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Phylogenetic study of disease-resistant genes *in silico*

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Abstract

Despite singnificant advances in plant disease control strategies, but also a large number of pathogens and pests are bringing a lot of damage. By comparing the amino acid sequences of proteins encoded by the resistance genes in plants with different evolutionary origin has been shown that resistance genes based on structural similarity to the protein products of the NBS-LRR (Nucleotide binding Site-Leucine Rich Repeats), LRR extracellular, LRR-kinase receptors are classified. This study investigate the relationship between protein sequences of gene in various plants, their intraction was determined Bioinformatics software. The protein sequence cluster analysis identified which these genes are located in three groups. *Arabidopsis thaliana* plant containing the resistance gene *RLM*1 located in a seprate group . Evolutionary distance matrix results showed that most distance (0.99) between the plant *Populus trichocarpa* and *Solanum lypersicum* and the minimum distance (0.287) between *Solanum lypersicum* and *Vitis vinifera*. The results of the simulated rank protein that resistance genes are far apart and have not a similar origin. Functional results Rlm1 gene in *Arabidopsis thaliana* revealed that this gene is located on chromosome 1 and united with intercellular or extracellular signals in transmission of signals from a membrane to the other works.

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Introduction

Plants often use a defense mechanism based on the gene-for-gene principle,which means that the product of a resistance (R) gene recognizes a specific avirulence gene product specified by the pathogen (Flor, 1971). A number of these receptor-like R genes have been cloned and characterized. Based on these genes, several classes of R genes can be distinguished. Most R genes identified up till now are members of the cytoplasmic nucleotide- Binding site-leucine- rich repeat (NBS-LRR)- containing R gene family. The NBS region is thought to be important for ATP binding and overall functionality of the R-gene product (Walker *et al.,* 1982, Saraste *et al.*,1990). The LRRs may be the main determinantin recognition of the avirulence gene product(Kobe and Deisenhofer, 1995, Jones and Jones, 1996). The NBS-LRR gene family is divided in two subclasses. One class has an upstream conserved domain that resembles the Toll-interleukin receptor (TIR) domain. This class includes the N gene from tobacco, M and L6 from flax, and RPP5 from *Arabidopsis thaliana* (Whitham *et al.*,1994, Anderson *et al.,* 1997).

Members of the other group (including RPM1 from Arabidopsis, Dm3 from lettuce, Rx1 and Gpa2 from potato, and Prf and Mi from tomato)often contain the consensus for a coiled-coil domain structure at a similar position (Vossen *et al.,* 2000). NBS-containing R genes are numerousin plant genomes 163 in Arabidopsis and over 600 in rice (TAGI, 2000, Goff *et al.,* 2002) and are often organized in clusters. Within these clusters, repeats of similar genes (paralogues) as well as several different genes can be recognized (Michelmore and Meyers, 1998, Young, 2000).

To date, more than 10 major loci (Rlm1-10 and LepR1 to LepR4) conferring resistance to *Leptosphaeria maculans* have been catalogued in *Brassica napus*, *B. rapa spp syvestris, B. juncea, and B. carinata* and most of them were mapped by different molecula markers (Delourme *et al.,* 2008). Blackleg, caused by the fungal pathogen *Leptosphaeria maculans* (Desm.) Ces. et de Not., (anamorph: *Phoma lingam*), is a serious disease that affects both yield and quality of canola (*Brassica napus* L). This disease is highly prevalent in various parts of the world including in Australia and Canada. *L. maculans* is highly variable for pathogenicity which is caused by a number of virulence genes. Utilization of host resistance to blackleg is an effective approach to control yield losses in canola.

In the last 10 to 15 years the computer has become an essential companion for cell and molecular biologists. Bioinformatics is an emerging scientific discipline that uses information technology to organize, analyze, and distribute biological information in order to answer complex biological questions. Bioinformatics has become an essential tool not only for basic research but also for applied research in biotechnology and biomedical sciences (Naghavi *et al.,* 2009). In this study we use an in silico analysis to compare the protein sequences of genes in NBS-LRR group for resistance to disease and to determine their phylogenetic relationships. Rlm1 belong to NBS-LRR group of disease resistance gene and determinate genes similar to Rlm1 and compare them.

Materials and methods

Receive Data

In current study, firstly the Rlm1 gene was received from the TAIR site (http://www.Arabidopsis.org), then performed in site NCBI (http://www.ncbi.nlm.nig.gov/) for BLAST and detected several protein sequences which have resistance gene to disease (Table 1).

