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS LFDIYHPRKHSKS FFDIYDPRSHSIS	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK EMYH-LLDLSNLK ERYLSSLDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023273 400023288 400024231 400032572 400032576 401010614 400006533	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV- VQVVDTFWERVTECRVHDL VQVVDTFWEKVTCRVHDL VQVANTFWEKVTECRVHDL IQVAKTFWEKVTECRVHDL IQVAETFFDKILTCRIHDL VOVAOTFWERVTECRVHDL	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVN LRDLA VQKAMEVN LRDLA I QNALEVN	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS LFDIYHPRKHSKS FFDIYDPRSHSIS LFDIYDPR	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRHAFHGQG SSCIRHAIHSOG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSLDLSNLK ERYLS-FDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV- VQVVDTFWERVTECRVHDL VQVVDTFWEKVTECRVHDL VQVANTFWEKVTECRVHDL IQVAETFFDKILTCRIHDL VQVAQTFWERVTECRVHDL ILVVHTFWERVTACRVHDL	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVK LRDLA I QNALEVK	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS LFDIYHPRKHSKS LFDIYDPRSHSIS LFDIYDPR FFDIYDPRKHSIS FFGIYDPRKHSIS	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHVIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK ERYLS-FDLSNLK
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QNA LEVK LRDLA I QNA LEVK	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS LFDIYHPRKHSKS FFDIYDPRSHSIS LFDIYDPR FFDIYDPR	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRHAIHSQG SLCIRHVIHSQG SLCIRHVIHSQG	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-FDLSNLK ERYLS-FDLSNLK ERYLS-SLDLSNLK
400011520 400011529 400011528 400032547 400032581 400032582 400023273 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QNA LEVK LHDLA I QEALKVN 60 170	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS FFDIYHPRKHSKS FFDIYDPRSHSIS LFDIYDPR FFDIYDPRKHSIS FFGIYDPKHSIS FFGIYDPKRSIS	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SLCIRHAIHSQG SLCIRHVIHSQG 190	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK ERYLS-SLDLSNLK 200
400011520 400011529 400011528 400032547 400011523 400032581 400032582 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QNA LEVK LRDLA I QNA LEVK LHDLA I QEA LKVN 60 170	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS FFDYPPRKHSKS FFDIYDPR FFDIYDPR FFDIYDPR FFGIYDPRKHSIS FFGIYDPKHSIS 180	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSQG SLCIRHVIHSQG 190	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK ERYP-SLDLSNLK 200
400011520 400011529 400011528 400032547 40001523 400032581 400032582 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLAVQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVK LRDLA I QNALEVK LHDLA I QEALKVN 60 170	FFDIYDPRKNLKS ¹ FFDIYDTRYHSIS ¹ FFDVYDPRSHSIS ¹ FFDYPPRSHSIS ¹ FFDIYDPRSHSIS ¹ FFDIYDPR FFDIYDPR FFDIYDPR FFGIYDPRKHSIS ¹ FFGIYDPRKHSIS ¹ FFGIYDPRKHSIS ¹ FFGIYDPR	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHVIHSEG SCIRLAFHGQG SCIRHAIHSQG SCIRHAIHSQG 190	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK ERYP-SLDLSNLK 200
400011520 400011529 400011528 400032547 40001523 400032581 400023273 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVK LRDLA I QEALKVN 60 170 	FFDIYDPRKNLKS ¹ FFDIYDTRYHSIS ¹ FFDVYDPRSHSIS ¹ FFDYDPRSHSIS ¹ FFDIYDPRSHSIS ¹ FFDIYDPR FFDIYDPR FFDIYDPRKHSIS ¹ FFGIYDPRKHSIS ¹ FFGIYDPRKHSIS ¹ 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHVIHSQG 190 	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK 200
400011520 400011529 400011528 400032547 400032581 400032582 400023288 400023288 400024231 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QKALEVN 60 170 VFRHLYVLYI FRSVFQHLYVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDVYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHVIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK 200
400011520 400011529 400011528 400032547 40001523 400032581 400023288 400023288 400024231 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536324	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVK LHDLA I QEALKVN 60 170 	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDYDPRSHSIS FFDYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRKHSSS FFGIYDPRKHSIS FFGIYDPRKHSIS 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL PDAIGSLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK 200
400011520 400011529 400011528 400032547 400011523 400032581 400032581 400023288 400023273 400023273 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536324 Rpihjti.1-GU563971	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLAVQK LRDLAI QKSLEVN LRDLAI QKSLEVN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QMALEVK LHDLA I QEALKVN 60 170 VFRHLYVLYI FRSVFQHLYVLYI LRSEFQHLYVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDYDPRSHSIS FFDYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHVIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL PDAIGSLYHLKL PDAIGCLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-FDLSNLK ERYLS-FDLSNLK 200
400011520 400011529 400011528 400032547 40001523 400032581 400032581 400023288 400023273 400023273 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563972	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLAVQK LRDLAI QKSLEVN LRDLAI QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QMALEVK LHDLA I QMALEVK CONTON 1	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDYDPRSHSIS FFDYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHVIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL PDAIGCLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK ERYP-SLDLSNLK 200
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400011520 400011529 400011528 400032547 40001523 400032581 400032581 400023288 400023273 400023273 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536327 Rpihjt1.1-GU563971 Rpihjt1.2-GU563973 Rpiedn1.1-GU563975 Rpisnk1.2-GU563975	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN CONTROLOGICON LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRSEFQHLVVLYI LRSEFQHLVVLYI LRSEFQHLVVLYI LRSEFQHLVVLYI LRSEFQHLVVLYI LRSEFQHLVVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDIYDPRSHSIS FFDVYDPRSHSIS FFDIYDPRSHSIS FFDIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPRKHSIS FFGIYDPKRHSIS 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHVIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG 190 PDAIGSLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-FDLSNLK ERYLS-FDLSNLK 200
400011520 400011529 400011528 400032547 40001523 400032581 400032581 400032582 400023273 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536327 Rpihjt1.2-GU563971 Rpihjt1.2-GU563973 Rpiedn1.1-GU563975 Rpisnk1.2-GU563976 400024234	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL 	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN LRDLA I QKALEVN CONTROLOGICON LRDLA I QKALEVN LRDLA I QKALEVN LRSE FQHLVVLYI LRSE FQHLVVLYI LRSE FQHLVVLYI LRSE FQHLVVLYI LRSE FQHLVVLYI LRSE FQHLVVLYI LRSE FQHLVVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS' FFDVYDPRSHSIS' FFDVYDPRSHSIS' FFDIYDPRSHSIS' FFDIYDPRKHSIS' FFGIYDPRKHSIS' FFGIYDPKRHSIS' 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHVIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLSSLDLSNLK ERYP-SLDLSNLK 200
400011520 400011529 400011528 400032547 400032581 400032581 400032582 400023273 400023273 400023288 400024231 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536326 Rpiblb3-FJ536324 Rpihjt1.1-GU563971 Rpihjt1.2-GU563972 Rpisnk1.1-GU563973 Rpisnk1.1-GU563975 Rpisnk1.2-GU563976 400024234 400011527	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN 60 170 FRSVFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI LRSEFQHLYVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS' FFDVYDPRSHSIS' FFDVYDPRSHSIS' FFDIYDPRSHSIS' FFDIYDPRKHSIS' FFGIYDPKHSIS' 180 	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SLCIRHVIHSEG SCIRLAFHGQG SLCIRHGIHSEG SCIRHAIHSQG SLCIRHGIHSEG SLCIRHVIHSQG 190 PDAIGSLYHLKL PDAIGSLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL PDAIGCLYHLKL	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLSSLDLSNLK ERYP-SLDLSNLK 200
400011520 400011529 400011528 400032547 400032581 400032581 400032582 400023273 400023273 400023273 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiabpt-FJ536326 Rpiabpt-FJ536324 Rpihjti.1-GU563971 Rpihjt1.2-GU563972 Rpihjt1.3-GU563973 Rpiedn1.1-GU563973 Rpiedn1.1-GU563975 Rpisnk1.2-GU563976 400024234 400011527 400011525	VQVVDTFWEKVTECRVHDL VQVVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVN LRDLA I QNALEVN LHDLA I QEALKVN 60 170 FRSVFQHLVUYI LRSEFQHLVUYI LRSEFQHLVUYI LRSEFQHLVUYI LRSEFQHLVUYI LRSEFQHLVUYI FRSVFQHLVVI FRSVFQHLVVI FR	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDYDTRYHSIS FFDYDPRSHSIS FFDYDPRSHSIS FFDIYDPRSHSIS FFDIYDPR FFDIYDPR FFGIYDPRKHSIS FFGIYDPRKHSIS 180 DTNFGYVSMVI DMNFGYVSMVI	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG 190 PDAIGSLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI PDAIGCLYHLKI	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-FDLSNLK ERYLS-FDLSNLK ERYP-SLDLSNLK 200
400011520 400011529 400011528 400032547 400032581 400032581 400032582 400023273 400023288 400024231 400032572 400032576 401010614 400006533 400006531 210 R2-FJ536326 Rpiabpt-FJ536326 Rpiabpt-FJ536324 Rpihjti.1-GU563971 Rpihjt1.2-GU563972 Rpihjt1.3-GU563973 Rpiedn1.1-GU563973 Rpiedn1.1-GU563975 Rpisnk1.2-GU563976 400024234 400011527 400011525 400011525	VQVVDTFWEKVTECRVHDL VQVAKTFWEKVTECRVHDL IQVVRTFWEKVSKCRIHDL AV	LRDLA I QKALEVN LRDLA I QKALEVN LRDLA VQK LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKSLEVN LRDLA I QKASDTN LRDLA I QKASDTN LRDLA I QKALEVN LRDLA I QNALEVN LRDLA I QNALEVN MODELA I QNALEVN LRDLA I QNALEVN LRSEF QHLYVLYI LRSEF QHLYVLYI LRSEF QHLYVLYI FRSVF QHLYVLYI FSSVF RHI YVLYI	FFDIYDPRKNLKS' FFDIYDTRYHSIS FFDIYDPRYHSIS FFDVYDPRSHSIS FFDIYDPRKHSKS FFDIYDPRKHSKS FFDIYDPRKHSKS FFDIYDPRKHSKS FFGIYDPRKHSKS FFGIYDPRKHSKS 180 DTNFGYVSMVI DMNFGYVSMVI DMNFGYVSMVI	SCIRHAIHSEG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG SCIRHAIHSQG 190 	ERYLSSLDLSNSK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLS-LDLSNLK ERYLSSLDLSNLK ERYLS-FDLSNLK 200

400011521	VSYVLRSRFSHESYKLQCVPTYVCVILGYXLVELYLMPEVYTTSSTDQVSIVFPP
400011920	LRSIMFFDPDFR-KMSLINFRSVFQHLYVLYLDMRFGNVSIVPDAIGSLYHLKLLRLRV-IRDLPSS
400011518	LPSS
400011517	IPSS
400011520	LRSIMFFDPHFR-NLFQHIHVFQHIYVLYLDINYGNVIPDAIGSLYHLKLLSLRG-VRNIPSS
400011529	LRSIMVFDHNFS-NTSLIKFSSVFRHLYVLYLDIEVGFTP-GAIGSLYHLKFLHLRG-VCYIPSS
400011528	
400032547	
400011523	
400032581	
400032582	
400023273	
400023288	LRSIMFFDPKFC-NVFPHIDVFQHIYVLNIKGSGAIP-DAIGSLYHLKFLSLRG-IYRLPSS
400024231	
400032572	LRSIMFFNQDFRNVFQHIDVFRHIYVLYLHIKGCGAIPDAIGSLYHLKFLSLRA-NRDLPSS
400032576	LRSIMFFDPDIC-NVFQHIDVFRHLYVLYLDIK-GSVIPEAIGSLYHLKFLRLRG-IRDLPSS
401010614	
400006533	LRSIMFFNRDFC-NVFQHIDVFRHLYVLYLDIKEGGVIPDAIGSLYHLKLLSLRG-IDNLPSS
400006531	LRSIMFFDR-KISLINFSSVFQHLYVLYLEMCVDKNPHPHLVPDAIGSLYHLKFLRLRGRIHDLPTS

220 230 240 250 260 270

	290	300	310	320	330	340	
400006531	IGNL K NLQTLVV	N-GYSCKL	PQETADLINLR	HLDARYSESLK	LSKLTSLQV	LKGVYCDQWKL	VDPVD
400006533	IGNLKNLQTFVV	VNEDGSFCQL	PPNTANLINLR	HLVAPYSEPLVI	RINKLTSLQV	VDGIACDQWKL	VDPVD
401010614							
400032576	IGNLKNLQTLAV	VN <mark>EGR</mark> SFCQL	PPKTVDLINLR	HLVARYSEPLEI	RISKLTSLQV	LKGVCCDQWEL	VVD PVD
400032572	IGNLKNLQTLVV	N <mark>EGGYYSCK</mark> I	PRETANLINLR	HLVVWYSKPLA	ISKLTSLQV	LERVCCDQWKL	VDPVD
400024231							
400023288	IGNLKNLQIL-V	VDAGGFTCHI	PCEAADLINLR	HLDARYSEPLA	RISKLTSLRV	L <mark>KGVGCD</mark> QWKL	VVD PVD
400023273							
400032582							
400032581							
400011523							
400032547							
400011528							
400011529	IGNLKNLQTL-H	ANSNFFSCHL	PPETADLINLR	HLVAPYSKPLKI	RISKLTSLQV	LKDIYCDQWKL	VDPVD
400011520	IGNLKNLQTLVV	VNID-LLCQL	PPETADLINLR	HLVV			
400011517	IGNLKNLQTLVV	VNMDRRFCQL	PPETADLINLR	HLVVPYSIPLAC		LR-LDCDQWKE	VD PVD
400011518	IRNLKNLQTLY-	VNDDIQYCEI	PHETTDLINLR	HLVSLYSKPLES	SQYQKX-LMP	IW-EYCQ	
400011920	IGNLKNLQTLVV	DTGGYTCQLP	QKIADLINLRH	LVARYYSKPLV	VNKLTSLQV	LEGVGCDQWKL	OVDPID
400011521	PFATSRIYRHFV	ST K T <mark>GAYA</mark> NY	XRETADLIDLR	YLDAPYSKPLK(INKLSSLQV	LKGLACDQWKN	IVDPVD
400011522	PFATSRIYRHFV	ST <mark>KTGAYA</mark> NY	XRETADLIDLR	YLDAPYSKPLK(LKGLACDQWKN	IVDPVD
400011524							
400011525	ISNLKNLRTLLV	DDHG-GFSRL	PQKTADLINLR	HLVAPYSEPLK(LKGFRCDQWKL	OVDPVD
400011527							
400024234	IGNLKNLQTLVV	NEGK-YTCQL	PRETADLINLR	HLVARYSKPLVI	ISKLTSLQV	VDGIHCDQWKL	OVDPVD
Rpisnk1.2-GU563976	IGNLKNLQTLVV	VNGYSLFCQL	PCKTADLINLR	HLVVQYSEPLK(LDGVACDQWKL	OVDPVD
	IGNLKNLQTLVV	VNGYTFFCQL	PCKTADLINLR	HLVVQYS E PLK	INKLTSLQV	LDGVACDQWKL	OVDPVD
Rpiedn1.1-GU563963	IGNLKNLQTLVV	IN <mark>GH</mark> PS <mark>YC</mark> QL	PRETVDLINLR	HLVAPYAEPLV	ISKLTSLQV	LDGVACDQWKL	OVDAVD
Rpihjt1.3-GU563973	IGNLKNLQTLVV	VNGYSLFCQL	PCKTADLINLR	HLVVQYSEPLK	INKLTSLQV	LDGVACDQWKL	VDPVD
Rpihjt1.2-GU563972	IGNLKNLQTLVV	VNGYSLFCQL	PCKTADLINLR	HLVVQYS <mark>E</mark> PLK(INKLTSLQV	LDGVACDQWKL	VDPVD
Rpihjti.1-GU563971	IGNLKNLQTLVV	VNGYSLFCQL	PCKTADLINLR	HLVVQYS <mark>E</mark> PLK(INKLTSLQV	LDGVACDQWKL	VDPVD
Rpiabpt-FJ536324	IGNLKNLQTLVV	VNGYTFFCEL	PCKTADLINLR	HLVVQYTEPLK(LDGVACDQWKL	OVDPVD
Rpiblb3-FJ536326	IGNLKNLQTLVV	INGHPSYCQL	PRETVDLINLR	HLVAPYAEPLV	ISKLTSLQV	LDGVACDQWKL	OVDAVD
R2-FJ536326	IGNLKNLQTLVV	VNGYTFFCE I	PCKTADLINLR	HLVVQYTEPLK	INKLTSLQV	LDGVACDQWKL	VDPVD
				.			

