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Study on resistance genes to potato late blight in the White Lady variety

Fitoftóra rezisztencia gének vizsgálata White Lady burgonyafajtán

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Abstract

One of the most important and destructive disease in potato is *Phytophthora infestans* causing late blight disease. The agent of this disease has 11 races which some of them may exist in different parts of the country and cause losses in potato field every year. There are different solutions for controlling the disease, amongst them use of resistance clones and variety of potato is an effective solution and could prevent disease spread and losses in potato yield and quality, also it is cost effective. In the scientific point of view, exploring the resistance gene in the plant materials has a great deal of value. So far, 11 major genes, R1– R11, have been recognized in potato originated from *Solanum demissum* and are responsible for *P. infestans* 1-11 virulence genes. Among the different varieties of potato in Hungary, White lady showed resistance to different diseases like PVX, PVY,PVA viruses and also has a good resistance to late blight. During this research, existence of some the Resistance genes to late blight in White Lady compared to Black R lines was studied. For this purpose, genomic DNA of White Lady and R lines was extracted by using of Walbot & Warren's protocol, then Screening of R genes to late blight in the samples was carried out by specific primers. The extracted fragments was amplified by pcr, and after gel electrophoresis , those have the same size with known R genes were cloned by using of PGEM-T vector in competent cell. Colony pcr of the white clone with inserted fragment was done and pcr condition was adjusted according to each specific primer. Plasmids containing the insert of interest were extracted and sent for sequencing. the results of homology sequence showed that amplified fragments in White Lady and black R line which have the same size of band in gel profile with R2, R3a and R3b genes had the most coverage and similarity with these gene sequence data in NCBI database. Therefore, it was confirmed that major resistance genes R2, R3a and R3b are present in White Lady variety.

Introduction

One of the most important and destructive diseases of potato in the world is Late Blight caused by Phytophthora infestans. High humidity and cool weather condition is favorite for disease progress, so that prolonging this situation may rise the risk of pathogen and give the fungus opportunity to destroy susceptible plants during a short time. This fungus have capability to infect all parts of plant containing foliage and underground tissues. Today, commercial potato crops are mainly protected by the use of disease-free seed sand frequent fungicide application(Struik and Wiersema, 1999; Fry, 2007). However, considerable financial and environmental costs are incurred for fungicides and their application (Vleeshouwers *et al.*, 2011b). Production of disease-free seeds causes considerable additional costs for there fined infrastructure and regular operation of the facilities.

Furthermore, the capacity of the pathogen to develop resistance to modern fungicides(Goodwin *et al.*, 1996; Grünwald *et al.*, 2001) necessitates the development of durably resistant varieties. Therefore, breeders have been extremely interested in creating resistant cultivars.

Two forms of genetic resistance to *P. infestans* in potato have been classified as race-specific (qualitative) and race-nonspecific (quantitative) resistance. The race-specific resistance is associated with dominant resistance (R) genes (Shi *et al.*, 2011). Eleven Dominant resistance (R) gene were initially identified in

the Mexican species *Solanum demissum* and introgressed by crossing and backcrossing methods into cultivated potato. by the way, a set of 11 differential potato clones, each carrying a different single R-gene, was used to identify complementary avirulence/virulence phenotypes in collections of *P. infestans* (Black *et al.*, 1953; Malcolmson and Black, 1966).

Deployment of these single genes did not provide durable resistance in the field due to the rapidly changing populations of *P. infestans* and appearance of new virulent races of the pathogen overcame the resistance encoded by single race-specific R genes (Wastie, 1991; Fry and Goodwin, 1997). Subsequent population development and mapping have led to the localization of several Rpi (for Resistance to *P. infestans*) genes from other wild *Solanum* species These efforts have shown that wild and primitive cultivated potato germplasm are a rich source of novel resistances to *P. infestans* that can be exploited in potato breeding programs (Hein *et al.*, 2009).