Data analysis

For Alignment datas be used Editseq and MegAlign softwares. Then, by MEGA 5 software draw the phylogenetic tree (Fig 1). To assessment of similarity between group sequences the first saved by software editseq then alignment with software Megalign. To measurement aminoacid content be used of site http://www.expasy.org/Tools/ParamProt)

Results and discussions

Cluster analysis

Result of phylogenetic tree (Fig 1) showed that plants species classificated in three groups, group 1 consist of 5 species (*Populus trichocarpa*, *Vitis vinifera*, *Medicago truncatula, Ricinus communis* and *Solanum tuberosum*), group 2 consist of 2 species (*Nicotiana tabacum* and *Solanum lycopersicum*) and group 3 consist of 1 specie (*Arabidopsis thaliana*). The evolutionary matrix was calculated based on Poisson correlation matrix results showed that the maximum distance (0.99) between *Populus trichocarpa* and *Solanum lycopersicum* ,least distance (0.287) between *Solanum lycopersicum* and *Vitis vinifera*.

The result showed that RLM1 gene in Arabidopsis thaliana little related with other disease resistance genes and this relationship needs to be investigated further.

Table 1. Disease Resistance gene in studied Plants.

Accession number	Resistance gene and It's product	Plants	Protein Sequence Length
AT1G64070	TIR-NBS-LRR class disease resistance protein, Rlm1	*Arabidopsis thaliana*	997
ABO21407	TMV resistance protein N, CN	*Nicotiana tabacum*	1141
ABF 81419	TIR-NBS-LRR type disease resistance protein	*Populus trichocarpa*	1121
AAR21295	bacterial spot disease resistance protein 4, Bs4	*Solanum lycopersicum*	1146
AAP44392	nematode resistance-like protein, Gro1-2	*Solanum tuberosum*	1136
XP-002277166	TMV resistance protein N-like	*Vitis vinifera*	1250
XP_003622506	TMV resistance protein N	*Medicago truncatula*	1137
XP_002523481	TMV resistance protein N	*Ricinus communis*	994

*Rlm1= Resistance to *maculans Leptosphaeria*1; Bs4= bacterial spot disease resistance protein 4.

Table 2. Content of Leucine and Serine Amino acids in sequences of disease resistance protein.

Plant / Amino acid	Arabidopsis thaliana	Medicago truncatula	Nicotiana tabaccum	Populus trichocarpa	Ricinus communis	Solanum lycopersicum	Solanum tuberosum	Vitis vinifera
Leucine	12.8%	1 3.7%	12.6%	13.1%	12.0%	12.3%	13.9%	13.3%
Serine	8.4%	9.6%	9.0%	8.7%	8.8%	9.4%	8.4%	10.7%

Table 3. Dissimilarity matrix for disease resistance gene in plant species in this study.

	Arabidopsis thaliana	*Medicago truncatula*	*Nicotiana tabacum*	*Populus trichocarpa*	*Solanum lycopersicum*	*Solanum tuberosum*	*Vitis vinifera*
Arabidopsis thaliana							
Medicago truncatula	0.702						
Nicotiana tabaccum	0.360	0.541					
Populus trichocarpa	0.581	0.756	0.852				
Ricinus communis	0.610	0.522	0.510	0.920			
Solanum lycopersicum	0.713	0.397	0.296	0.999	0.499		
Solanum tuberosum	0.943	0.643	0.909	0.669	0.939	0.885	
Vitis vinifera	0.575	0.393	0.577	0.426	0.287	0.678	0.836

Content of serine and leucine amino acids

According to (Hulbert *et al.,* 2001) amounts of leucine and serine amino acids in proteins because of the LRR domain placed in the protein sequences which create more resistance against pathogens that with the results indicated in table 3 showed that the resistance genes contain sequences similar to the amino acids serine and leucine are used (Table 2).