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	! ! !
R2-FJ536326	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpib1b3-FJ536326	LVNLQELSMDHIRSSYSLNNISSLKNLSTLTLSSELEQSPFPSLEFLISCQKLQKLWLKGRINKLP-
Rpiabpt-FJ536324	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpihjti.1-GU563971	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpihjt1.2-GU563972	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpihjt1.3-GU563973	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpiedn1.1-GU563963	LVNLQELSMDHIRSSYSLNNISSLKNLSTLTLSSELEQSPFPSLEFLISCQKLQKLWLKGRINKLP-
Rpisnk1.1-GU563975	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH
Rpisnk1.2-GU563976	LVNLRELSMDRIRSSYSLNNISSLKNLSTLKLICGERQSFASLEFVNCCEKLQKLWLQGRIEELPH

400024234	LVNLGELSMHRVKN	KNSFKYV				
400011527						P L
400011525	LVNLRELSMDYINR	SYSLNNISSL	KNLSTLI	RLLCYADESE	PSL <mark>KFV</mark> NS C Q	KLQKLWLR <mark>G</mark> KI E KLP-
400011524						
400011522	LVNLRELSMHDITK	SYSLNNISSL	NLSTVI	LCCEDDESF	PALEFLTSCO	KLOKLWLEGEIEKLPL
400011521	LVNL.RELSMHDTTK	SYSTANTSSL	INT.S TV	LCCEDDESE	PALEFLTSCO	KI.OKI.WI.EGETEKI.PI
400011920		TVCLNNTCCL	NT.S TT		DSTEEVNCCE	KIOKIMI FCKIFKIP
400011520						
400011518						
400011517	LVNLRELEMRXYIQ	ILLPKQHQL <mark>E</mark> I	(PHS)	2IVYFAWSTI	YISIPIPICL	KAPEIVVRWEIRETAC
400011520						
400011529	LVNLRELSMTNITR	FYSLNNIS K LF	KNLSTLRLI	CPGDGPF	PSLEFVNCCV	KLQKLFLNGRI E KLPD
400011528	LISIFKNXMISITK	SYSLNKISNL	KNLSTLE	KLLCYADESF	PSLEFLISCH	NL KK LLL EGRIEK LPL
400032547						
400011523					<i>CE</i>	KLQKLWL DGRIEK LPL
400032581						
400032582						
400023273						
400023273						
400023288	LVHLQELSMHDITK	SHSLNNISSL	(NLSTLKL)	CRSRASE	PSLEFVNCCE.	KLQKLWL D GRIVKLPH
400024231						
400032572	LVNLRELSMFHTSK	TYSLNNISSL	KNLSTLI	KLICGETESF	PSLEFVNCCE	KLQKLRL <mark>EGRIGK</mark> LP-
400032576	LVNLRELSMHYISD	SYSLNNISSL	NLSTLTLS	SS <mark>ELNH</mark> SSPF	PSL <mark>EF</mark> LNS C Q	KLQKLWLK <mark>GRINKLP</mark> -
401010614						
400006533	LVNLRELSMHYINK	SYSLKNISSL	NLSTLRLS	SGEYGNSSPF	PSLEFVNCCE	KLOKLWLDGGVEKLP-
400006531	T.VNT.RET.TMCSTWN	SYST.KNTSST	NT.STT.RT.FC		PSTEFTYSCE	KI.OKI.WI.KGRVEKI.P-
1000000001	2112122211002111	010111110011				
	260	270	200	200	400	410
100	360	370	380	390	400	410
420						
				· · · · · · · ·		
R2-FJ536326	LFSNSITMMVLS	FSELTEDPMP1	LGRFPNLRN1	LKLDG-AYEG	KEIMCSDNSF	SQLEFLHLRDLWKLER
Rpiblb3-FJ536326	LFPNSITMIALR	NSGLT ED P M PI	L <mark>GMLPNLR</mark> NI	LKLEN-AYEG	KEIMCSDNNF	SQL <mark>EFLHLRDLWNLK</mark>
Rpiabpt-FJ536324	LFSNSITMMVLS	FSELTEDPMP	LGRFPNLRNI	KLDG-AYEG	KEIMCSD NSF	SOLEFLHLRDLWKLER
Rpihiti.1-GU563971	LFSNSITMMVLS	FSELTEDPMP	LGRFPNLRNI	KLDG-AYEG	KEIMCSDNSF	SOLEFLHLRDLWKLER
$B_{pihit1} 2 - GU563972$	T.FSNSTTMMVT.S	FSELTEDPMP	T.GREPNT.RNI	KIDG-AVEG	KETMCSDNSE	SOT.EFT.HT.RDT.WKT.ER
$P_{ribi+1} = 00000000000000000000000000000000000$				KIDC-AVEC	KETMCCDMCF	
Rpinjci.3-G0503973		I SELLEDPMP		KLDG-AIEG	KEINCODUSE	SQLEF LILKDLWKLER
Rpiedni.1-G0563963	LEPNSITMIALR	NSGLIEDPMP	LIGMLPINLRINI	KLEN-AYEG	KEIMCSDNNE	SQLEFLHLRDLWINLKL
Rpisnkl.1-GU563975	LFSNTITMMVLS	FSELTEDPMPI	LGRFPNLRNI	KLDG-AYEG	KEIMCSDNSF	SQL EFLHLRDLWKLE R
Rpisnk1.2-GU563976	LFSNSITMMVLS	FSELTEDPMPI	LGRFPNLRN1	LKLDG-AYEG	KEIMCSDNSF	SQL EFLHLRDLWKLE R
400024234						
400011527	LFPNSITMMVLW	RSQLTKDPMP1	LGMLPNL <mark>RD</mark> I	LILQ <mark>E-AYNG</mark>	KEIMCSDNSF	RQLEFLHLYHLWNLER
400011525	LFPDSITMMVLW	KSKLMEDPMP	LGMLPNLRNI	ELEE-AYEG	KEITCSDNNF	SRLEFLRLHRFDKLEI
400011524						
400011522		KCKT MKDDMD	T CMT DNT DNI	DITE-AVEC		SOLFELPLOOLFKLEP
400011522		KOKI MKDEME I			NELICODINSE!	SOLET INIDDIENIEN
400011521	SDAFPNSITMMVLV.	KSKLMKDPMP			NELICSDISE	SQLEFLRLDDLERLER
400011920	LFPNSITMMVLR	KSVLKEDPMP1	LGMLPNLRNI	LNLFS-AYEG	KEIMCSDNSF	SQLEFLHLTELEKLER
400011518				RAYE G	KEIMCSDNSF	SQLEFLRLYDLEKLER
400011517	VSRFHHNDISLGVK	TDKRSDA	FGNVTKPKES	SRIRSXAYEG	EEIMCSDYSF	SQLELLHLYGLDNLER
400011520						
400011529	MFSNSITMMILQ	YSQLI ED PMP1	IL <mark>GML</mark> PNL <mark>RD</mark> I	LQL <mark>RG-AYKG</mark>	KDITCNDNSF	SQLEFLRLDSLGRLER
400011528	FPNSITMLTLV	DSKLMEDPME	LGMLPNLRNI	CLFR-AYOG	KEIICSDNSF	SOLKFLSLDCLWNLER
400032547						
400011523					NGW	ST. FVFVNT.PDT.FS
400011525		DSRUMEDEMEL				
400032581						
400032582						
400023273						
400023288	MFPNSITMMVLW	FSVLTEDPMP	L <mark>GMLPNLR</mark> NI	LYLVV-AYEG	KEIMCSDN	
400024231						
400032572	LFPNSITMMLLE	NSKLTEDPMP	LGMLPNLRNI	HLVG-AYEG	KEIMCSD NSF	SOLEFLHLYDLSKLER
400032576		KSSTTEDPMP	T.GMT.PNT.RNI	ELEE-AVEG	KETACSDNSE	SOT.ET.T.TT.NDT.YNT.EE
401010614						
100006522	UEDNOTENOS					
400000533	VEPNSITMMVLI.	DSKLMEDPMPI	LIGMLPNLRN1	IELLVGAYEG	MCSDNSF	SQUEFURLYDLENLEI
400006531	LFPNSITMMVLW	QSRLMEDPMP1	LI <mark>GK</mark> LPNLK <mark>D</mark> I	LILQ E-AYKG	KEIMCSDNTF	SQLESLRLYDLSNLER
	430	440	450	460		
R2-FJ536326	WDLGTSAMPLIKGL	GIHNCPNLKE	PERMKDVELI	KRNYML		
Rpiblb3-FJ536326	W					
Rpiabpt-FT536324						
-r-aspo 1000024	WDLG'I'SAMPLIKCI.	GTHNCPNI.KH	PRRMKINA	KRNYMI		
Bnibiti 1_00563071	WDLGTSAMPLIKGL	GIHNCPNLKEJ GIRNCPNIKEJ	IPERMKDVELI IPERMKDVELI	KRNYML		
Rpihjti.1-GU563971	WDLGTSAMPLIKGL WDLGTSAMPLIKGL	GIHNCPNLKEI GIRNCPNLKEI GIRNCPNLKEI	PERMKDVELI PERMKDVELI	LKRNYML LKRNYML		

Rpihjt1.3-GU563973	WDLGTSAMPLIKGLGIRNCPNLKEIPERMKDVELLKRNYML
Rpiedn1.1-GU563963	WI
Rpisnk1.1-GU563975	WDLGTSAMPLIKGLGIHNCPNLKEIPERMKDMELLKRNYML
Rpisnk1.2-GU563976	WDLGTSAMPLIKGLGIHNCPNLKEIPERMKDVELLKRNYML
400024234	
400011527	WHLATSAMPLIKGLAIDRCPKLKEIPERMKDVE
400011525	WHLSTSAMPSIKGLDIKYCPHLSHIPKRMQDVD
400011524	
400011522	WHLGTSAMPLIKGLAIYHCPKLKEIPERMKDVE
400011521	WHLGTSAMPLIKGLAIYHCPKLKEIPERMKDVE
400011920	WDLGTSAMPLIKGLGIHDCPNLKEIPERMKDVELLKRNYM
400011518	WDLGTSAMPLIKGLGIHDCPNLKEIPERMKDVELLKRNY
400011517	WHLGTNAMPLIKDLVIHHCPKLREIPERMKDVKHFKRXMKSYMI
400011520	
400011529	WHLGTSAMPLIKGLYIYD
400011528	WNLATSAMPLIKALRIDRCLKLNQIPERMKDVEPL
400032547	
400011523	WHSATSAMLVIKGLGRHECLKLHQIPKRMKSM
400032581	
400032582	
400023273	
400023288	
400024231	
400032572	WDLGTSAMPLIKGLGIHDCPNLKEIPE
400032576	WHLGTSAMPLIKGLHIYDCPKLKEIPK
401010614	
400006533	WHLATSAMPLIKSLAISRCPKLKEIPE
400006531	WHLDTSAMSLIKGLHIHACPELKEIPK

Protein sequence alignment of the NBS region of R3a homologs

R3agene-AY845382 I2gene-AF118127	10 . <i>RQND</i> <i>RQSE</i>	20 . IEDLIDRLLS	30 EDASGKKRT EGASGKKLT	40 . VVPIVGMGGLG VVPIVGMGGQG	50 . KTTLAKAVYN KTTLAKAVYN	60 . I <mark>DERVQIHFGL</mark> I <mark>DERVKNHFD</mark> L	70 <i>KAWF</i> <i>KAWY</i>
400014047 400018570 402018576 400019668	STSLVDDSDIFGRQTN	IEELIDRLLS IEDLIDRLLS	EDANGKKLT EDASGKKLT EDASBCNLT	VVSIVGMGGVG	KTTLSKVVYN	DEKVKDHFDL	KAWF KAWF
400018694 400007344 400018574	RHKE RQND RQNE	KKDLIDRLLS IENLIDHLLS IENLIGCLLS	ESASGEKLT EDVNGNFLT KDTKGKNLA	IVPIVGMGGAG VVPIVGMGGVG VVPIVGMGGLG	KTALAKVVYN KTTLAKAVYN KTTLAKEVYN	IDEKVKNHFGL IDEKVKNHFGL IDERVQKHFGL	KAWY KAWF KAWF
	80	90	100	110	120	130	140
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	CVSEAFDAFRITKGLL CVSEGFDALRITKELL CVSEAFDAFRITKGLL CVSEAYDAFRITKGLL CVSEAYDAFRITKGLL CVSEPYDALRITKGLL CVSEAYDAFRITKGLL CVSEAYDAFRITKGLL	. QEIGSFDLK- QEIGSFDLK- QEIGSFDLK- QEIGSFDLKD QEIGSFDLKD QEIGSFDLKV QEIGSFDLKV QEIGSFDLK-	ADDNLNQLQ VHNNLNQLQ VDDNLNQLQ DD-NLNQLQ DG-NLNQLQ DDKNLNQLQ GD-NLNQLQ VDDNLNQLQ	VKLKERLKGKK VKLKESLKGKK VKWKESLKGKK VKLKESLKGIK VKLKESLKGIK VKLKNSLTGKK AKLKESLKGKK	GELIVLDDVWA GELIVLDDVWA GELIVLDDVWA GELIVLDDVWA GELVVLDDVWA GELVVLDDVWA GELVVLDDVWA GELIVLDDVWA	IDNYNKWDELR IDNYNEWDDLR IDNYNEWDDLR IDNYNEWDDLR IDTYSEWDDLR IDNYSEWDDLR IDNYNEWDDLR IDNYNEWDDLR	NVFV NIFA NLFV ECFV NVFV SVFV NLFV NLFV NLFV
	150	160	170	180	190	200	210
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	QGDIGSKIIVTTRKES QGDIGSKIIVTTRKES QGDIGSKIIVTTRKES QGDIGSKIIVTTRKES QGDIGSKIIVTTRKES QGVMGSKIIVTTRKES QGDIGSKIIVTTRKEN QGDMGSKIIVTTRKES	VALMMGNE VALMMGNE VALMMANE VALMMGNE VALMM VAQMMCADHC VALMMGSG VASMMGSR VALMMGNE	QISMDNLST QIRMGNLST QISLDNLSI QISMDNLSI QISMDNLSI AITMGTLSS AINVGTLSD QISMDNLSI	ESSWSLFKTHA ESSWSLFQRHA EVSWSLFKRHA EVSWSLFKRHA EDSWALFKRHS EVSWDLFRRHS EVSWDLFKRHS EASWSLFKRHA	FENMGPMGHE FENMDPMGHE FENMDPMGHE FENMDPMGHE SLENRDHE SLENRDPKEHE SLENRDPEEHE FENMDPMGHE	ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA ELEEVGKQIA	AKCK AKCK AKCK AKCK AKCK AKCK DKCK DKCK
	220	230	240	250	260	270	280
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576	GLPLALKTLAGMLRSK GLPLALKTLAGMLRSK GLPLALKTLAGMLRSK GLPLALKTLAGMLRSK	SEVEEWKRIL SEVEEWKRIL SEVEGWKRIL PEVEGWKRIL	 RSEIWELPH RSEIWELPH RSEIWELPH RSEIWELPH		SYNDLPAHLR SYNDLPAHLR SYNDLPAHLR SYNDLPAHLR SYNDLPAHLR	RCFSFCAIFP RCFSFCAIFP RCFSYCAIFP RCFSYCAIFP	KDYP KDYP KDYP KDYA KDYP
400019668 400018694 400007344 400018574	GLPLALKTVAGTLRGK GLPLALKALAGILRCK GVPLALKTLAGILRCK GLPLALKTLAGMLRSK	SEVEEWRNIL SDVDEWRDIL SEVEEWRDIL SEVEGWKRIL	RSEIWDQ-H KSEIWELPS SRKIWDQPS RSEIWELQD	CLNGILPALKI HLNGILPALMI CLNGILPALMI KDILPALMI	SYNDLPAHLK SYNDLPAHLK SYNDLPPDLK SYNDLPAHLK	QCFAFCAIYP QCFAFCSIYP RCFAYCAIYP RCFSFSAIFF	KDYE KDYP KDYL KDYL
	290	300					
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344	FRKEQVIHLWIANGLV FRKEQVIHLWIANGLV FRKEQVIHLUVANGLI FRKEQVIHLWSANGLI FCKDLLIYLWIANGLV FCKDQVIHLWIANGLV FCKDQVIYLWIANGLV						
400018574	<i>FRKE</i> QV						
Protein sequence alignment of the LRR region of R3a homologs