Recently over 20 functional late blight R genes, including the *Solanum demissum* genes R1, R2, R3a, and R3b, have been cloned (Ballvora *et al.*, 2002; Huang *et al.*, 2004; Lokossou *et al.*, 2009; Li *et al.*, 2011).The cloned genes all belong to the CC-NB-LRR class and, besides *S. demissum,* they were derived from wild *Solanum* species like *S. bulbocastanum* (Song *et al.*, 2003; Van Der Vossen *et al.*, 2003; Vossen *et al.*, 2005) *S. stoloniferum* and *S. papita* (Vleeshouwers *et al.*, 2008) *S. venturii* and *S. mochiquense* (Foster *et al.*, 2009; Pel *et al.*, 2009)

Each kind of R genes with origin of *Solanum demissum* was located in different chromosome of potato except for R4 that is located on XI chromosome in which R3 resides in also . R3 locus is composed of two genes with distinct specificities. These two subfamily of R3 gene called *R3a* and *R3b* are 0.4 cM apart and have both been introgressed from *S. demissum* (Huang *et al.,* 2004).

The apparent resistant of White Lady potato cultivar to Late Blight disease in screening greenhouse studies and also good resistance in the field motivate us to study the molecular basis of resistance in this cultivar as a resistant Hungarian cultivar with good characteristics. Actually, screening tests in greenhouse with different races of Pathogen which carry different Avr genes trigger cognate major resistance (R) genes from *S. demissum* , showed a high degree of resistance in White Lady against different races of pathogen exist in Hungary. so, we decide to use molecular genetic marker to reveal the genes with origin of *S. demissum* in this cultivar that involve in resistance to late bligh in this research. And test the White Lady variety and susceptible clone with other differential Black R lines to check which special R genes exist in the genetic background of this variety.

Materials and methods

Genomic DNA of White Lady as Resistant, S440 as susceptible phenotype to Late Blight and other Mastenbroek & Black Rlines was extracted using Walbot & Warren's protocol (Walbot and Warren, 1988). PCR reactions were carried out in a total volume of 12µl, comprising the following: 1.5 µl of the template DNA (100 ng/µl); 1.2 µl of dNTP (0,2mM of each dNTP), 0.1 µl Dream Taq Polymerase with 1.5 µl of the adjacent 10 x Buffer, 0.6 µl (100pmol/µl) of each of specific primers (table 1).

PCR were optimized on Eppendorf 384 amplifier. All PCR products were separated on 1.5 % agarose gel post stained with ethidium bromide visualized and analyzed on GeneGeinous (Syngene, UK) gel documentation system. after gel electrophoresis , those have the same size with known R genes were cloned by using of PGEM-T vector in competent cell Cloning PCR Products with pGEM-T

PCR product of amplified fragments were cloned by PGEM_T kit and Dh5α Z-Competent *E. coli* competent cells each transformation cultured onto duplicate LB/ampicillin/IPTG/X-Gal plates and plates were put in the Incubator adjusted at 37°C overnight. After growing the colonies of Bacteria only

white colonies was selected and sub cultured to another LBA medium by using a loop, simultaneously the loop were put in to the wells of pcr plate filled with master mix to do pcr for each colony.

Colony PCRs on white colonies were run using primers M13f (CGCCAGGGTTTTCCCAGTCACGAC) and M13r (GTCATAGCTGTTTCCTGTGTGA) applying the following conditions : 3 cycles of 2 min at 95°C, 1 min at 65°C, 3 min at 72°C followed by 41 cycles of 30 sec at 95°C, 30 sec at 56°C, 2.5 min at 72°C.

Plasmids contatining the insert of intrest were extracted using GeneJET Plasmid Miniprep Kit for preparation of high quality plasmid contain inserted DNA from recombinant *E.coli* cultures. During the process cell bacteria were lyses and plasmid vector was extract and purified by using of spin column. Plasmid then was sent to the Bay Zoltán Institute for sequencing.