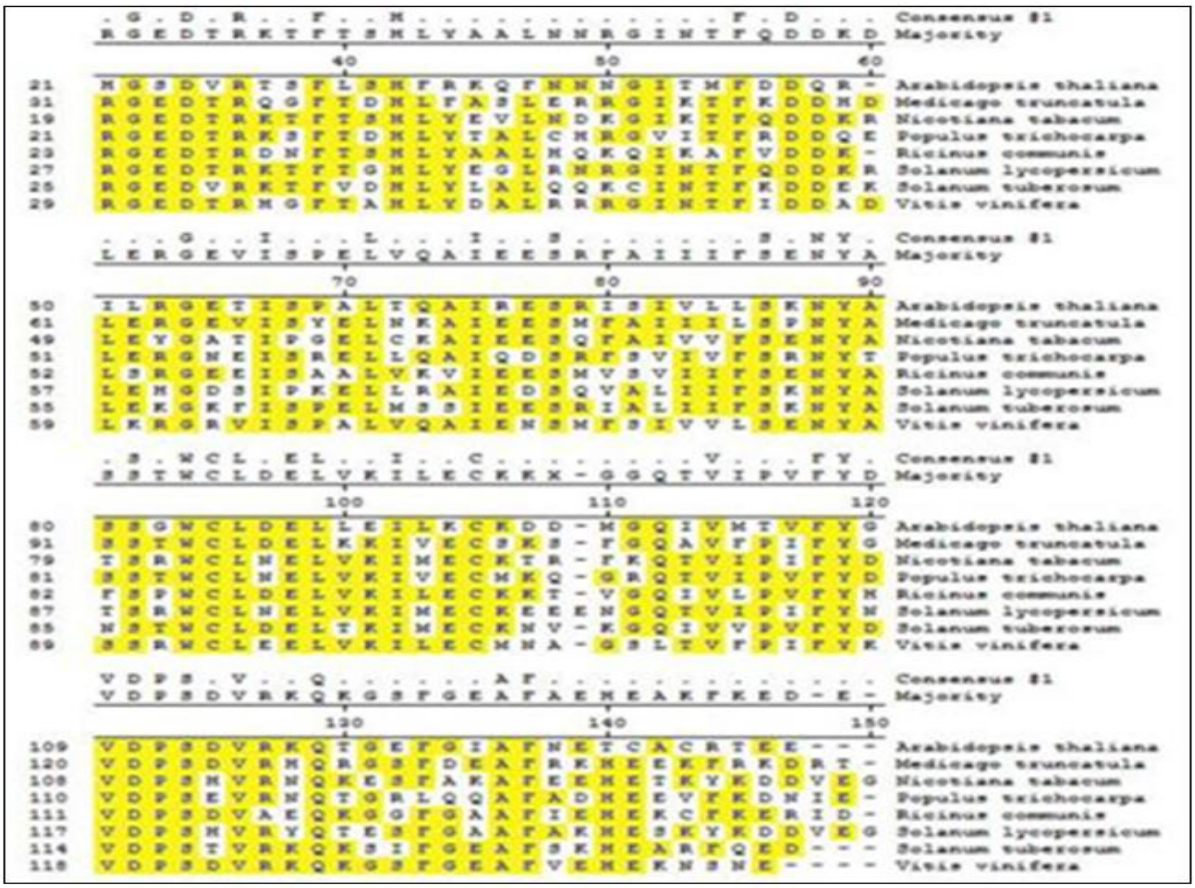

Fig. 1. Result of Alignment the protein sequences in disease resistance. Indicates that there is little similarity among the resistance protein and they are far apart.

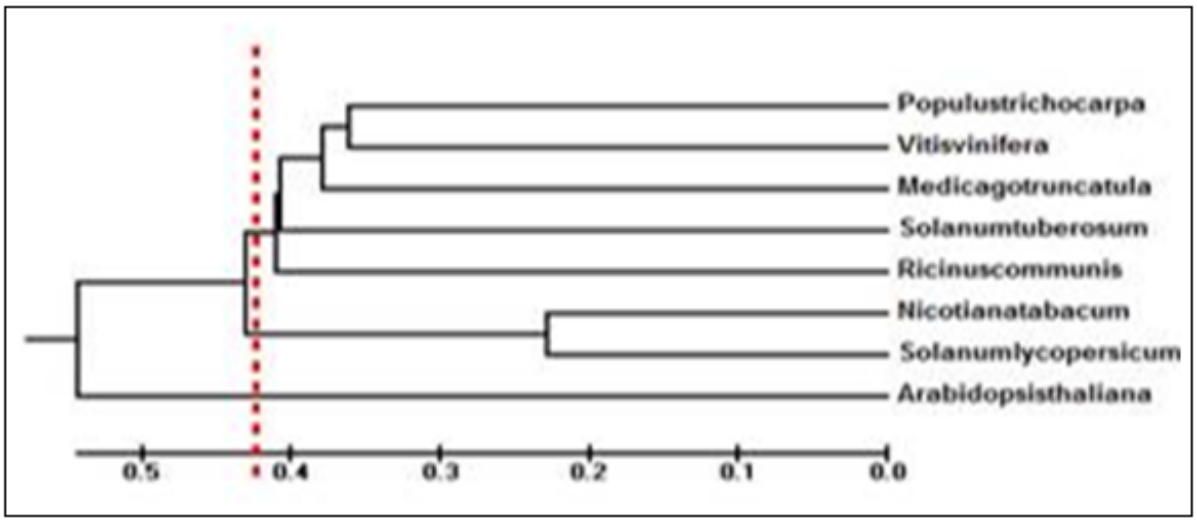

Fig. 2. Phylogenetic tree of the plant species in this study based on disease resistance gene.

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