	10	20	30	40	50	60	70
D 2							
RJagene-A1845382	LPALMLSYNDLPAHL	KRCFSFCALL	PKDYPERKE	QVIHLWIANGI OVTHLWIANGI	VPQEDVIIEI VPVKDETNOI	DSGNQYFLELF DI.GNOYFI.FI.F	SRSLF SRSLF
400014047	LPALMLSYNDLPAHL	KRCFSYCAT	PKDYAFRKE	OVTHI.I.VANGI	TLOEDRITO	DSGNOYFLEL	SRSLF
400018570	LPALMLSYNDLPAHL	KRCFSYCAI	PKDYPFRKE	QVIHLWSANGI	ILOEDKIIO	DSGNQYFLEL	SRSLF
402018576	MAALMLSYNDLPAHL	K Q CFAYCAI	PKDYQFRKD	QVIHLWIANGI	$V_Q \widetilde{Q} \widetilde{L}$	HSGNQYFLEL	RSRSLF
400019668	LPALKLSYNDLPAHL	KQCFAFCAI	PKDYEFCKD	LLIYLWIANGI	VKQF	CLGKEYFDEL	RSRSLF
400018694	LPALMLSYNDLPAHL.	KQCFAFCSI	PKDYPFCKD	QVI <mark>H</mark> LWIAN <mark>G</mark> I	<i>VQQF</i>	HSGNQYFLEL	RSRSLF
400007344	LPALMLSYNDLPPDL	KRCFAYCAI	PKDYLFCKD	QVIYLWIANGI	VQQ F	H	
400018574	LPALMLSYNDLPAHL.	KRC FSFSAII	PKDYPFRKE	Q V			
	80	90	100	110	120	130	140
				 		<u>1</u> 50	
R3agene-AY845382	ERVPNPSQGNTENLF	LMHDLVNDLA	OIASSKLCI	RLEESOGS-HN	ILEOSOHLSY	SMGYGGEFEKI	TPLYK
12gene-AF118127	EKVPNPSKRNIEELF	LMHDLVNDL2	QLASSKLCI	RLEESQGS-HN	ILEQCRHLSY	SIGENGEEKKI	TPLYK
400014047	ERVPNPSQGNIEK-F.	LMHDLVNDLA	QIASSKLCI	RLEESQGS-HM	IL <mark>EKGRH</mark> LSY	SMES-IDFEKI	LTPL-K
400018570	ERVPNPSQGNIET-F.	LMHDLVNDL2	QIASSKLCI	RLEESQGS-RM	ILEKSQHLAY	SMGY-GDFEKI	LTPL <mark>DK</mark>
402018576	ERVPES-EWKPEG-F.	LMHDLVNDLA	QIASSNLCI	RLEENKGS-HM	MLEQCRHMSY:	SIGKDGDFEKI	KSLFK
400019668	ERVPES-EWEWER-F.	LMHDLINDL2	QIASSKLCI	RLEESKGSDDN	ILEQSRHMSY:	SMERGGEFEKI	KQLS K
400018694	EMVSESSERDVEK-F.	LMHDLVNDLA	QVASSNLC1	RLEENKGS-H1	LEQCRHISY	SIGQYGEFEKI	,
40000/344 400018574							
100010374							
	150	160	170	180	190	200	210
					••••		••••
R3agene-AY845382	LEQLRTLLPTCIDLP.	DCCHHLSKR	LHNILPRLT:	SLRALSLSCYE	IVELPNDLF.	IKLKLLRFLD	IS R T E I
I2gene-AF118127	LEQLRTLLPIRIEFR	-LHN-LSKRV	LHNILPTLR	SLRALSFSQYF	IKELPNDLF'	TKLKLLRFLD	ISRTWI
400014047	LKHLRTLLPISFQ	-HGAPLSKR	LHSILPRLT	SLRALSLSNYN	TVELPDALF.		SQTAI
400018576	SEKI.PTI.DTTI.DDV	VNNCOLSKR	URITLPRIK T.HNTT.PRI.T	SLRALSLSKI ST.RAT.ST.SRVB		TELELLERE LDI	SQIRI
400019668	SEOLRTLLPINIG	-YSSDLSKR	T.HNTLPGLT	SLRALSLSGY	DTEEFPDN-F	TKI.KI.I.RFI.DI	SOTCT
400018694							
400007344							
400007344 400018574							
400007344 400018574	220	230	240	250	260	270	 280
400007344 400018574	 220 	230 	240	250	260 	270 	280
400007344 400018574 R3agene-AY845382	220 KRLPDSICALYN	230 	240	250 <i>LRHLDISN</i> 1	260 	270 SKLKSLQVLV(280 SAKFLI
400007344 400018574 R3agene-AY845382 12gene-AF118127	220 KRLPDSICALYN TKLPDSICGLYNLET.	230 LLLSSCADLE	240 ELPLQMEKL.	250 - <i>LRHLDISN</i> 1 INLRHLDVSN1	260 "RLLKMPLHL "RRLKMPLHL	270 SKLKSLQVLV SRLKSLQVLV	280 AKFLI PKFFV
400007344 400018574 R3agene-AY845382 12gene-AF118127 400014047	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS	230 LLLSSCADLE	240 ELPLQMEKL.	250 <i>LRHLDISN</i> INLRHLDVSN	260 RLLKMPLHL	270 SKLKSLQVLV(SRLKSLQVLV(280 GAKFLI CPKFFV
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS	230 LLLSSCADLE	240 ELPLQMEKL. ELPLQMEKL.	250 LRHLDISN INLRHLDVSN INLRHLDISN	260 RLLKMPLHL RRLKMPLHL	270 SKLKSLQVLVC SRLKSLQVLVC SNLKSLQVLVC	280 GAKFLI GPKFFV GAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS EKLPDSICGLYNLET.	230 LLLSSCADLE	240 ELPLQMEKL.	250 LRHLDI SN1 INLRHLDV SN1 INLRHLDI SN1	260 RLLKMPLHL RRLKMPLHL	270 SKLKSLQVLVQ SRLKSLQVLVQ SNLKSLQVLVQ	280 GAKFLI GPKFFV GAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS EKLPDSICGLYNL EKLPDSVCALYKLET.	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE	240 ELPLQMEKL. ELPLQMEKL.	250 LRHLDI SNI INLRHLDV SNI INLRHLDI SNI	260 RLLKMPLHL RRLKMPLHL SLLKMPLHL	270 SKLKSLQVLVV SRLKSLQVLVV SNLKSLQVLVV SKLKSLQVLVV	280 GAKFLI SPKFFV GAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS EKLPDSICVLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET.	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE	240 ELPLQMEKL. ELPLQMEKL.	250 LRHLDISNI INLRHLDVSNI INLRHLDISNI	260 RLLKMPLHL: RRLKMPLHL: SLLKMPLHL:	270 SKLKSLQVLVQ SRLKSLQVLVQ SNLKSLQVLVQ SKLKSLQVLVQ	280 SAKFLI SPKFFV SAKFLL SAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDS EKLPDSICGLYNL EKLPDSICGLYNL EKLPDSVCALYKLET.	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE	240 ELPLQMEKL. ELPLQMEKL.	250 LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN	260 RLLKMPLHL: RRLKMPLHL: SLLKMPLHL:	270 SKLKSLQVLVQ SRLKSLQVLVQ SNLKSLQVLVQ SKLKSLQVLVQ	280 3AKFLI 3AKFLI 3AKFLL 3AKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICVLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET.	230 LLLSSCADLE LLLSHCHHLE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL.	250 LRHLDISNI INLRHLDISNI INLRHLDISNI	260 RLLKMPLHL RRLKMPLHL	270 SKLKSLQVLVC SRLKSLQVLVC SKLKSLQVLVC	280 SAKFLI SPKFLI SAKFLL SAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICVLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320	260 RLLKMPLHL, SLLKMPLHL, WVLKMPLHL, 330	270 SKLKSLQVLVC SRLKSLQVLVC SNLKSLQVLVC SKLKSLQVLVC	280 2AKFLI 2AKFLI 3AKFLL 3AKFLL 34KFLL 350
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 290 	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300 	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 .	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 	260 RLLKMPLHL, SLLKMPLHL, SLLKMPLHL, WVLKMPLHL, 330 	270 SKLKSLQVLVC SRLKSLQVLVC SNLKSLQVLVC SKLKSLQVLVC 340	280 2AKFLI 2PKFFV 2AKFLL 3AKFLL 350
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127	220 KRLPDSICALYN TKLPDSICGLYNL EKLPDSICGLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 290 GGLRMEDLGEVHI DGURMEDLGEVHI	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE 300 NLYGSLSVVE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LENVVDRRE	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE	260 	270 SKLKSLQVLVO SRLKSLQVLVO SNLKSLQVLVO SKLKSLQVLVO 340 SSSADNSQTI	280 2AKFLI 2PKFFV 2AKFLL 3AKFLL 350 2RDILD 2RDILD 28DILD
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 290 GGLRMEDLGEVH DGWRMEDLGEAQ	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300 NLYGSLSVVE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LLENVVDRRE	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKN	260 RLLKMPLHL, RRLKMPLHL, SLLKMPLHL, WVLKMPLHL, 330 WDRLYLEWS(VEQLSLEWS)	270 SKLKSLQVLVC SRLKSLQVLVC SNLKSLQVLVC SKLKSLQVLVC 340 GSSSADNSQTI ESSIADNSQTI	280 2AKFLI 2AKFLI 2AKFLL 3AKFLL 350 2RDILD 2SDILD 2SDILD
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570	220 KRLPDSICALYN TKLPDSICALYN TKLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 290 GGLRMEDLGEVH DGWRMEDLGEVH GGCGGLRMEDLGEVQ	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE 300 NLYGSLSVVE NLYGSLSVVE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LLENVVDRRE LLENVVDRRE	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKN AVKAKMREKN	260 	270 SKLKSLQVLVV SRLKSLQVLVV SKLKSLQVLVV SKLKSLQVLVV GSSSADVSQTF ESSIADNSQTF RSS-ADNSQTF	280 2AKFLI 2PKFFV 2AKFLL 3AKFLL 350 350 350 2RDILD 2SDILD 2SDILD 2SDILD
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576	220 <i>KRLPDSICALYN</i> <i>TKLPDSICGLYNLET</i> <i>KRLPDSICGLYNLET</i> <i>KRLPDSICGLYNL</i> <i>EKLPDSICGLYNL-</i> <i>EKLPDSVCALYKLET</i> <i>290</i> <i>GGLRMEDLGEVH</i> <i>DGWRMEDLGEAQ</i>	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE 3000 NLYGSLSVVE NLYGSLSVVE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LQNVVDRRE DLQNVVDRKE.	250 -LRHLDI SN INLRHLDI SN INLRHLDI SN INLRHLDI SN 320 AVKAKMREKNE AVKAKMREKNE	260 	270 SKLKSLQVLVO SRLKSLQVLVO SKLKSLQVLVO SKLKSLQVLVO GSSSADNSQTE ESSIADNSQTE RSS-ADNSQTE	280 2AKFLI 2AKFLI 2AKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICGLYNLET. EKLPDSICGLYNL EKLPDSVCALYKLET. 290 GGLRMEDLGEVH DGWRMEDLGEAQ 	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300 NLYGSLSVVE NLYGSLSVVE NLYGSLSVLE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE DLQNVVDRRE LQNVVDRRE	250 -LRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE AVKAKMREKNE AVKAKIREKNE	260 RRLKMPLHL: SLLKMPLHL: WVLKMPLHL: WVLKMPLHL: WVLKMPLHL: VURLYLEWS VERLSLEWS	270 SKLKSLQVLVQ SRLKSLQVLVQ SKLKSLQVLVQ SKLKSLQVLVQ SKLKSLQVLVQ GSSSADNSQTE ESSIADNSQTE ESSSADNSQTE	280 28KFLI 28KFLI 28KFLL 38KFLL 350 28DILD 28DILD 28DILD 28DILA 28DILA
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694	220 KRLPDSICALYN TKLPDSICGLYNLET. KRLPDSICGLYNLET. EKLPDSICGLYNL EKLPDSVCALYKLET. 290 GGLRMEDLGEVH DGWRMEDLGEAQ GGCGGLRMEDLGEAQ	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300 NLYGSLSVVE NLYGSLSVVE NLYGSLSVLE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LQNVVDRRE LQNVVDRRE	250 INLRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE AVKAKMREKNE AVKAKIREKNE	260 RRLKMPLHL SLLKMPLHL WVLKMPLHL 330 VDRLYLEWS VEKLSLEWS VEKLSLEWS	270 SKLKSLQVLVQ SRLKSLQVLVQ SKLKSLQVLVQ SKLKSLQVLVQ GSSSADNSQTE ESSIADNSQTE ESSIADNSQTE	280 3AKFLI 3AKFLI 3AKFLL 3AKFLL 350 350
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344	220 <i>KRLPDSICALYN</i> <i>TKLPDSICGLYNLET</i> <i>KRLPDSICGLYNLET</i> <i>KRLPDSICGLYNL</i> <i>EKLPDSICGLYNL-</i> <i>EKLPDSVCALYKLET</i> <i>Georgenergenergenergenergenergenergenerge</i>	230 LLLSSCADLE LLLSSCADLE LLLSSCTSLE 300 NLYGSLSVVE NLYGSLSVVE NLYGSLSVLE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . ELQNVVDRRE ELQNVVDRRE ELQNVVDRRE	250 INLRHLDISN INLRHLDISN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE AVKAKMREKNE AVKAKIREKNE	260 RRLKMPLHLA SLLKMPLHLA WVLKMPLHLA 330 VDRLYLEWSA IVEKLSLEWSA	270 SKLKSLQVLVQ SRLKSLQVLVQ SKLKSLQVLVQ SKLKSLQVLVQ GSSSADNSQTF ESSIADNSQTF ESSIADNSQTF	280 3AKFLI 3AKFLI 3AKFLI 3AKFLL 350 350
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400019668 400018694 400007344 400018574	220 RRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNL EKLPDSVCALYKLET. 	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE 300 NLYGSLSVIE NLYGSLSVIE NLYGSLSVIE	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 . LQNVVDRRE LQNVVDRRE LQNVVDRRE	250 -LRHLDISN INLRHLDVSN INLRHLDISN INLRHLDISN INLRHLDISN AVKAKMREKN AVKAKMREKN AVKAKMREKN AVKAKIREKN	260 	270 SKLKSLQVLVO SRLKSLQVLVO SKLKSLQVV SKLKSLQVV SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVO SKLKSLQVVO SKLKSLQVVO SKLKSLQVO SKLKSLVO SKLKSLQVO SKLKSLQVO SKLKSLQVO SKLKSLQVO S	280 GAKFLI GAKFLI GAKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. CRLPDSICGLYNLET. CRLPDSVCALYKLET. CRLPDSVCA	230 LLLSSCADLE LLLSHCHHLE LLLSSCTSLE 300 NLYGSLSVVE NLYGSLSVVE NLYGSLSVE NLYGSLSVE	240 ELPLQMEKL. ELPLQMEKL. 310 LQNVVDRRE LQNVVDRRE LQNVVDRRE 380	250 -LRHLDISN INLRHLDVSN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE AVKAKMREKNE AVKAKIREKNE AVKAKIREKNE AVKAKIREKNE	260 RRLKMPLHL SLLKMPLHL WVLKMPLHL WVLKMPLHL WVLKMPLHL VEQLSLEWS VEXLSLEWS VEKLSLEWS	270 SKLKSLQVLVC SRLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SSLSQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLSQVLVC SSLQVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVLVC SSLQVC SSLQVC SSLQVLVC SSLQVLVC SSLQVC SSLQVC SSLQVLVC SSLQVC SSLQVC	280 2AKFLI 2AKFLI 2AKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	220 KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNL- EKLPDSVCALYKLET. 290 GGLRMEDLGEVH DGWRMEDLGEAQ SGLRMEDLGEVQ SGLRMEDLGEVQ SGLRMEDLGNLH 	230 LLLSSCADLA LLLSSCADLA LLLSSCTSLA 300 NLYGSLSVVI NLYGSLSVVI NLYGSLSVVI NLYGSLSVVI NLYGSLSVVI NLYGSVSVLA 370 	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 ELQNVVDRRE. ELQNVVDRRE. ELQNVVDRRE. 2LQNVVDRRE. 380 	250 -LRHLDISN INLRHLDVSN INLRHLDISN INLRHLDISN 320 AVKAKMREKNE AVKAKMREKNE AVKAKIREKNE AVKAKIREKNE AVKAKIREKNE AVKAKIREKNE	260 RRLKMPLHL SLLKMPLHL SLLKMPLHL WVLKMPLHL WVLKMPLHL VEQLSLEWS VEXLSLEWS VEKLSLEWS VEKLSLEWS VEKLSLEWS	270 SKLKSLQVLVC SRLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SSLSSLQVLVC SSLSSLC SSLSSLC SSLSSLC SSLSSLC SSLSSLC SSLSSLC SSLSSLC SSLS SSLSSLC SSLS SSLSSLC SSLS SSSLS SSLS SSSLS SSS	280 2AKFLI 2AKFLI 2AKFLL
400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574 R3agene-AY845382 I2gene-AF118127 400014047 400018576 400019668 400019668 400018574	220 KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. KRLPDSICGLYNLET. CRLPDSICGLYNLET. CRLPDSICGLYNLET. CRLPDSVCALYKLET. CRLPDSVCA	230 LLLSSCADLA LLLSSCADLA LLLSSCTSLA 300 NLYGSLSVVI NLYGSLSVVI NLYGSLSVVI NLYGSLSVVI NLYGSVSVLA 370 YRGTNFPNMI	240 ELPLQMEKL. ELPLQMEKL. ELPLQMEKL. 310 LQNVVDRRE LQNVVDRRE LQNVVDRRE 2LQNVVDRRE 380 380 	250 -LRHLDISN INLRHLDVSN INLRHLDISN INLRHLDISN 320 AVKAKMREKN AVKAKMREKN AVKAKIREKN AVKAKIREKN AVKAKIREKN AVKAKIREKN AVKAKIREKN	260 RRLKMPLHL SLLKMPLHL 330 VDRLYLEWS VEXLSLEWS VEKLSLEWS VEKLSLEWS VEKLSLEWS SLLKMPLHL SLEWS	270 SKLKSLQVLVC SRLKSLQVLVC SNLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC SKLKSLQVLVC A10 FLKFLSLE	280 2AKFLI 2AKFLL

400014047 400018570 402018576 400019668 400018694 400007344 400018574	ELHPHRNIKELQITC ELRPHKNIKQLQITC	YRGTKFPNWL SYRGTNFPNWL	ADHSFLKLV(VDVSFLKLVI	2LSLSNCKDC	DSLPAVGQLPC	LKYLSIRGM	HRITEV
	430	440	450	460	470	480	490
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	TEEFYGSWSSKKPFN TEEFYGRLSSKKPFN TEEFYGSSSSKNPFF	 ICLEKLEFKDM ISLEKLEFEDM CSLQKLEFEDM	PEWKQWDLLC TEWKQWHALC PEWKQWHALC	SSGEFPILEK GIGEFPTLEN GNGEFPALEN	LLIENCPELSI LSIKNCPELSI LSIENCPKLMG	ETVPIQLSS E-IPIQFSS K-LPENLCS	 LKSFDV LKRLEV LIELRI
R3agene-AY845382 12gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	500 <i>IG</i>	510 RSQLEAMKQIE "SQHEEMKQIE	520 SPLN EIDICDCNSY GLFITDCNSI	530 VINFPLSILP VTSFPFSILP LTSFPFSILP	540 TTLKRIKISDC TTLKRIQISRC	550 ?QKLKLEQPT ?PKLKLEAPV ?QKFKLEASV	560 GEIS GEM GEMSYY
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	570 -MFLEELTLIKCDCJ -FVEYLRVNDCGCV DVFLEDLILEECDCJ	580 DDISPELLPR DDISPEFLPT DDISPALLPR	590 ARKLWVQDWI ARQLSIENCQ AHKLSVSRCI	600 HNLTRFLIPT 2NVTRFLIPT HNLSRFLIPT	610 ATETLDIWNCE ATETLRISNCE ATERLYILNCE	620 INVEILSVACC INVEKLSVACC	630 G-GTQM GGAAQM G-GTQM
R3agene-AY845382 12gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	640 TSLTIAYCKKLKWLE TSLNIWGCKKLKCLE TYLYIDDCEKLKWLE 	650 PERMQELLPSL EHMQELLPFL EHMQELLPFL 720	660 KELHLSNCPI KELRLSDCPI KKLELCNCPI	670 	680 PFNLQQLAIRY PFNLEILRIIY PFNLQQLKIWN	690 CKKLVNGRKI CKKLVNGRKI ICKKLVNGRKI	700 EWHLQR EWHLQR EWRIQR 770
R3agene-AY845382 I2gene-AF118127 400014047 400018570 402018576 400019668 400018694 400007344 400018574	/10 RLCLTALIIYHDGSI LTELWIDHDGSI LPCLRELFIVHDGSI	/20 DEEIVGGENWE DEDIEHWE DEDI	730 LPSSIQRLT: LPCSIQRLT:	/40 . IVNLKTLSSQ IKNLKTLSSQ	/ JU HLKNLTSLQYI HLKSLTSLQYI	/ GU 	/// 2PMLE2 2S