Sequences of fragments related to each target gene was assembled by using of codon code aligner and a contig was made for each gene for analyzing in nucleotide database.

Table 1: *characteristics of specific primers used in the pcr*

Name	Sequence	Tm (°C)	Target gene	Product length (kb)	References
76-2SF2	CACTCGTGACATATCCTCACTA	50	*R1*	1.4	(Ballvora *et al.*, 2002)
76-2SR	CAACCCTGGCATGCCACG				
SHa-F	ATCGTTGTCATGCTATGAGATTGTT	56	*R3a*	0.982	(Huang *et al.*, 2005)
SHa-R	CTTCAAGGTAGTGGGCAGTATGCTT				
*R3b*F4	GTCGATGAATGCTATGTTTCTCGAGA	55	*R3b*	0.378	(Rietman, 2011)
*R3b*R5	ACCAGTTTCTTGCAATTCCAGATTG				
R2F	ATGGCTGATGCCTTTCTATCATTTGC	55	*R2*	2.5	(Kim *et al.*, 2011)
R2R	TCACAACATATAATTCCGCTTC				
184-81F,	RRAGATTCAGCCATKGARATTAAGAAA		*R8*	0.500	(Jo *et al.*, 2011)
*184-81*R	ACTCGATTCTCAACCCGAAAG				

Results

Amplification of fragments by PCR

Gel electrophoresis of samples after pcr with specific primers of R gene showed that not only in the Mastenbroek & Black R line but alson in the White Lady there are bands in the size that is identical to the size of R2, R3a and R3b gene fragment (fig.1-a,b,c). in the case of R8, normal pcr with primers 184-81F, 184-81R (table 1) showed monomorphism without any differential pattern between samples. But after digestion with *RsaI* restriction enzyme (caps marker) and gel electrophoresis, only R8 and R9 has a band in a region with 450 kb size (fig. 2).

PCR with R1 specific primer resulted a band with around 1400 bp only in profile of some Mastenbroek & Black R line like 5, 6 and 9. and there isn't any amplified band in pcr production of White Lady sample after using this primer.

A) M s440 WL B) M WL s440 C) M s440 WL

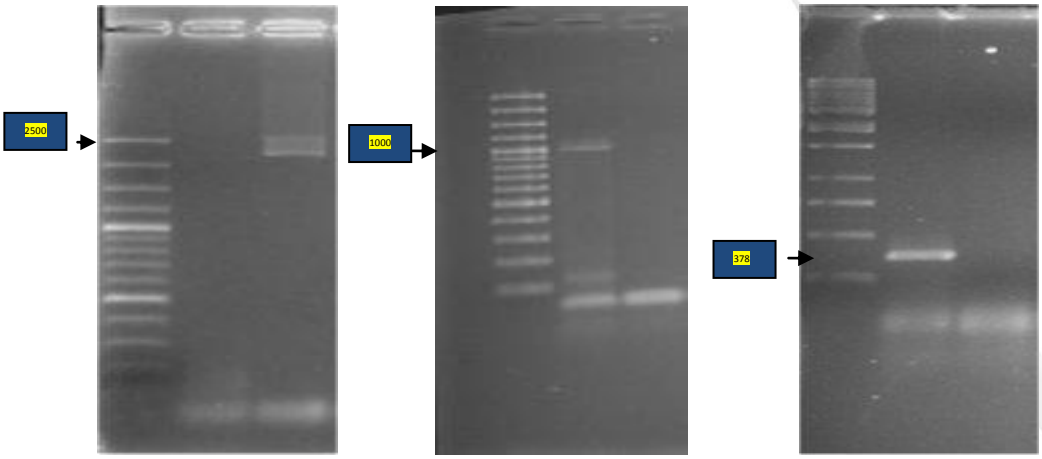

Fig1: *specific markers for detecting of R genes. pcr product in White Lady by using of a. R2, b.R3a and c. R3b specific markers*