Protein sequence alignment of the NBS region of *R3b* homologs 50 60

11000	n sequence u	-Simene (region of		UI055	
	10	20	30	40	50	60	70
			.	1		1	1 1
R3b gene-JF900492	RONEIEGLIDRLI	SEDGKNLT	VVPVV <mark>GMGG</mark> VO	KTTLAKAVYI	N DEKVKNHFGF	KAWICVSEPY	DILRIT
T2NBS3b	ROSETEDITORI	SEGASGKKUT	VVP TVGMGGO	KTTT.AKAVYI	DERVKNHEDT	KAWYCVSEGE	
401030236	1	T.T.EWAC-SI.T	VTPVVCMCCV	KTTT. AKAVVI		KAWECUSEON	
400030230	-					KANECUCEOL	DATRIA
400030238			GAL	KIILAKAVII	VDERVINDHE DL	KAWFCVSEQI	DAFRIA
400030239	ROSETEELVGRLI	SVDANGRSLT	VIPVVGMGGVG	KTTLAKAVYI	NDERVNDHEDL	KAWFCVSEQY	DAFRIA
	80	90	100	110	120	130	140
			.	1	.	1	1 1
R3b gene-JF900492	KELLQEFGLM	VDNNLNQLQ	VKLKESLKGKF	FLIVLDDVW	NENYKEWDDLR	NLFVQ <mark>GD</mark> VGS	KIIVTT
I2NBS3b	KELLQEIGKFDSF	DVHNNLNQLQ	VKLKESLKGKF	FLIVLDDVW	NENYNEWNDLR	NIFAQGDIGS	KIIVTT
401030236	KGLLOEIVRO	-VDDNINOIO	IKLKESLKGK	FLIVLDDVW	NDNYNEWDDLR	NLEVOGDLGS	KIIVTT
400030238	KGLLOETGLO		TKLKESLKRK	FT.TVT.DDVW	NDNYNEWDDLR	NLEVOGDLGS	KTTVTT
400030239	KCLLOFICLO		TKT.KEST.KRK				KTTVTT
400050255	NOTION TODO	vooningig.			Do Indiana de Carto d		
	150	160	170	180	190	200	210
			.	1	.	1	1 1
R3b gene-JF900492	RKE SVALMMGCGA	INVGTLSSEV.	SWDLFKRHSFE	NRDPKEHPE	L <mark>EEIGIQIAYK</mark>	CKGLPLALKA	LAGILR
I2NBS3b	RKDSVALMMGNE	IRMGNLSTEA	SWSLFQ <mark>RHAF</mark>	NMDPMGHPE	L <mark>EEVGR</mark> QIAAK	CKGLPLALKI	LAGMLR
401030236	RKESVALMMGGGA	MNVGILSNEV	SWALFKRHSL	NRDPEEHLEI	LEEIGKKIAEK	CKGLPLAIKI	"LAGMLR
400030238	RKESVALMMGGG	MNVGTLSNEV	SWALFKRHSL		.EETGKKTAEK	CKGLPLATK	
400030239	RKESVALMMGGGZ	MNVGTLSNEV	SWALFKRHST.		EETGKKTAEK	CKGLPLATK	T.AGMI.R
400050255	ICLES VALIA10000		SWALL MICHOL			CRODI DAIM	Inonin
	220	230	240	250	260	270	280
			.		.	1	1 1
R3b gene-JF900492	SKSEVDEWRHILF	RSEIWELQSRS	NGILPALMLS	NDLPPQLKR	CFAFCAIYP <mark>KD</mark>	YLFCKEQVVE	LWIANG
I2NBS3b	SKSEVEEWKRILF	RSEIWELPH	DILPALMLS	NDLPAHLKR(CFSFCAIFPKD	YPFRKEQVIE	ILWIANG
401030236	SKSTIEEWKRILF	RSEIWELPD	GILPALMLS	NELPPHLKR	CFSYCAIFPKD	YQFYREQIIÇ	LWIANG
400030238	SKSTIEEWKRILE	RSEIWELPD	GILPALMLS	NELPPHLKR(CESYCALEPKD	YOFYREOIIC	LWIANG
400030239	SKSTIEERKRILE	SEIWELPD	GILPALMLS	NELPPHLKR	CESYCALESKD	YOFYREOIIC	DLWIANG
						~ ~ ~	
R2h gapa- TE900492	TROOT HS						
TONDOOL	TAČČTUP						
TZNDDODD	TA						

 401030236
 LVQKLQ

 400030238
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Prote	in sequence ali	gnment o	f the LRI	R region of	f <i>R3b</i> hon	nologs	
	10	20	30	40	50	60	70
	••••		••••				
R3bgene-JF900492				LPALMLS	(NDLPPQLKR)	CFAFCAIYPK CFSFCAIFPK	DYLFCK
401030236	LPLAIKTLAGMLRSI	KSTI EEWKR II	RSEIWELPD	NGILPALMLS	NELPPHLKR	CFSYCAIFPK	DYOFYR
400030238				LPALMLS	NELPPHLKR	CFSYCAIFPK	DYQFYR
400030239				LPALMLS	NELPPHLKR	CFSYCAIFSK	DYQFYR
	80	90	100	110	120	120	140
		90 	100	1		130	140
R3bgene-JF900492	E QVVHLWIANGLVQ	2LHS	ANQYFLELR	SRSLFEKVRES	<u>5</u>	S K W	NSGE-F
I2gene-AF118127	EQVIHLWIANGLVP-	VKDEINQDI	LGNQYFLELR	SRSLFEKVPNE	·	<i>SKR</i>	NIEELF
401030236	EQIIQLWIANGLVQ	KLQKDETVEEI	LGNQYILELR	SRSLLDRVPDS	SFAWKRGVI	RODLDIYSEM	DGEK-F
400030238	EOTTOLWIANGLVO	KLOKDETVEEI KLOKDETVEEI	GNQIILELK	SRSLLDRVPD: SRSLLDRVPD-		SOM	DGEK-F
100030235	<u>19119101019</u>					Ug.	
	150	160	170	180	190	200	210
D2h							
T2gene-AF118127	IMHDLVNDLAQIAS:	SNLCMRLEENG SKLCTRLEESO	GSHMLERTR GSHMLEOCR	HLSYSTGFN	GE	FKKLTPLYKL	EQLRTL EOLRTL
401030236	VMHDLVNDLAQIAS	SKHCTRLEDI	GSHMLERTR	HLSYSTGDGN	SPFTIGDGD	FGKLKTLYKL	EQLRTL
400030238	VMHDLVNDLAQIASS	SKHCTRLEDI	GSHMLERTR	HLSYST <mark>GDGK</mark> I	LSYSSGDGD	FGKLKTLYKL	<u>EQLRTL</u>
400030239	FMHDLVNDLAQIASS	SKHCTRLEDI	GSHMLERTR	HLSYITGDSN1	LSFSGGDGD	FGKLKTLHKL	EQLRTL
	220	230	240	250	260	270	280
R3bgene-JF900492	LPINIQ RR P CH LE	KKRML <mark>HDIFP</mark> I	RLISLRALSL	SPYDIEELPNI	DLFIKLKHLK	FL D LSWTQI K	KLP <mark>D</mark> SI
I2gene-AF118127	LPIRIEFRLHNLS	SKRVLHNILP	LRSLRALSF	SQYKIKELPNI	DLFTKLKLLR	FLDISRTWIT	KLPDSI
401030238	LPINIOLHLFSVNL	SKRVLHNILPI SKRVLHNILPI	RLTT LKALSF RLTVLRALSF	S RIDIKEV PNI SGYDIKDVPNI		LDLSWILIK FLDLSWIMIK	OLP D LI OLP D SI
400030239	LPINFQHHWCSVNLS	SKRVLHNILPI	RLTFLRALSE	SGYYIKDVPNI	DLFIKLKLLR	FL D LSWTHIK	QLP <mark>D</mark> SI
	290	300	310	320	330	340	350
R3bgene-JF900492	CELYSLEILILSHCS	SHLNEPPLQM	KLINLHHLD	VSDAYFLKTP1	HVSKLKNLH	VLV <mark>GAKFF</mark> LT	GSSGLR
I2gene-AF118127	CGLYNLETLLLSSC	ADLEELPLQM	EKLINLRHLD	VSNTRRLKMP1	HLSRLKSLQ	VLVGP KFF VD	GWR
401030236	CVLYNLETLIVSSCI	DYLEELPLQM	SNLINLRYLD	IRRCSQLKLPI	HPSKLKSLQ	VLLGVKCLQS	GLK
400030238	CVLYNLETLIVSSC	CLEELPLOM DIFEIPLOM	NLINLHYLD	TRHCSRLKMP1	HPSKLKSLQ	VILGVRCL	GLK
100030235	0,11,121,111,0000						0
	360	370	380	390	400	410	420
B3bgana - 15000402						 COTTER DI L DE	
I2gene-AF118127	MEDLGEAONLHGSL	SVVKLENVVDI	REAVKAKMR	EKNHVERLSLE	WG-GSFADN.	SOTESDILDE.	LCPHKN
401030236	LKDLGELHNLYGSLS	SIVELQNVVDI	RREALKSNMR	EKEHIERLSLS	SWG-KSIADN	SQTERDIFDE.	LQPNTN
400030238	LKDLGELHNLYGSLS	SIAELQNVVDI	RREALKANMR	EKEHIERLSLI			
400030239	LKDLGEQCNLYGSLS	SIVELRNVVDI	REALKANMR	EKEHIKRLSLS	SWG-ISIAEN:	SQT ERDILDE	LQPNTN
	430	440	450	460	470	480	490
				1 1 1			1 1
R3bgene-JF900492	IKELRITGYRGTKFI	PNWLADHSFH	KLIEMSLSYC	KDCDSLPALGQ	LPCLKSLTI	RGMHQITEVS	EEFYGR
I2gene-AF118127	IKKVEISGYRGTNFE	PNWVADPLFL	KLVNLSLRNC	KDCYSLPALG	LPCLKFLSV	KGMHGIRVVT	EEFYGR
401030238							
400030239	<i>IQDLEISGYRGTKF</i> S	SNWLA <mark>D</mark> YSYL	LETLSLSHC	NNCDSLPALG	LPSLKVLTI	GYIDRITEVT	EEFYGS
	500	510	520	530	540	550	560
R3bgene-JF900492	FSSTKPFNSLEKT.EI	TAEMPEWKOW	INTERNET	III L <u>EELLIYRC</u> PF	····I····· KLIGKLPENV	· · · · · · · · SSLRRLRII.KI	CPELSI
I2gene-AF118127	LSSKKPFNSLEKLE	FEDMTEWKQWI	ALGIGEFPT	LENLSIKNCP	LSLEIPIQF	SSLKRLEVSD	CPVVFD
401030236	PSSIKPFNSLEWLE	ENWMNGWKQWI	IVLGSGEFPA	LQILSINNCP	KLMGKLPGNL	CSLTGLTISN	CP <mark>EF</mark> IL

400030238 400030239	PSSIKPFNSLELLGF	N RMNGWK QWE	VLGRGEFPA1	LQNLSI EGC PI	LMGKLPGNL	CSLTGLSISDC.	P KF IL
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	570 ETPIQLSNLKEFEVA D ETPIQLSSLKWFKVFG ETPIQLSNLKRFKVA	580 GSLKVGVLFL	590 AQLFTSQLI AQLFTSQLI HAELFASQLQ HAELFASQLQ	600 CGMKQIVKLDI EAMKQIEEIDI CGMTQLESLII	610 TDCKSLTSLH CCDCNSVTSFH RSCHSLTSFH	620 SISILPSTLKR FSILPTTLKR SFSILPTTLKR SSLPKTLKK	630 IRIAF IQISR IEISD IKIDY
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	640 CGELKLEASMNAMFLI CPKLKLEAPVGEMFVI CDQLKLEPSASEMFLI CQKLKLEPSASEMFLI	650 EKLSLVKCD- EYLRVNDCGC KSLVLTECNS ERLELSGCNS	660 VDDISPEFLY MNEISPELY INDISPELY	670 PRARNLSVRSC PTARQLSIENC PRAHYLSVNRC	680 CNNLTRLLIPI CQNVTRFLIPI CHSLTRLLIPI	690 ATERLSIRDY ATETLRISNC IGTEDLHISGC IGTEDLRISEC	700 DNLEI ENVEK ENLEM ESLEI
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	710 LSVARGTQMTSLN LSVACGGAAQMTSLN LLVASRTPTLR-NLH LLVASRTPTLLRKKK	720 IYDCKKLKSI IWGCKKLKSI IHSCKKLKSI	730 .PEHMQELLPS .PEHMQELLPS 	740 SLKKLVVQACE SLKELRLSDCE SLNYLNLYDCE SLSHLFLQSCE	750 PEIESFPEGGI PEIKSFPEGGI PEIKSFPEGGI	760 _PFNLQALSIW _PFNLEILRII _PFSLEVLQIW 	770 NCKKL YCKKL NCEKL
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	780 VNGRKEWHLQRLPSL VNGRKEWHLQRLT ENDRKEWHLQRLPCL ENDRKEWHLQRLPCL	790 IDLTIYHDGS -ELWIDHDGS RELKIFHHST 	800 DEEVLAGER DEDIEHU DEEIDU CDEGIHU	810 WELPCSIRELT WELPCSIKSLA WELPCSIRELT	820 TISNLKTLSSQ TIKNLKTLSSQ VI YNMKTLSSQ	830 2LLKSLTSLEY. 2HLKSLTSLQY. 2LLKSLTSLES.	840 LDAR- LCIEG LSTD-
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	850 <u>YLSQIQSQGQLSSFS</u>	860 HLTSLQTLQ1	870 WNFLNLQSL	880 AESALPSSLSF	890 [LEIDDCPNL] 	900 2SLLEEGLPFS 2SLFESALPSS 2SLIEEG	910 LSQLF
R3bgene-JF900492 I2gene-AF118127 401030236 400030238 400030239	920 IQDCPNLQSLPFKGM	930 PSSLSKLSIF	940 NCPLLTPLL	950 EFDKGEYWPQ1	960 TAHIPIINID		

Protein	sequence ang	nment o	I the ME	ss region	01 <i>Kpi-b</i>	nome	nogs
	10	20	30	40	50	60	70
Rpibt1-FJ18841	-RDKEKDEIVKILIN	INVSNAQTLPV	LPIL <mark>GMGGL</mark>	GKTTLAQMVFN	DQ <mark>RVIEHF</mark> HF	KIWICVSED	NEKRL
Rpistolgene-EU884421	RDKEKDEIVKILINN	IVS <mark>D</mark> AQHLSVI	PIL <mark>GMGGLG</mark>	KTTLAQMVFN <mark>E</mark>	QRVTEHFHSK	IWICVSEDFI	DEKRLI
Rpigene-AY4262	RDKEKDEIVKILINN	IVS <mark>D</mark> AQHLSVI	PILGMGGLG	KTTLAQMVFNL	QRVTEHFHSK	IWICVSEDFI	DEKRLI
Rpiptagene-EU884422	RDKEKDEIVKILINN	IVS <mark>D</mark> AQHLSVI	PILGMGGLG.	KTTLAQMVFNL	QRVTEHFHSK	IWICVSEDFI	JEKRLI
400009324	RDKEKDETVKTLTNN	IVSDAOELPVI	PTVGMGGLG	KTTLAOMVENE	ORVTEHEYPE	TWVCVSGDFI	DEKRIT
400030855	RDKEEDEIVKILINN	VRDSQKISVI	PILGMGGLG	KTTLAQMVFNL	QRVTEHFYPK	IWICVSDDFI	DEKRLI
400017060	RAKEKDEIVKILINN	IVS <mark>DA</mark> Q E LSVI	PIL <mark>GMGGLG</mark>	KTTLAQMVFN <mark>E</mark>	QTVT <mark>EHLYP</mark> K	IWICVSNDF1	DEKR LI
400017062	RGKEEDEIVKIL	INNAQQLSVI	PIL <mark>GLGGLG</mark>	K TTLAQVVFNL	PRVTEHFHPR	IWICVSDDFI)EKRLI
400018050	RDKEEDEIVKILINY	VSDAQQLSVI	PIVGMGGLG	KTTLSQMVFNL	QRVIQHF D PK	IWVCVSDNF	EKKLI
400029590		VI	PIVGMGGLG.	KTTLAQMIFNL	QSVTT <mark>H</mark> FNLK	IWVCVSDDF1	JEKRLI
400007609							
400029588							
	80	90	100	110	120	130	140
Bnibt1-F.T18841	TKETVESTEEKSLGG	MDT. APT.OKKI	RDT.T.NCKKY			T.KVCASCASI	
Rpistolgene-EU884421	KAIVESIEGRPLLGE	MDLAPLOKKI	OELLNGKRY	LLVLDDVWNEL	OOKWANLRAV	LKVGASGAS	LTTTR
Rpigene-AY4262	KAIVESIEGRPLLGE	MDLAPLQKKI	ELLNGKRY	LLVLDDVWNEL	QQKWANLRAV	LKVGASGAS	LTTTR
Rpiptagene-EU884422	KAIVESIEGRPLLGE	MDLAPLQKKI	QELLNGKRY	LLVLDDVWNEL	QQ KWANLRA V	LKVGASGAS	/LTTTR
400009324							
400019804	KAIVESIE						
400030855	KAIVESIEGKSLS-D	MDLDPLQKKI	QELLNGKRY		QQKWDNLRAV	LKVGASGAS	LITTR
400017060	KATTESTEGKSLS-D		OKT.I.NRERV		OOKWDNI.RAV	T.KVCATCAS	TT.TTTR
400018050	KAIVESAEGRPLLGE	MDLAPLOKKI	OELLNGKRY	LLVLDDVWNEL	OEKWD		
400029590	KAIVESIERRPLG-D	MDLAPLQKKI	QELLNGKRY	FLVLDDVWNEL	<u>, EKWAKIKAV</u>	LNVGAQGSS	ILATTR
400007608							
400007609							
400029588							
	150	160	170	180	190	200	210
Rpibt1-FJ18841	L EKVG SIMGTLQPY E	LSNLSQ EDC M	ILLFMQ RAFG	HQ EE INLNLVA	IGKEIVKKC	GVPLAAKTL	GILRF
Rpistolgene-EU884421	LEKVGSIMGTLQPYE	LSNLSQEDC	ILLFMQRAFG.	HQEEINPNLVA	IGKEIVKKSG	GVPLAAKTLO	GILCF
Rpigene-A14262	LEKVGSIMGTLQPIE	LSNLSQEDC	ILLEMQRAEG.	HQEEINPNLVA	TCKETVKKSG	GVPLAAKTL(CILCE
400009324							
400019804			Q K SLG	DMDL SP	FOKKLOELLN	GKKYLL	/L <mark>DD</mark> VW
400030855	L EKVG SIMGTLQPYE	LSHLSQ <mark>EDC</mark> S	LLFMQRAFG	HQ EE I NPN FV S	MGKEIVKKC	GVPLAAKTLO	GLLRF
400017060	L EKVG SIMGTLQPYE	LSNLSQ <mark>EDC</mark> M	ILLLMQ RAFG	YQEE INPNLVA	IGMEIVKKC	GVPLAAKTL(GILRF
400017062	LQ KVC SIMQTLHPYE	LSNLSQEDCI	SLFNQRAFE	HLEEINPNLEA	IGKEIVKKCG	GVPLAAKTLO	SILHF
400018050			ILLEMQRALG.	HQEEINPNLVV	TCKKTVKKCG	GVPLAAKTL(GILRE
400029390		C					
400007609	QP YE	LSNLSQANC	ILLFMQRALG	HQ EE INPNLVV	IGKEIVKKCG	GVPLAAKTL	GILQF
400029588						<i>TL</i> (GLLRF
			040	050	0.00	070	
	220	230	∠40 l	∠50 II I	∠o∪ 	270	280
Rpibt1-FJ18841	KREEROWEHVRDSEI	WKLPOEESSI	LPALRLSYH	HLPLDLROCFI	YCAVFPKDTE	MEKGNLISL	MAHGF
Rpistolgene-EU884421	KREERAWEHVRDSPI	WNLPQDESSI	LPALRLSYH	QLPLDLKQCFA	YCAVFPKDAR	MEKEKLISL	MAHGF
Rpigene-AY4262	KREERAWEHVRDSP1	WNLPQ <mark>DE</mark> SS1	LPALRLSYH	QLPL D L K Q CFA	YCAVFPKDAK	MEKEKLISL	MAHGF
Rpiptagene-EU884422	KREERAWEHVRDSP1	WNLPQDESS1	LPALRLSYH	QLPL D L K Q CFA	YCAVFPKDAK	MEKEKLISL	VMAHGF
400009324		WSLPQDESSI	LPALRLSYH.	HLPVDLRQCFA	YCAVFPKDTK	MIKENLITL	MAHGF
400019804	REDUERWONLRAV	WNT.DOH F STI	LTTTRSERV	HIDIDI POCEA	VCAVEDEDC-	MEKEKTTST	MALCE
400017060	KREEREWEHVRDSEI	WNLPODESS1	LPALRLSYH	HLPLDLROCFV	YCAVFPKDTE	MKKEELIAF	MAHGE
400017062	KREERVWKHVRDSEI	WNLPQDESSI	LPALRLSYH	HLPLDLRQCFA	YCAVFPKDTK	MEKENLISL	MAHGE
400018050	KREEREWEHVRDSEI	WKLPQDESS1	LPALQLS YH	HLPLDLRQCF1	YCAVFPKDTE	MEKENLISL	MAHGF
400029590	KREESEWEHVKDSEI	WNLPQDENSV	LPSLRLSY <mark>H</mark>	HLPLDLRQCFA	YCAVFPKDTK	TEKEYLITL	VMAHGF
400007608					YCAVFPKDTE	MEKENLISL	MAHGE
400007609	KREEREWEHVRDSEI	WKLPLDESSI	LPALRLSYH	HLPLDLRQCF1	LLCSIPK		GY
100023300	MARCORNERVEDSEL	. HINDE QUEINS V	LE SURIS III.	ILLE LIDURQUEA	CAVE PRDTK		miniter
Dmib+1_ET10041							
Pristolgono-FU904421	LICKC						

Protein sequence alignment of the NBS region of *Rpi-bt1* homolog

 Rpibt1-FJ18841
 ILSKG

 Rpisto1gene-EU884421
 LLSKG

 Rpigene-AY4262
 LLSKG

 Rpiptagene-EU884422
 LLSKG

 400009324
 LLSKG

 400019804

400030855	LLS KG
400017060	LLS KG
400017062	LSS <mark>KG</mark>
400018050	LLS KG
400029590	L
400007608	LLS KG
400007609	
400029588	LLS KG

Protein sequence alignment of the LRR region of *Rpi-bt1* homologs

	10) :	20	30	40	50	60	70
			 	1				
Rpibt1-FJ18841	LRVLNLSD	KLKQLPSS.	IGDLVHLRY1		RSLPNQLCKL	QNLQTL <mark>DLHG</mark>	CHSLCCLPK	TSK
Rpisto1-EU884421	LRVLNLGDS	STFNKLPSS.	IGDLVHLRY1	LNLYGS-GM	RSLPKQLCKL	QNLQTL <mark>D</mark> LQ Y (CTKLCCLPK	TSK
Rpigene-AY4261	LRVLNLGDS	STFNKLPSS.	I <mark>GD</mark> LVHLRYI	LNLYGS-GM	RSLPKQLCKL	QNLQTL <mark>D</mark> LQ Y (CTKLCCLPK	TSK
Rpiptal gene-EU884422	LRVLNLGDS	STFNKLPSS.	I <mark>GD</mark> LVHL <mark>R</mark> YI	LNLYGS-GM	RSLPKQLCKL	QNLQTL <mark>D</mark> LQ Y (CTKLCCLPK	TSK
400009324	LRVLNLSYS	SKLEQLPSS.	I <mark>GDLLHLRY</mark> I	LDLSRN-NF1	RSLPERLCKL	QNLQTL <mark>DVH</mark> NO	CYSLNCLPKQ	2TS K
400019804					FCKL (QNLQTLALNN	CCSLCCLPK	RTNK
400030855	LRVLNLSDI	L <mark>GLK</mark> QLPSS.	IGDLVHLRY1	LNLSGNWNM	RSLPKELCKL	QNLQTL <mark>DI Y</mark> NO	CQSLCCLPK	2TSQ
400017060	LRVLNLSNI	L KLG QLPSS.	IGDLVHLRY1	INLSSN-SM	RSLPKQLCKL	QNLQTL D LQ D (CLPLRCLPK	2TS K
400017062	LRVLNLRYI	ELNQLPSS.	IGDLVHLRYN	IDLSYNREM(CSLPKQLCKL	QNLQTL D LQY	CISLCCLPK	TSK
400018050	LRVLNLSYTEI	LELEQLPSS.	IGDLVHLRYI	INLSSNLRI	RSLPKQLCKL	QNLQTLDLHD	CWSLSCLPK	2TST
401009305	LRVLNLSNS	SEFEQLSSS	VGDLVNLRYI	DLSGN-KI	CSLPKRLCRL	QNLQTLDLHN	COSLSCLPK	2ISK
400007608		<u>KLKQL</u> PSS.		INLSSNLRI	RSLPRQLCRL	2NLQTLDLHD(2TSK
400029588		SKEDOLSSS		NT.HCS-ST	PST.PKPT.CKT.		CEST.VVTPK	0779 K
400029588		DOLSSS		NT.HCS-ST	RSI.PKRI.CKI.	ONLOTIDISCO	FSL.VVTPK	213K
400023330		DQ10000		111105 511			JE OLI I I E RY	2101
	80) (90 1	00	110	120	130	140
		, I	 					
Rpibt1-FJ18841	LGSLRNLLLD	CYGLTCMP	RIGSLTCL	TLSRFVVG	IOKKSCOLGE	LRNLNLYGSI	EITHLERVK	NDMD
Rpisto1-EU884421	LGSLRNLLLD	SOSLTCMP	RIGSLTCL	TLGOFVVG	R-KKGYOLGE	LGNLNLYGSI	KISHLERVK	NDKD
Rpigene-AY4261	LGSLRNLLLD	SOSLTCMP	RIGSLTCL	TLGOFVVGI	R-KKGYQLGE	L <mark>GNLNLYG</mark> SI	KISHLERVK	NDKD
Rpiptal gene-EU884422	LGSLRNLLLD	SOSLTCMP	RIGSLTCL	TLGOFVVGI	R-KKGYQLGE	L <mark>GNLNLYG</mark> SI	KISHLERVKI	NDRD
400009324	LSSLRNLVLD	CP-LTSTP	PRIGLLTCL	TLGFFIVG	S- KKGY QLGE	L <mark>K</mark> NLNL <mark>CG</mark> SI	SITHLERVK	NDTD
400019804	LGSLQNLLLD	CYGLNSMP	RIVSLTCL	TLGRFAVG	R-KKGYKLGE	LRNLNLRGSI	SIT <mark>HLERVK</mark> I	NGTE
400030855	LGSLRNLLLE	CYRLTFMP	PRFGSLTCLE	TLDFFAVG	E- <u>KE</u> GSQLSE	L <mark>GNLNLYG</mark> SI:	SIKHLERVKI	NDKD
400017060	LVSLRNLLLD	IN-LLKSMP	PRIGSLTCLE	KTL <mark>GQFIVG</mark>	R-KKGYQLGA	L <mark>GSLNLYG</mark> SI	EITHLERVKI	NDKD
400017062	LVSLRNLLLD	CP-LDCMP	PRIGSLTCLE	KTLS RFVVG	W- KKGG QLG E I	L <mark>GNLNL YG</mark> SI	EISHLERVKI	NDKD
400018050	LGSLRNLLFD	CP-LTCMP	PRIGSLTCLE	TLSYFVVG	R-KKSYQLGE	L <mark>RNLDLHG</mark> SI:	SITNL <mark>ERVK</mark>	KHTE
401009305	LGSLRNLVFD	CP-LTAMP	PRIGLLTCLE	TLSYFLVG	E- RKGYQLGE I	LRNLNLRGAI	SIT <mark>HLERVK</mark> I	NDME
400007608	LGSLRNLLLD	CP-LTCMP	PRIGSLTCLE	TLSYFVVG1	R- <i>KKGCQLGE</i>	LRNLDLHGTI:	SITHLERVK	KDTE
400007609								
400029588	LSSLRNLVFK	CQ-ITSMP	PRIGSLTCLE	TLDYFIVG	E-G <mark>K</mark> GYQLGE	LQNLNL <mark>HG</mark> SL:	SITHLERVK-	
400029590	LSSLRNLVFK	CQ-ITSM-						
	1 5 4	. 1		70	1.0.0	1.00	200	010
	150	, , ,	1 10		180	190	200	210
Prih+1-F119941		IT NOT CMPH			T KDHCMT TC			
Ppisto1-FU884421	AKEANLSAKE	IT.HST.SMCW	INF-COHTVE	SERVEVLE	ALKPHSNLICI	LETVCERCTH	LP DWMMHSVI	LENT
Rpigene-AY4261	AKEANLSAKG	ILHSLSMSW	NNF-GPHTY	SEEVKVLE	ALKPHSNLTS	LKTYGFRGTH	LPEWMNHSVI	LKNT
Rpiptal gene-EU884422	AKEANLSAKG	LHSLSMSW	NNF-GPHIYE	SEEVKVLE	ALKPHSNLTS	LKIYGERGIH		LKNI
400009324	A-EANLSAKAN	LOSLSMSW	DND-GPNRY	SEEVKVLE	ALKPHPNLKY	LEIIAFGGFR	PSWINHSVI	LEKV
400019804	AKEANLSAKAI	NLHSLSMIW	NGP RRY	SEEIKVLE	ALKPHPNLKY	LKIFGFGGFR	FP DWMNHSVI	L <mark>EK</mark> V
400030855	ANEANLSAKG	ILHSLSMIW	KQLHRY	SEEVKVLE	ALKPHPNLTS	LTIIGFRGFR	P DWINQSVI	LINV
400017060	AKKANLSAKAN	ILHSLSMRW.	D <u>E</u> P Y G Y E	SEEVKVIE	ALKPHPNLKF	LEIIGFRGIH	LP <mark>EWMNH</mark> SVI	LKNI
400017062	AKEANLSAKE	NLRSLIIDW.	DWL-EPRRYE	SEEVEVLE	ALKPHSNLTS	LKINGFKGIR	LPYWMN <mark>H</mark> SVI	LKNV
400018050	AKEANLSAKAN	√ L H−−−−−			<i>S</i> i	LTII <mark>GFRGFR</mark>	LP <mark>D</mark> WMNHSVI	L <mark>KR</mark> V
401009305	AKEANLSAKAN	IL <mark>H</mark> SLS M SW	DGPH- Y	SEEVKVLE	ALKPHPNLKY	LEIIDFCGFC	LP <mark>DWMNH</mark> SV1	L KKV
400007608	AKEANLSAKE	ILHSLRMSW	YKYGP YC	CLHETDVLE	ALKPHPNLTS	LTIIDFRGFR	LP <mark>DWMNH</mark> SV1	L <mark>KR</mark> V
400007609								
400029588								
400029590								
		· ·			250	260	070	
	220	, 2.	30 2	40	250	260	270	280
Prib+1_FI19941	VETETTECEN							
Pristo1-FU1884421	VSTLTSUKN	SCLEPPEGE.	LPCIELI	WCSAEVEY		CEDTREKEPS		
Rpiscor-20004421	VSTLTSNERN	SCIPPEGD.		WCSADVEI		CEDTRICES		SIK
Rointal gene-EU884422	VSTLTSNEPM			WCSADVEV	VEEVDIDVHS	CEDTRICES		SLK
400009324	ISIRIKSCKM	CLCLPPFCE	LPCLESLEL	NGSAEVEVI	VEEDDVHSI	RESTREEPS	LKKLRTWFFF	RNI.K
400019804	ISIEIYGCEN	CLRLPPFGE	LPCLESLEI.	DGSGEVEV	VEEDDVHF	REPTKRREPS	LRKLCICHFF	RNLK
400030855	VSIVIEGCENO	SCLPPFGE	LPCLESLET.	KGSAEV	VEEADVHS	GEPTRKWEPS	LRKLSIGKFO	CNLK
400017060	VSIVIKDCRNO	CLCLPPFGD	LPCLESLKLS	WGSADMEY	VEEVDIDVDS	GFPTRIRFPS	LRKLAIWGFO	GNLK
400017062	VSIEISSCANO	CSCLPPFGE	LPCLESLRL	WGSVDVEY	VDVD	VDSGRFPS	LRTLVIVNES	SNLK