D) M s440 WL E) M WL R8

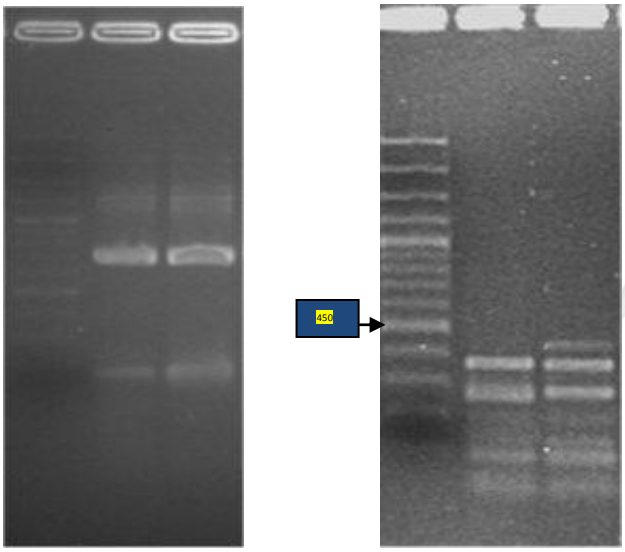

Fig2: *CAPS markers for detecting of R8. D. before digestion E.after digestion with Rsa1*

Homology of DNA sequences

The sequencing of inserted fragments of R genes in White Lady and Black R lines was done and the homology of corresponding fragments in two type of samples and with known sequence data of genes in NCBI database were examined. The results showed that fragment R2 from WL has the 97 % identity with other samples of WL and Black R2 lines and also 97% identity with known sequence in NCBI.

Sequence identity between R3a taken from different samples of WL and between a contig made of the alignments with known sequence of disease resistance protein subfamily R3a was 100 percent.

In the case of R3b the sequence similarity between the fragment taken from samples was 100% and between a contig made of different samples of WL and known sequence of Disease resistance protein subfamily R3b was 100 percent (table 2).

Table2: *percent of identity and coverage of amplified fragments with known R sequences in NCBI*

fragment	Potato Species	Fragment size	Sequence Identity	Sequence coverage
R2	*Solanum sp*	2500	97	*77*
R3a	*S. demissum*	1000	100	78
R3b	*S. demissum*	378	100	96

Discussion

In this research according to the results of the pcr and homology of sequences it was proven that there are at least three Major R gene in White Lady and more investigating may reveal existing of more major R gene in genetic background of this cultivar. Theoretically, broad spectrum or multi-R gene-based resistance, would result in enhanced durability because it is increasingly less likely that all cognate Avrs are lost simultaneously in one Pi spore(Kim *et al.,*2011). broad spectrum R gene stacks potentially provide enhancement of durability for future application in new late blight resistant varieties by breeding program. it was shown that stacking of multiple R genes strongly delayed the onset of late blight symptoms(Vleeshouwers *et al.,* 2011a).

The genomic region of R3 is rich in functional diversity for *P. infestans* resistance the R3 locus harbors two R genes with distinct resistance specificities to the oomycete pathogen(Huang *et al.*, 2004). Also, R2 gene is a representative of a highly diverse gene family located at a major late blight (MLB) resistance locus on chromosome IV of potato. Eleven R2 orthologs conferring resistance against *P. infestans* have been identified so that R2GH conferring resistance to *P. infestans* originate from diverse Solanum species(Vleeshouwers et al., 2011a). As it was shown in this research White Lady has at least these diverse gene in its genetic background that confers resistance to some local *P. infestans* populations and maybe has other major R gene, therefore cultivation of this variety not only has some advantages in durability of resistance and delaying of infection but also using it in breeding program as a parent with multiple stocking R gene is more advantageous because of developing new cultivars with good agronomical traits and resistance to light blight.

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