	VSIRIEGCE	INCSCLPPI	FGDLPCLE	SLVLANGSG	EVEYLEED-	- D VS	TRRWFP:	SL RK LSIWD	FCNLK
401009305	VSTLTNGC	NCSCLPP	GELPCLE	SLELODGSV	EVEYVEDS-	-GFP		ST.RKT.HTGG	FCNLK
400007608	VSTRIEGCE	NCSCLPPI	GDLPCLE	SLVLANGSG	EVEYVEED-	-DVS	TRRWEP	STRKLSTWD	FRNTK
400007609									
400029588									
400029590									
	2	290	300	310	320	3	330	340	350
		1		1			1		I
Rpibt1-FJ18841	GLLKKEGEE	QCPVLEE	EIKCCPN	FVIPTLSSV	KKLVVSGDR	SDAIGES	SSISNLMA	LTSLQI RY N	KEDA S
Rpisto1-EU884421	GLLKKEGEE	<u>COFPVLEEN</u>	MIIHECPI	LTLSSN			LRA	LTSL <mark>RICY</mark> N	KVATS
Rpigene-AY4261	GLLKKEGEE		MIIHECPI	LTLSSN			LRA	LTSLRICYN	KVATS
Rpiptal gene-EU884422	GLLKKEGEE		LIIHECPE	LTLSSN			LRA	LTSLRICYN	KVATS
400009324	GLVKQEGE	KFPMLEE	MAILHCP1	FVFPILSSV	KKLEVHGN1	KAR-GLS	SSISNLST	LTSLRIGAN	YRATS
400019804	GLLKKETEE		MKI IHCPI	FVFSALSSV	KELEIE G	DTGLC	SISNLST	LTSLNISSY	DEVTS
400030855	GLLKNEGE	HFPVLEEN	MTIS <mark>DC</mark> PN	FVYTTLSLV	v G	ERK-GF	2 LGE	LRNPNLYG-	KIE IT
400017060	GLLKKEGEB	COFPVLEE	MTINGCPN	FVIPTLSSV	KTLKVLGDR	SEAIVL	RSIYKLTT	LTSLYIINN	YEATS
400017062	GLLKKEGEB		MIILYCPN	FVIPTLPSL	KVCN	SDASSL	STSNLSA	LTSLNIS <mark>G</mark> N	YEATS
400018050	GLLKKGGEB								
401009305	GLORMEGEE	OFPVLEEN	MKISDCPN	FVFPTLSSV	KKLEIWGEA	DAR-GLS	SSISNLST	LTSL KIF SN	HTVIS
400007608	GLIKKGGER	OFPVLEE	NCP	FVTP					
400007609						ATSL	ISTSNLCA	I.TYLHTSST	TKATS
400029588									
400029590									
400029390									
	3	860	370	380	390	4	100	410	420
		1		1					I
Rpibt1-FJ18841	LP EEMFK SI	ANLKYLNI	ISFYFNLF	ELPTSLASI	NALKHLEIF	SCY-AL	SLPEEGV	KG LISLTQL	SITYC
Rpisto1-EU884421	FPEEMFKN1	ANLKYLTI	ISRCNNL	ELPTSLASI	NALKSLKIC	LCC-ALL	SLPEEGL	EGLSSLTEL	FVEHC
Rpigene-AY4261	FPEEMFKN1	ANLKYLTI	ISRCNNLF	ELPTSLASI	NALKSLKIQ	LCC-ALE	ESLPEEGL	EGLSSLTEL	FVEHC
Rpiptal gene-EU884422	FPEEMFKN1	ANLKYLTI	ISRCNNL	ELPTSLASI	NALKSLKIC	LCC-ALL	SLPEEGL	EGLSSLTEL	FVEHC
400009324	LP EEMF TS1	TNLEYLS	FFDFKNLF	ELPTSLTSL	NALKRLQIE	S-CDSL	ESLPEQGL	EGLTSLTQL	FVKYC
400019804	LP EEMFK S1	VNLKYLK							
			LSFFKNLF	ELPASLVSL	TALKRLEII	GCD-ALE	CLPERL	EGLTSLTEL	SVEYC
400030855	HLERVKKD	E-AKEANI	LSFFKNLF LS <mark>GKM</mark> NLF	ELPASLVSL SLSMSW	TALKRLEI1	GCD-ALE		EGLTSLTEL	SVEYC
400030855 400017060	HLERVKKDI LPEEMFKSI	T <mark>E-AKEA</mark> NI LANLKYLNI	LSFFKNLF LS <mark>GKM</mark> NLF LSFFKNLF	ELPASLVSI ISLSMSW GLPTSLASI	TALKRLEII NALKHLRIQ	WCD-ALE	SCLPEERLI SLAEEGLI	EGLTSLTEL DGLTSLTEL	SVEYC FVEHC
400030855 400017060 400017062	HLERVKKDI LPEEMFKSI LPEEMFKSI	T <mark>E-AKE</mark> ANI LANLKYLNI LANLTYLEI	LSFFKNLF LS <mark>GKM</mark> NLF ISFFKNLF ISVFYNLF	ELPASLVSI ISLSMSW GLPTSLASI ELPSSLASI	TALKRLEII NALKHLRIQ NALKRLDIY	GCD-ALE WCD-ALE YCD-TLE	SLAEEGU SLAEEGU	EGLTSLTEL DGLTSLTEL KGLTSLTQL	SVEYC FVEHC SLHYC
400030855 400017060 400017062 400018050	HLERVKKD LPEEMFKS1 LPEEMFKS1	TE-AKEANI LANLKYLNI LANLTYLE	LSFFKNLF LS <mark>GKM</mark> NLF ISFFKNLF ISVFYNLF	ELPASLVSI SLSMSW GLPTSLASI ELPSSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY	WCD-ALE	ESLAEEGLI ESLPEEGV	EGLTSLTEL DGLTSLTEL KGLTSLTQL 	SVEYC FVEHC SLHYC
400030855 400017060 400017062 400018050 401009305	HLERVKKD LPEEMFKS1 LPEEMFKS1 LLEEMFKS1	TE-AKEANI LANLKYLNI LANLTYLE LENLKYLSV	LSFFKNLF LS <mark>GKM</mark> NLF LSFFKNLF LSVFYNLF VSYL E NLF	ELPASLVSI SLSMSW GLPTSLASI ELPSSLASI ELPTSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI	ICLPEERLI ISLAEEGLI ISLPEEGVI IRRSSASP(EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608	HLERVKKDI LPEEMFKSI LPEEMFKSI LLEEMFKSI	TE-AKEANI LANLKYLNI LANLTYLEI LENLKYLSV	LSFFKNLF LS <mark>GKM</mark> NLF ISFFKNLF ISVFYNLF 	ELPASLVSI SLSMSW GLPTSLASI ELPSSLASI ELPTSLASI	TALKRLEII NALKHLRIQ NALKRLDIY NALKRLDIY	WCD-ALE	ESLAEEGLI ESLAEEGVI ESLPEEGVI	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609	HLERVKKDI LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI	E-AKEANI ANLKYLN ANLTYLE ENLKYLS ANLKYLK	LSFFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF	ELPASLVSI SLSMSW GLPTSLASI ELPSSLASI ELPTSLASI ELPSSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHI NALKSLEIE	GCD-ALE WCD-ALE YCD-TLE LEWNAVI	IRRSSASP	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588	HLERVKKDI LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI	E-AKEANI ANLKYLN ANLTYLE ENLKYLS ANLKYLK	LSFFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF	ELPASLVSI (SLSMSW (GLPTSLASI ELPSSLASI ELPTSLASI ELPSSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHI NALKSLEIE	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI	IRRSSASP	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590	HLERVKKDI LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMIKSI	E-AKEANI LANLKYLNI LANLTYLE LENLKYLSU	LSFFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF	ELPASLVSI ISLSMSW GLPTSLASI ELPSSLASI ELPTSLASI ELPTSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHI NALKSLEIE	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI	SLAEEGL SLAEEGL SLPEEGVI IRRSSASP(SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI	TE-AKEANI ANLKYLNI ANLTYLE ENLKYLSV ANLKYLK	LSFFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF	ELPASLVSI ISLSMSW GLPTSLASI ELPSSLASI ELPTSLASI	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI	IRRSSASP	EGLTSLTEL DGLTSLTEL KGLTSLTQ QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590	HLERVKKD LPEEMFKS1 LPEEMFKS1 LPEEMLKS1	ISO	LSFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF	ELPASLVSI SLSMSW	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI	IRRSSASP ISLAEEGU IRRSSASP IRRSSASP	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LEEMFKSI	ISO	LSFFKNLF LSGKMNLF ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF 440	ELPASLVSI SLSMSW SLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI 450 	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE 460	GCD-ALE WCD-ALE YCD-TLE LEWNAVI	IRRSSASP SSLAPEGU IRRSSASP SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 40000305 400007608 400007609 400029588 400029588 400029590	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI 	TE-AKEANI LANLKYLNI LANLTYLE LENLKYLS LANLKYLKI LANLKYLKI SLOHLTALI	LSFKNLF LSGKMNLF ISFFKNLF ISVFYNLF ISGLCNLF ISGLCNLF 440 	ELPASLVSI SISMSW GLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI PTLAKREE	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE 460 . GIGEDWYKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE	ITTO	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590 Rpibt1-FJ18841 Rpist01-EU884421	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI EMLQCLPEC NMLKCLPEC	TE-AKEANI LANLKYLNI LANLTYLE ENLKYLS LANLKYLKI LANLKYLKI LANLKYLKI SLQHLTLI SLQHLTLI	LSGKMNLE ISGKMNLE ISFFKNLF ISVFYNLE VSYLENLF ISGLCNLF 440 	ELPASLVSI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI 450 1	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE 460 GIGEDWYKI GIGEDWYKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE SCY-ELE AHIPRVI SHIPNVI	SLAEEGLI SLAEEGLI SLEEEGVI IRRSSASP(EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400007609 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI LPEEMLQLPE NMLKCLPE(NMLKCLPE(re-akeani Lanlkylni Lanlkylni Lenlkylsv Lanlky	LSGRMNLH LSGRMNLH ISVFYNLH USVFYNLH ISGLCNLH ISGLCNLH ISGLCNLH USVEPC TSLKIRGC TSLKIRGC	ELPASLVSI [SLSMSW [GLPTSLASI ELPSSLASI ELPSSLASI ELPSSLASI CONTRACTOR PTLAKRCEK PQLIKRCEK PQLIKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE 460 GIGEDWYKI GIGEDWHKI GIGEDWHKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI	SLAEEGLI SSLAEEGLI SSLPEEGVI IRRSSASP(SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 4000029588 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpigene-AY4261 Rpigene-EU884422	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI HPEEMLKSI HLECLPEC NMLKCLPEC NMLKCLPEC	TE-AKEANI LANLKYLNI JANLKYLNI LENLKYLSV LANLKYLSV LANLKYLK SLQHLTLI SLQHLTLI SLQHLTTLI SLQHLTTLI	SFFKNLE LSGRMNLF ISFFKNLE ISGLCNLF ISGLCNLF 440 ISGLCNLF ISLKIRG ISLKIRG ISLKIRG	ELPASLVSI SLSMSW GLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI PTLAKREK PULIKREK PQLIKREK PQLIKREK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHI NALKSLEIE GIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI	SLAEEGLI SSLAEEGVI IRRSSASP SS SS SS SS SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 4000029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpipta1 gene-EU884422 400009324	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LEEMFKSI LPEEMLKSI EMLQCLPE(NMLKCLPE(NMLKCLPE(KMLECLPE(TE-AKEANI ANLKYLNI ANLKYLNI ENLKYLSI ENLKYLSI ISO ISO ISO IQHLTALI SLQHLTTLI SLQHLTTLI SLQHLTTLI SLQHLTTLI SLQHLTTLI	LSGRMNLH LSGRMNLH LSGRMNLH ISVFYNLH VSYLENLH ISGLCNLH LSGLCNLH TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC	ELPASLVSI SLSMSW GLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI 	TALKREEII NALKHLRIÇ NALKRLDIY NNLTFIKHL A60 	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI	SLAEEGLI SSLAEEGVI IRRSSASP 55	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 4000029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpipt1 gene-EU884422 400009324 400019804	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LEEMFKSI LPEEMLKSI MLKCLPE NMLKCLPE KMLECLPE EMIKCLPE EMIKCLPE	TE-AKEANI LANLKYLNI LANLTYTEI LENLKYLSU LENLKYLSU LANLKYLKI SLOHLTTLI SLOHLTTLI SLOHLTTLI SLOHLTTLI SLOHLTTLI	LSCRMNL LSCRMNL ISFFKNLF ISVFYNLF VSYLENLF ISGLCNLF ISGLCNLF ISGLCNLF TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG	ELPASLVSI ELPSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI 450 PTLAKRCEK PQLIKRCEK PQLIKRCEK PEVEKRCDK PELEKLYEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NNLTFIKHL GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI RIGEDWIKI RIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI AHIPNLS ARITNLI	SLAEEGLI SLAEEGLI SLAEEGVI IRRSSASP 55 571Y VIYIN- VIYIN- VIYIN- VIY 51Y DIY	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC
400030855 400017060 400017062 400018050 401009305 400007608 4000029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpiptal gene-EU884422 400009324 400019804 400030855	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI MLKCLPE NMLKCLPE MMLKCLPE EMIKCLPE	TE-AKEANI LANLKYLNI LANLKYLNI LENLKYLSU LANLKYLSU LANLKYLSU LANLKYLSU LANLKYLSU LANLKYLSU SLOHLTTLI SLOHLTTLI SLOHLTTLI SLOHLTTLI	LSGRMNLF LSGRMNLF ISGFRNLF ISVFYNLF ISGLCNLF ISG	ELPASLVSI [SLSMSW [GLPTSLASI ELPSSLASI ELPSSLASI ELPSSLASI PTLAKRCEK PQLIKRCEK PQLIKRCEK PPLEKLYEK	TALKREEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE 460 . GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI RIGEDWIKI RIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI	SLAEEGLI SLAEEGLI SLPEEGVI IRRSSASP SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpigta1 gene-EU884422 400009324 400019804 400030855 400017060	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LEEMFKSI MLCLPEC NMLKCLPEC NMLKCLPEC KMLECLPEC EMIKCLPEC EMIKCLPEC	TE-AKEANI LANLKYLNI LANLKYLSI LENLKYLSI LANLKYLSI LANLKYLSI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI	LSGRMNL LSGRMNL ISFFKNL ISVFYNL ISGLCN	ELPASLVSI GLPTSLASI ELPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI PULKRCEK PQLIKRCEK PQLIKRCEK PQLIKRCEK PQLIKRCEK PELEKLYEK PELEKLYEK	TALKREEII NALKHLRIQ NALKRLDIY NNLTFIKHI NALKSLEIE GIGEDWIKI GIGEDWIKI GIGEDWIKI RIGEDWIKI RIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE SCY-ELE SHIPNVN SHIPNVN SHIPNVN AHIPNLS ARITNLI AHIPNVN	SLAEEGLI SSLAEEGVI IRRSSASP SS SS SS SS SS SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpipta1 gene-EU884422 400009324 400019804 400019804 400017060 400017062	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI MLKCLPE NMLKCLPE KMLECLPE EMLKCLPE KLLKCLPE	TE-AKEANI ANLKYLNI ANLKYLNI ENLKYLSU ENLKYLSU ANLKYLKI SLQHLTALI SLQHLTTLI SLQHLTTLI SLQHLTTLI SLQHLTALI SLQHLTALI SLQHLTALI	SFFKNLF LSGRMNLF SFFKNLF ISVFYNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC TSLTTGC	ELPASLVSI SLSMSW SLSMSW ELPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI ELPSSLASI PTLAKRCEK PQLIKRCEK PQLIKRCEK PUVEKRCEK PUVEKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI SHIPNVI ARITNLI ARITNLI ARITNLI	SLAEEGLI SSLAEEGVI IRRSSASP SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpigene-AY4261 Rpiptal gene-EU884422 400009324 400019804 400030855 400017060 400017062 400018050	HLERVKKD LPEEMFKSI LPEEMFKSI LPEEMFKSI LPEEMLKSI LPEEMLKSI MLKCLPE KMLKCLPE KMLKCLPE KILKCLPE	TE-AKEANI LANLKYLNI LANLKYLNI LENLKYLSU LANLKYLKI SLQHLTALI SLQHLTALI SLQHLTTLI SLQHLTALI SLQHLTALI	LSGENNLE LSGENNLE LSGENNLE ISVFYNLE USVFYNLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE USVLENLE	ELPASLVSI SILSMSW SILPTSLASI ELPTSLASI ELPTSLASI ELPSLASI ELPSLASI PTLAKREK PQLIKREK PQLIKREK PEVEKRCEK PIVEKRCEK PIVEKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE GIGEDWKKI GIGEDWHKI GIGEDWHKI GIGEDWHKI GIGEDWHKI GIGEDWHKI GIGEDWHKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SCY-ELE SHIPNVN SHIPNVN SHIPNVN SHIPNVN AHIPNLS ARITNLI AHIPNVI	SLAPEGLI SLAPEGLI SLPEEGVI IRRSSASP SS SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpipta1 gene-EU884422 400019804 400030855 400017060 400017062 400018050 401009305	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LPEEMLKSI LPEEMLKSI MLKCLPE NMLKCLPE EMIKCLPE EMIKCLPE KILKCLPE FRGFCLPD	TE-AKEANI LANLKYLNI LANLKYLNI LENLKYLSU LENLKYLSU LANLKYLSU LANLKYLSU LANLKYLSU LANLKYLSU SLOHLTALI SLOHLTALI SLOHLTALI SLOHLTALI SLOHLTALI	LSGRMNL LSGRMNL LSGRMNL ISVFYNL USVLENL USVLENL ISGLCNLF USVLENL TNLSVEFO TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG TSLKIRG	ELPASLVSI ELPSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI PDLIKRCEK PQLIKRCEK PQLIKRCEK PELEKLYEK PIVEKRCEK PIVEKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NNLTFIKHL GIGEDWIKI GIGEDWIKI GIGEDWIKI RIGEDWIKI RIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI AHIPNLS ARITNLI	SLAEEGLI SLAEEGLI SLAEEGVI IRRSSASP SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpigta1 gene-EU884422 400009324 400019804 400030855 400017060 400017060 400017062 400007608	HLERVKKD LPEEMFKSI LPEEMFKSI LLEEMFKSI LEEMFKSI LEEMFKSI MLCLPE NMLKCLPE NMLKCLPE KMLECLPE KMLKCLPE KILKCLPE FRGFCLPD	TE-AKEANI ANLKYLNI ANLKYLSI ENLKYLSI ENLKYLSI LANLKYLSI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI SLQHLTALI	SFFRNLE LSGRMNLF ISFRNLF ISVFYNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG ISLKIRG INFGVTG ISLKIRG INFGVTG ISLKIRG INFGVTG ISLKIRG INFGVTG ISLKIRG INFGVTG ISLKIRG INFGVTG ISLKIRG ISS ISS ISS ISS ISS ISS ISS ISS ISS IS	ELPASLVSI SLSMSW GLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI PTLAKREEK PQLIKREEK PQLIKREEK PQLIKREEK PELEKLYEK PIVEKREEK PIVEKREEK	TALKREEII NALKHLRIÇ NALKRLDIY NNLTFIKHI NALKSLEIE GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI AHIPNLS ARITNLI AHIPNVI SHIPNVI	2	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpipta1 gene-EU884422 400009324 400019804 400019804 400017060 400017062 400018050 400007608 400007609	HLERVKKD LPEEMFKSI LPEEMFKSI LPEEMFKSI LPEEMIKSI EMLQCLPEC NMLKCLPEC NMLKCLPEC NMLKCLPEC EMLKCLPEC EMLKCLPEC FRGFCLPD	TE-AKEANI ANLKYLNI ANLKYLSI ENLKYLSI ENLKYLSI ENLKYLSI SLQHLTALI SLQHLTTLI SLQHLTTLI SLQHLTTLI SLQHLTTLI SLQHLTALI SLQHLTALI SLQHLTALI	SFFKNLF LSGRMNLF SFFKNLF ISVFYNLF ISVFYNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF ISGLCNLF TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC TSLKIRGC	ELPASLVSI SLSMSW GLPTSLASI ELPTSLASI ELPTSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI ELPSSLASI PTLAKRCEK PQLIKRCEK PQLIKRCEK PQLIKRCEK PEVEKRCEK PELEKLYEK PIVEKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL NALKSLEIE GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SHIPNVI SHIPNVI SHIPNVI SHIPNVI ARITNLI ARITNLI ARITNLI	SLAEEGLI SSLAEEGVI IRRSSASP SS	EGLTSLTEL DGLTSLTEL KGLTSLTQL QTTPQFLKS	SVEYC FVEHC SLHYC LTISG
400030855 400017060 400017062 400018050 401009305 400007608 400029588 400029590 Rpibt1-FJ18841 Rpisto1-EU884421 Rpigene-AY4261 Rpiptal gene-EU884422 400019804 400019804 400017060 400017062 400017062 400017062 400017063 400007608	HLERVKKD LPEEMFKSI LPEEMFKSI LPEEMFKSI LPEEMLKSI LPEEMLKSI MLKCLPE NMLKCLPE MLKCLPE EMLKCLPE EMLKCLPE EMLKCLPE	TE-AKEANI LANLKYLNI LANLKYLNI LENLKYLSI LENLKYLSI LANLKYLKI SLQHLTALI SLQHLTALI SLQHLTTLI SLQHLTTLI SLQHLTALI SLQHLTALI SLQHLTALI	SFFKNLF LSGRMNLF LSGRMNLF ISFFKNLF ISVFYNLF ISGLCNLF ISGL	ELPASLVSI SLSMSW GLPTSLASI ELPSLASI ELPSLASI ELPSLASI ELPSLASI PLLAKREK PQLIKREK PQLIKREK PEVEKRCDR PEVEKRCDR PEVEKRCDR PEVEKRCEK PUVEKRCEK	TALKRLEII NALKHLRIÇ NALKRLDIY NNLTFIKHL A60 GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI GIGEDWIKI	GCD-ALE WCD-ALE YCD-TLE DLEWNAVI SCY-ELE SCY-ELE SHIPNV SHIPNV SHIPNV SHIPNVS SHIPNVS SHIPNVS SHIPNVS SHIPNVS SHIPNVS SHIPNVS SHIPNVS	SLAPEGLI SLAPEGLI SLPEEGVI IRRSSASP SS SS	EGLTSLTEL DGLTSLTUE KGLTSLTQL QTTPQFLKS	SVEYC

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RpiB1b2-DQ122125	FGEETNLILRKL	TSGPADLDVIS	SIIGMPGLGK	TTLAYKVYN <mark>E</mark>	KSVSSHFDLR	WCTVDQVYDE	KKLLDKI
400023253					HFDIC	WCTVRQGYDE	KKMLDKI
400020736					-SVSTHFDLC	WCTVDQEYDE	NNLLNKI
400025511	FEEETNWIIRKL	TSGPADLDVIS	SIT <mark>GMPGSGK</mark>	TTLAYKVYN <mark>E</mark>	KSVSSHFDLR	WCTVGQEYDE	KKLLHKI
400020749	FEEETNLILREL	T <mark>RGPAELDVIS</mark>	SIT <mark>GMPGSGK</mark>	TTLAFKVYN <mark>E</mark>	KSISCHFDLR	WCTVDQEYDE	KKERGIL
400010895						CTVDQEYDE	KNLLNKI
400020741	FEEETKWILRKL	TGGPT DLDVI S	SIT <mark>GMPGSGK</mark>	TTLAYKVYN <mark>E</mark>	KSISSHFDLR	WCTVDQEYEE	KNLLDKI
400020732	FEEETKWIVRKL	TGGPT DLDVI S	SIT <mark>GMPGSGK</mark>	TTLAYKVYNN	KSVSSHFDLR	WCTVDQEYDE	KKLLDKI
400021987							
400004561							
	80	90	100	110	120	130	140
RpiB1b2-DQ122125	FNQVSDSNSKLS	ENIDVADKLR	QLFGKRYLI	VLDD-VWDTN	TWDELTRPFPI	GMKGSRIIL1	TREKKVA
400023253	FNQVHDKDLKLS	EIINVADKLQF	QLY-KRYLI	VLDD-MWDTT	TW <mark>DKLTRPFP</mark>	KV <mark>EKGCR</mark> IILI	TQEKEVA
400020736	LNQVNGS D SKSS	ENIDVADKLRF	QL <mark>YGKRY</mark> LI	XXXDDVWDTT	TWDELTRPFPI	VGTKGSRIIL1	TRKKDVA
400025511	FNQVSDLDSKLS	ENIDVADMLRF	QL <mark>FGKRY</mark> LI	V-IDDVWDTI	TWVDLTRPFP	rv <mark>ekg</mark> sriili	TRELEVA
400020749	LSMMCG	ILL F	RGMSQDLFLM	VKX		SRIILI	TREKEVA
400010895	FNQVNGS D SKLI	KNI DVVDKLRF	QL <mark>YGKRY</mark> LI	VLDD-VWDTI	TWDELTRPFP	EVEKGSRIILI	TREKEVA
400020741	FNQVNGSDSNLI	ENIDVADKLRF	QLF <mark>GKRY</mark> LI	IL <mark>DD</mark> -VWDTI	TWDALTRHFP1	V <mark>GMKG</mark> SRIIL1	TREKEVA
400020732	FNQVSDSDSTLS	ENIDVADKLRF	QL <mark>FGKRY</mark> LI	VLDD-VWDTT	TWGELTRPFP1	EVKKG SRIILI	TREKEVA
400021987							
400004561							
	150	160	170	190	190	200	21.0
						200	
BpiB1b2-D0122125	I.HGKI.YTDPI.NI.	RLIRSEESWEI	T.EKRAFGNE	SCPDELLDVG	KETAENCKGL		AGREKKK
400023253	LHGKLYTDPINL	RLLRSERSLEI	FEKRGFGKE	SCPDELLDVG	KEIAONCKGLI	LVVDLIAGV	ARKEK
400020736	LHGKVYTAPLNL	RLLRSEESWEI	LEKRAFGNE	SCPNELLDVG	KEIAENCKGLI	LVADLIAGVI	AGREKKR
400025511	LHGKRTTDPLNL	RLLRPEESWEI	FEKRAFGKE	SCPDELLNVG	KELAQNCKGL	LVADLIAGV	ARKEKKK
400020749	LHGKRYTDPLEL	RLLRSEESWEI	LEKRAFGNE	SCPDELLDVG	KE IAENCKGL	LVADLIAGVI	AGREKKK
400010895	LHGMIYTDPLNL	RLLRSEESWEI	LEKRAFGNE	SCPDELLDVG	KEIAENCKGL	LVV <mark>D</mark> LIAGVI	AGRENKK
400020741	LYGKLYTDPLNL	RLLRSEESWEI	LEKRAFGNE	SCPNELLDIG	KE IAENCKGLI	LVV <mark>D</mark> LIAGVI	VVR <mark>EKKK</mark>
400020732	LHGKLYTAPLNL	RLVRSEESWEI	LEKRAFGNE	SCPDELLDVG	KE IAENCKGHI	WVV <mark>D</mark> LISGVI	AGREKKK
400021987					K GL1	LVADLIAGVV	SGREKKK
400004561					K GLl	PLVADLIAGVV	'S <mark>GREKKK</mark>
	220	220	240	250	260	270	290
	1 1	230	240	250	200	270	280
BpiB1b2-D0122125	SVWLEVVNNLHS	FTLKNEVEVM	VTETSYDHU		FASAPKDWVT	PTHELKI,TWGF	EGEVEKT
400023253							
400020736	TAWLEVONNLSS	FILNSEVEVM	VIELSYDHL	PHOLKPCFLY	LASCRKDNAM	IISVL <mark>K</mark> SAWSZ	EGLVEOT
400025511	TVWLEVRNNLSS	FTLNSEVEVM	VTELSYDHT	PNHLKPCFLY	TARFPKDSPM	TT.AT.KDFWRA	EGLVEOT
400020749	TVWLEVONNLSS	FILNSEVEVKE	VIELSYDHL	PHHIKPCFLY	LXKXFRR	LHOYMOMVYC	M
400010895	SVWLEVVNNLHS	FILKNEVEVM	VIEISYDHL	PDHLKPCLLY	FASRPKDKEL	TYELKCVWG	EGEVEKT
400020741	SVWLEVVNNLHS	FIFKNEVTF	VIEISYDHL	PDHLKPCLLC	FASWPKDSAM	CIDELRDVWVZ	EGEVEKT
400020732	TVWLEVONNLSS	FILNSEVEVM	VIELSYGHL	PHHIKPCLLS	LASSPKDTTIS	SILNFKVLSS	EGFMEKT
400021987	TVWLEVRNNLSS	FILNSOVEVM	VIELSYDHL	PHHLKPCFLY	LASFPKDTAI	ISSTLKDFWRA	EGLVEOA
400004561	TVWLEVRNNLSS	FILNSQVEVM	VIELSYDHL	PHHLKPCFLY	LASFPKDTAI	ISSTLKDFWRA	EGLVEQA

Protein sequence alignment of the NBS region of *Rpi-blb2* homologs

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RpiB1b2-DQ122125	DM
400023253	
400020736	EM
400025511	EM
400020749	
400010895	EM
400020741	EI
400020732	EM
400021987	EM
400004561	EM

Protein	sequence align	ment of t	he LKK r	egion of <i>R</i>	<i>pi-blb2</i> he	omologs	
	10	20	30	40	50	60	70
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Rpib1b2-DQ122125	LRTLHLESSFIMVKD	SLLN <mark>EICML</mark> N	I <mark>HLRY</mark> LSIGT	EVKSLPLSFSN	ILWNL <mark>E</mark> ILFVD	NKESTLILLP.	RIWD L
400023253	AED	TNLE	NLRYLG-	NLMLSYS-			
400025511	DPSFIKVKD	SLLN <mark>EICML</mark> N	IHLRFLCIGT	EVKSLPTSLSN	ILWNL <mark>E</mark> TLLVS	NIGSTLVLLP.	RIWDL
400010895	SFIMVKD	TLLN <mark>EICML</mark> N	IHLRYLRIGT	Q VK SLPLS F SN	ILWNL <mark>EFLRVD</mark>	NKESTLILLP.	RIWD L
400020741	SFIKVKD	SLL <mark>HEICML</mark> N	IHLRFLCIGT	EVESLPLSFSN	ILWNL <mark>E</mark> TLLVI	NEELMLLP	RIWD L
400021987	LRVLYLHPSFIMVND	SLLN <mark>EICML</mark> N	IRLRLLRIAT	KVKYLPLSFSN	ILWNL <mark>E</mark> TLLL <mark>D</mark>	NEGSTLVLLP.	RIWDL
400020732	DPSFIKVKD	SLLN <mark>EICML</mark> N	IHLRFLCIGT	EVKSLPTSLSN	ILWNL <mark>E</mark> TLLVS	NIGSTLVLLP.	RIWDL
400004561	LRVLYLHPSFIMVND	SLLN <mark>EICML</mark> N	RLRLLRIAT	KVKYLPLSFSN	ILWNLETLLLD	NEGSTLVLLP.	RIWDL
	80	90	100	110	120	130	140
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Rpib1b2-DQ122125	VKLQVLFTTACSFFD	MDADESILIA	EDTKLENLT	ALGELVLSYWR	DTEDIFKRLP	NLQVL <mark>HFKLK</mark>	ESWDY
400023253				R	CDTDHIFKRF P	NLHELVFDIM	ESWDY
400025511	VKLRVLFMSACSFFD	LNSDEPILIA	EDTKLENLR	QLENLELSYSK	<i>DAEDIFKRFP</i>	NLQGLAFRLK	ESWDY
400010895	VKLRLVAMNACSFFD	MDADESILIA	EDTKLEKLT	LL <mark>G</mark> QLLLS Y S B	DTEDIFKRFP	NLQHLTFELK	ESWDY
400020741	VKLRVLITTGCSFFD.	MDA			EHIFERF P	NIQ D LSFVLK	ESWDY
400021987	VKLRVLSVSACSFFD	LYADESILIA	EDTKLEKLR	ML <mark>GQLMLSYS</mark> K	CDTEDIFKRL P	NLQHLGF D LK	ESWDY
400020732	VKLRVLFMSACSFFD	LNS DE PILIA	EDTKLENLR	QLENLELSYSK	CDAEDIFKRF P	NLQGLAFRLK	ESWDY
400004561	VKLRVLSVSACSFFD	LYADESILIA	EDTKLEKLR	ML <mark>GQLMLSYS</mark> K	DTEDIFKRL P	NLQHLGFDLK	ESWDY
	150	160	170	180	190	200	210
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Rpiblb2-DQ122125	ST E QYWFPKLDFLTE	LEKLTVDFEF	SNTNDSGSS.	AAIN R PW DFH F	PSSL KR LQL <mark>H</mark>	EFPLTSDSLS	TIARL
400023253	STGQFWSPKLDFLTE	L K NLTVS FK S	SS				
400025511	ST EGYWFPKLDFLTE	LEDLRIVFES	SSNTN D SGPS	VATN R PW DFH F	PASLKSLWLG	VFPLSSDSLS	IIARL
400010895	ST EHYWFPKLDFLTE	L <mark>EDLK</mark> VSFAS	SSNTN D SGSS	AAIN R PW DFH F	PSSLKRLWLN	EFPMTSDSLS	TIARL
400020741	ST E QYWFPKLDFLTE	LEGLNVGFEF	SNTNDSGSS.	AAIN R PW DFH F	PSSL KK LSLG	DFHLASDSLS	TIARL
400021987	ST E QFWFPKLDSLTE	LEGLTVGFEF	SNTNDSGSS.	AAIN R PW DFH F	PSSLKRLWLN	EFPLTSDSLS	IIARL
400020732	ST EGYWFPKLDFLTE	LEDLRIVFES	SSNTN D SGPS	VATN R PW DFH F	PASLKSLWLG	VFPLSSDSLS	IIARL
400004561	STEQFWFPKLDSLTE	LEGLTVGFEF	SNTN D SGSS	AAIN R PW D FHF	PSSL KR LWLN	EFPLTSDSLS	IIARL
	220	230	240	250	260	270	280
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Rpib1b2-DQ122125	LNLEELYLYRTIIHG.	EEWNMGEEDI	FEN		LEKL	ELSDCHNLEE	IPSSF
400023253							
400025511	PNLENLTLKNTIIQG	EEWNMGEED	FENLKFLEL	DEVALAKWEVG	EESFAVLEKL	VLWRCRKLDE	IPPSF
400010895	PNLEELFLYETIIHG	EEWNMGEEDI	FENLKYLKL	YQVTLSKWEVG	EESFPSLEKL	KLEGCRKLEE	IPPSF
400020741	PNLEVLSLYDTIIEG.	EERNMEEED	FENLKCLKL	RQVTLSKWEVG	EESFPALEKL	KLEGCRKLEE	IPPSF
400021987	PNLEELTLYRTIIHG.	EEWSMGEED	FENLKCLKL	NQVTLSKWEVG	EESFPTLEKL	KLSGCRDLEE	IPSSF
400020732	PNLENLTLKNTIIQG	EEWNMGEEDI	FENLKFLEL	DEVALAKWEV@	EESFAVLEKL	VLWRCRKLDE	IPPSF
400004561	PNLEELTLYRTIIHG	EEWSMGEEDI	FENLKCLKL	NQVTLS KWE VG	EESFPTLEKL	KLSGCRDLEE	IPSSF
	290	300	310	320			
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Rpiblb2-DQ122125	GDIYSLKIIELVRSP	QL <mark>E</mark> NSALKIK	KEYAEDMR GG	DELQILGQKD1	IPLF K		
400023253							
400025511	GDIYSLKIIKVDYNR	HLKDSAMMIF	<i>COVVEDMTGE</i>	DKLQV			
400010895	GDIYSLKIIKLVKSP	QL ED SALKIF	EYAEDLR GG	DELQILGQKN1	IPL FK		
400020741	GDIWSLKIIKLVE SP	QL ED STMKIF	EYAEDMR GG	DELQVVGRKN1	P		
400021987	GDIYSLKIIKLVR TP	QL ED SALKIF	EYAEDMR GG	DELQVVGWKN1	PLLK		
400020732	GDIYSLKIIKVDYNR	HLKDSAMMIF	<i>QYVEDMTGE</i>	DKLQV			
400004561	GDIYSLKIIKLVRTP	QL <mark>ED</mark> SALKIF	KEYAEDMR GG	DELQVVGWKN1	PLL <mark>K</mark>		

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Protein seau	ience alignn	ient of t	he NBS re	egion of <i>R</i>	vi-vnt1	homologs
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Rpivnt1-FJ423044	LEDDENTLOAKLLD	HDLPYGVVSIV	GMPGLGKTT	LAKKLYRHVC	HOFECSGLVYV	SOOP RAGEIL E	IDIAK
Rpivnt1-FJ423045	LEDDENTLOAKLLD	HDLPYGVVSIV	GMPGLGK TT	LAKKLYRHVC	HOFECSGLVYV	SOOP RAGEIL	IDIAK
Rpivnt1-FJ423046	LEDDENTLOAKLLD	HDLPYGVVSIV	GMPGLGK TT	LAKKLYRHVC	HOFECSGLVYV	SOOP RAGEIL	IDIAK
Rpivnt1-745-1	LDDDFKMLOAKLLD	DLPYGVVSIV	MPGLGK TT	LAKKLYRHVR	OFECSGLVYV	SOOPRAGEIL	IDIAK
400024363		HDLPYGVVSTV	MPGLGKTT	T.AKKT.YROVR	DOFECSGLVYV	SOOPRAGETLE	IDTAK
401020585	LDDDFNMLKAKLLD	HDLPYGVVSIV	GMPGLGKTT	LAKKLYROVR	OFECSGLVYV	SOOPRAGEILE	IDIAK
400020584							TAK
400020587			MPGLGKTT	T.AKKT.VRHVR	OFFCSALVYV	SOOPRACETLI	
403020585							
	80	90	100	110	120	130	140
Bpivn+1-F.T423044	OVGLTEEERKENLE	NNT.RST.T.KTKR				SRTTTTSRNS	IVGRY
Rpivnt1-F.T423045	OVGLTEEERKENLE	NNT.RST.T.KTKR	VTT.T.DDTW		VIPECDSKIG	SRITITSRNSN	IVGRY
$R_{pi} = 1 - F_{1} + 23046$	OVGLTEEERKENLE	NNT.RST.T.KTKR	VTT.T.DDTW		VIPECDSKIG	SRITITSRNSN	IVGRY
$R_{pivne1} = 745 - 1$	OVGLMEVERKENLE	CNLRSLLKTKR	VTT.T.DDTW		VIPECDSKIG	SRITITSRNSN	IVGRY
400024363	OVGLMEEERKENLE	NNT.RST.T.KTKR	VTT.T.DDTW		VIPECDSKIG	SRITTTSRNSN	IVGRY
401020585	OVGLMEEERKENLE	NNT.RST.T.KTKR	VTT.T.DDTW		VIPECDSKIG	SRITITSRNSN	IVGRY
400020584	OVGLTEEERKENLE	NNT.RST.T.KRKR	VTT.T.DDTW	DVETWNYTKI	VIPEVDSKIG	SRITITSRNSN	IVGRY
400020587	OVGLTEEERKEHLE	DNLRSLLETKR	VTT.T.DDTW	DTKTWDALNR	VI.RPECDSKIG	SRTTTTSRYDE	IVGRY
403020585	2	T.KTKR	VTT.T.DDTW		VF-PECDSKIG	SRITTTSRNSM	IVVRY
	150	160	170	180	190	200	210
			I	1			
Rpivnt1-FJ423044	IGGDFSIHVLOPLD	S EKSFELFTKK	EFNFVNDN-	WANASPDLVN	IGRCIVERCGG	IPLAIVVTAGN	ILRAR
Rpivnt1-FJ423045	IGGDFSIHVLOPLD	SEKSFELFTKK	TENEVNDN-	WANASPDLVN	GRCIVERCGG	IPLAIVVTAG	ILRAR
Rpivnt1-FJ423046	IGGDFSIHVLOPLD	S EKSFELFTKK	TENEVNDN-	WANASPDLVN	IGRCIVERCGG	IPLAIVVTAGN	11.RAR
Rpivnt1-745-1	IGGDFSIHVLOPLD	SENSFELFTKK	IFTF <mark>D</mark> NNNN	WANASPDLVD	I <mark>GR</mark> SIV <mark>GRC</mark> GG	IPLAIVVTAGN	11.RAR
400024363	IGGDFSIHVLOPLD	SENSFELFTKK	LFTFD NNNN	WANASPDLVD	IGRSIVGRCGG	IPLAIEVTAGN	ILRAR
401020585	IGGDFSIHVLOPLD	SENSFELFTKK	LFTFD NNNN	WANASPDLVD	IGRSIVGRCGG	IPLAIEVTAG	ILRAR
400020584	IGGHSSLCVLOPLD	SDNSFELFSKK	TFNFDNNN-	WANASPDLVN	I <mark>GR</mark> SIV <mark>GRC</mark> GG	IPLAIVVTAGN	11.RAR
400020587	IGEDFSLHELOPLD	S EKSFELFTKK	IFIF <mark>D</mark> NNNN	WVNASPFLVD	I <mark>GK</mark> SIVQ <mark>RC</mark> GG	IPLAIVVTAG	/L <mark>RAR</mark>
403020585	IGGDFSIHVLQPLN	SENSFELFTKK	IFIF <mark>D</mark> NNNN	WANASPELVD	IGRNIVGRCGG	IPLAIEVTAG	1LRAR
	220	230	240	250	260	270	280
Rpivnt1-FJ423044	GRTEHAWNRVLESM	AHKIQDGCGKV	L <mark>ALSYND</mark> LP	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
Rpivnt1-FJ423045	GRTEHAWNRVLESM	AHKIQDGCGKV	L <mark>ALSYND</mark> LP	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
Rpivnt1-FJ423046	GRTEHAWNRVLESM	AHKIQDGCGKVI	LALSYNDLP	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
Rpivnt1-745-1	ERTERAWNRVLDSM	GHKVQDACAKV	LALSYN <mark>D</mark> LS	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
400024363	ERTERAWNRVLESM	GHKIQDGCAKVI	LALSYNDLP	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
401020585	ERTERAWNRVLESM	GHKIQDGCAKVI	LALSYNDLP	IALRPCFLYF(GLYPEDHEIRA	FDLTNMWIAEF	LIVV
400020584	ERTEHAWNRLLESM	SHKVQDGCAKV	LALSYNXFA	HCIKAMFLVL	WPFP <mark>RG</mark> PNS C F	FD	
400020587	ERTEHAWNRVLERI	GHNIQDGCAEV	LSLS <mark>YND</mark> LP	IASRPCFLYF	GLFPEDHEIRA	FDLINMWIAEF	LIVV
403020585	ERTERAWNRVLESM	SHKVQDGCAXG	IGSELQ-FA	NCIKAMFLVF	WPLP <mark>RG</mark> SNS CF	FD	

	• • •
Rpivnt1-FJ423044	NTG
Rpivnt1-FJ423045	NTG
Rpivnt1-FJ423046	NTG
Rpivnt1-745-1	NSG
400024363	N
401020585	N
400020584	
400020587	NS-
403020585	

Protein sequence alignment of the LRR region of *Rpi-vnt1* homologs

Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020587 403020585	10 GKULALSYNDLPIAL GKULALSYNDLPIAL GKULALSYNDLPIAL AKULALSYNDLPIAL AKULALSYNDLPIAL AKULALSYNDLPIAL AEVLSLSYNDLPIAS	20 RPCFLYFGL RPCFLYFGL RPCFLYFGL RPCFLYFGL RPCFLYFGL RPCFLYFGL	30 YPEDHEIRAFI YPEDHEIRAFI YPEDHEIRAFI YPEDHEIRAFI YPEDHEIRAFI	40 DLTNMWIAEKI DLTNMWIAEKI DLTNMWIAEKI DLTNMWIAEKI DLINMWIAEKI	50 LIVVN-TGNGR LIVVN-TGNGR LIVVN-SGNRR LIVVNYSGDRR LIVVNYSGDRR	60 EAESLADDVLI EAESLADDVLI EAESLAEDILI EAESLAEDILI EAESLAEDILI EAESLAEDILI	70 NDLVS NDLVS NDLVS NDLVS NDLVS NDLVS NDLVS
Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020584 400020587	80 RNLIQVAKRTYDGRI RNLIQVAKRTYDGRI RNLIQVAKRTYDGRI RNLIQVVERTYDGRI RNLIQVVERTYDGRI RNLIQVVERTYDGRI RHLIQVAKRTYDGRI	90 SSCRIHDLL SSCRIHDLL SSCRIHDLL SSCRIHDLL SSCRIHDLL SSCRIHDLL SSCRIHDLL	100 HSLCVDLAKES HSLCVDLAKES HSLCVDLAKES HSLCVDLAKES HSLCVDLAKES HSLCVDLAKES	110 INFFHTEHNAN INFFHTEHNAN INFFHTEHNAN INFFHTEHNAN INFFHTEHNAN	120 ?GDPSNVARVR ?GDPSNVARVR ?GDPSNVARVR ?GDPSNVARVR ?GDPSNVARVR	130 RITFYSDDNAI RITFYSDDNAI RITFYSDDNAI RITFYSDNNAI 	140 MNEFF MNEFF MNEFF MNEFF MNEFF
Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020587 403020585	150 HLNPKPMKLRSLFCF HLNPKPMKLRSLFCF HLNPKPMKLRSLFCF RSNTKLEKLRAMG RSNPKPRKLRALFCF	160 	170 MAHLNFKLLQV MAHLNFKLLQV MAHLNFKLLQV MAHLNFKLLQV	180 	190 ?QHVTFPKKIG ?QHVTFPKKIG ?QHVTFPKKIG ?QHVTFPKKIG ?QIVWMLRAR 	200 	210 EGAIR EGAIR EGAIR EGRIR LESMS EGDMY
Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020587	220 VKLPNSIVKLKCLET VKLPNSIVKLKCLET VKLPTSIVKLKCLET HKVQDGCAKVLALS- GKLPNCMVKLKRLET	230 LDIFHSSSK LDIFHSSSK LDIFHSSSK LDIFHSSSK LDIFHSSSK LDIFHSSSK	240 LPFGVWESKII LPFGVWESKII LPFGVWESKII LPFGVWESKKI	250 .RHLCYT .RHLCYT .RHLCYT .RHLCYT	260 	270 ASPFCRIMPPI ASPFCRIMPPI VSPFYRIMPPI VSPFYRIMPPI VFSNVYSLPP	280 NNLQT NNLQT NNLQT NNLQT NNLQT
Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020587 403020585	290 LMWVDDKFCEPRL LMWVDDKFCEPRL LMWVDDKFCEPRL LMWVDDKFCEPRL YNDLFIALRPCF ESNFFHTEHNA ESNFFHTEHNA LMWMYDNFFELRL DDKFFEATL	300 LHRLINLRT LHRLINLRT LHRLINLRT LHRLINLRT LYFGLFPED FGDP SNVAR FGDP SNVAR VHRFVNLRK LHRLMNLRK	310 LCIMDVSGSTI LCIMDVSGSTI LCIMDVSGSTI LCIMDVSGSTI HEIRAFDLTNM VRRITFY-SDN VRRITFY-SDN LGILSGSDSTV LGILGVSDSTV	320 	330 RALEVIKLRF PKALEVIKLRF PKALEVIKLRF PKALEVIKLRF WIKLEKLRALF WIKLEKLRALF ATTLEVIKLYF PTALEVIKLSV	340 FKNTSEQINL FKNTSEQINL FKNTSEQINL FKNTSEQINL AEDFLNDLVS CF FRDTSEQINL SRHMSEQINL	350 SSHPN SSHPN SSHPN RNLIQ TEDPC TEDPC SSYPN SSYPN
Rpivnt1 gene-FJ423044 Rpivnt1.2 gene-FJ423045 Rpivnt1.3 gene-FJ423046 Rpioka1 gene-7451 400024363 401020585 400020584 400020587 403020585	360 IVELGLVG-FSAMLL IVELGLVG-FSAMLL IVELGLFG-FSAMLL VAKRTYDGRISSCRI IFSQL IFSQL IVKLHLNGRMPL IVKLCLINVCGTMRL	370 NIEAFPPNL NIEAFPPNL NIEAFPPNL HDLLHS-LC AHLDFKLLQ NSEAFPPNL NSEAFPPNL	380 VKLNLVGLM VKLNLVGLM VKLNLVGLM VKLNLVGLM VLVVVTSRL VLVVVTSRL VLVVVTSRL VLVVTSRL VKLTLIRLM	390 NDGHLLAVLE NDGHLLAVLE NDGHLLAVLE ITEHNAFGDPS RYQHVITIPN DRYQHVITIPN RYQHVITIPN CNDGHVEAVLE NDGHVEAVLE	400 KKLPKLRILIL KKLPKLRILIL KKLPKLRKLTL SNVARVRRITF IKIGNMSCLRY IKIGNMSCLRY KKLPKLRILKM	410 LWCRHDAEKMI LWCRHDAEKMI LRCRHDAEKMI YSDNNAMNEF VR YRCNHMEEKMI	420 DLSG- DLSG- DLSG- DLSG- FRSN- -LEG- -LEG- DLSGD DLSG-

	430	440	450	460	470	480
Rpivntl gene-FJ423044	-DSFPQLEVLYIE	DAQGLSEVTCI	IDDMSMPKLKK	L F LVQ G PNISF	ISLRVSERL	AKLRISQVL
Rpivnt1.2 gene-FJ423045	-DSFPQLEVLYIE	DAQGLSEVTCI	MDDMSMPKLKK	L FLVQG PNISF	ISLRVSERL	AKLRISQVL
Rpivnt1.3 gene-FJ423046	-DSFPQLEVLYIE	DAQGLSEVTCI	MDDMSMPKLKK	L FLVQG PNISF	ISLRVSERL	AKLRISQVL
Rpiokal gene-7451	-DSFPQLEVLHIE	DAHGLSEVTCI	MDDMSMPKLKK	LLLVQ <mark>G</mark> PIISF	ISLRVSERL	AKLRISQVL
400024363	-PKPTKLRALFCF	N-NNSCLFSHI	AHLSFKLLQVI	LVVVTS <mark>RDRY</mark> Q	HVITIPNKI	<u>GNM</u>
401020585	RIRVK	LPNSIVKLKC				
400020584	RIRVK	LPNSIVKLKC				
400020587	GDSFLQLEVLHIQ	ESNGLYEVTC	RDVVSMPKLKK	LLLI <mark>ER</mark> -TDSN	IVRLSEKL	AKLRI
403020585	GDSFPQLEFLHI N	EPDGLSEVMC	RDDVSMPKLKK	LLLVQ <mark>G-SG</mark> SF	PISLSERL	AKLRIN

Code	Homolog ID	Function	Primer	Size	Tm (°C)	GC	Amp Tm	Amp length
				(qq)	()		()	(dd)
22GH2	1011522	HJRTR2GH1	TCCATTTGCAACCTCAAGAATTTA	24	59	33	78	64
			GGCATAAGCGCCCGTTT	17	58	59		
45B23	25545	R1B-23	CAGTGATCAAATGGCTAACCTTCAG	25	59	45	77	76
			ATCTAGTATTGGCAGATGAATGAGATTG	28	59	40		
Bt609	7609	Rpi-bt1	TTCACACCGCTTTTCCAGTGT	21	59	48	79	63
			GCACCTAACAGCCCTCACAAG	21	58	57	-	
Bo54	24754	Rboh-B	AATGTCGACGCCACTCTTAGC	21	58	52	80	63
2001			GCCCGGTCTGCACTAATCAC	20	59	60		
RbohA	12316	Rboh-A	TCTTCTCCTTATTTTCTACACGGTTTCT	28	59	36	72	53
			CCTTGTGATGGAAAAGAGTTTGTTT	25	59	36		
RbohC	14168	Rboh-C	CCAAGTCCAATGACGATGCTT	21	59	48	79	58
			AGCCACAGAGTCTTCACGAACA	22	59	50		
P10-94	2001494	PR10-protein	CGTTTTAATTACGAGCGCAGTTC	23	59	43	78	65
		·	GCGCAAGAAGGTTTGAAGCT	20	58	50		
235A3	2030235	R3-TC	TCTATTAGAGCTATGCATTTACGAGTGA	28	58	36	76	71
2007.10			TCAAGGACCAAGGAGAATGGA	21	58	48	,0	· -

Appendix 5. Characteristics of qPCR primer pairs of gene homologs selected from the TC database

Code	Homolog ID	g ID Function Primer		Size (bp)	Tm (°C)	GC	Amp Tm (°C)	Amp length (bp)
Pp54	20554	PPR1 protein	TGTCTAAGTTGGGAGGTGATATGC	24	58	46	77	70
ī			TTCGAGCCTCTTCTGAAATCCT	22	58	45		
53Bl2	23253	Rpi-blb2	TTCCATTCTCCCCCCTGAAT	20	59	50	78	64
			GCGAGACTGCCCAACTTGA	19	59	58		
glup78	2004978	Glutathione	GGACATAAAAGTGCTGTTGGGAAT	24	59	42	78	70
		peroxidase	CGAAATCGATCCACATTGTTGA	22	59	41		
P1-74	37874	PR1-protein	GGCTTTCCCCGCACTCA	17	59	65	85	57
			TGCTTCTCATCGACCCACATC	21	52	52		
Ktn67	15267	Kunitz-type	GGGTATCCACAACCAGGCTATC	22	58	55	77	72
κιμον	15207	protease inhib	TGGCAGGCTTTGGTCATAATAA	22	58	41		
Aspi	10120	Aspartic protease	CGGCGTGTTCCGTTACG	17	58	65	04	61
·	10129	inhibitor 10	TCTGCCACCCCTAGGCCTAT	20	60	60	84	91
Serpi	26953	Serine protease	TTTTGGTTGCATGAAACAAGCT	22	58	36	77	56
		inhibitor 1	TCTGTCTCTCATCTCCTTCTTTTCC	25	58	44		
Cyspi	10134	Cysteine protease	CCAAACGCCGTGTTGCA	17	60	59	80	59
		inhibitor 1	GGGTGTGCCTTTTCCCATAA	20	58	50		