

ABC-Transporter
CAPS (*Rhynchosporium secalis* (Oud.) Davis)
Mapping of an ABC-Transporter gene associated with barley scald disease
(*Rhynchosporium secalis* (Oud.) Davis) using a CAPS marker

CAPS (*Rhynchosporium secalis* (Oud.) Davis) ABC-Transporter
:()

ABC-Transporter

R. secalis ABC-Transporter

PCR / kb CAPS SNP

SNP

Harrington Chebec *NlaIII* SNP SNP

CAPS

Synteny

Synteny *NlaIII* CAPS SNP ABC-Transporter :

// :

()

CER5 (Yazaki, 2006).

ABC-T

NpABC1 (Pighin *et al.*, 2004)

ABC-T

(ATP-Binding Cassette Transporter) ABC-T

)

(

(Jasinski *et al.*, 2001)

PDR ABC-T

(Trans-Membrance Domain) TMD

(α -helix)

)

NpPDR1

(Nucleotide Binding Domain) NBD

ABC Walker B Walker A

(

ABC-T

Signature

NBD TMD

Gm (Sasabe *et al.*, 2002; Stukkens *et al.*, 2005)

(Jasinski *et al.*, 2003; Stukkens *et al.*, 2005)

PDR12

Pleiotropic Drug Resistance (PDR)

)

Multi-Drug Resistance (MDR)

(

Multi-Drug Resistance-associated Proteins (MRP)

At PDR8 (Eichhorn *et al.*, 2006)

ABC-T

(*Arabidopsis thaliana*)

(Kobae *et al.*, 2006)

ABC-T

(Fernandez *et al.*, 2001)

ABC-T

"...ABC-Transporter "

(Jasinski *et al.*, 2003)

SNP

(Deletion) (Insertion)

(Brookes, 1998)

DNA

(Alignment)

BAC kb SNP (Rostoks *et al.*, 2005)

(Bacterial Artificial Chromosome) DNA

BAC ABC-T

SSH

BAC *R. secalis* SSR

Primer3

(Giordano *et al.*, 1999; Rafalski, 2002)

BAC 7-1 5AATTGCTAGGTGAGATGCTTGTGGTCC SNP

BAC 7-2 5GCTCTTGATCTTTCCTTGATGTCACC (Gut, 2001; Kwok, 2000; Rafalski, 2002; Shi,

BAC 7-3 5AATGGGAGTACCATGCCCTTCCTTCTTG CAPS SNP .2001)

BAC 7-4 5GCCATGATTGGATAACACTGCTCTTCA

PCR (Rostoks *et al.*, 2005; Thiel

/ / PCR *et al.*, 2004)

/ dNTPs SSH

Taq DNA Polymerase DMSO % (Suppression Subtractive Hybridization)

DNA (Immolys, UK)

Rhynchosporium secalis (Oud.) Davis

ABC-T

PCR (Single Nucleotide SNP

Polymorphisms)

(Cleaved Amplified Polymorphic Sequence) CAPS

PCR

Table 1- Barley genotypes.

Genotype	Growth habit	No. of row
Chebece	Spring	Two rowed
Harrington	Spring	Two rowed
Clipper	Spring	Two rowed
Sahara	Spring	Two rowed
Galleon	Spring	Two rowed
Sloop	Spring	Two rowed
Alexis	Spring	Two rowed
Harana Nijo	Spring	Two rowed
Frankiln	Spring	Two rowed
Halcyon	Spring	Two rowed

Haruna Nijo

<http://wheat.pw.usda.gov/ggpages/links.shtml>
<http://genbank.vurv.cz/barley/pedigree/pedigree.asp>

(Min Elute PCR purification, QIAGEN, USA)

CAPS

Big Dye Terminator

Harrington Chebec

Terminator V3.1 Cycle

BAC7-3 BAC7-1

(Willsmore *et al.*, 2006)

Sequencing (Applied Bio Systems. USA)

(Manly *et al.*, Map Manager QTX

2001)

P=0.05-0.001

Contig Express

(Voorrips, 2002) Map Chart

SNP

SNP

NEBcutter2

In Silico

(<http://tools.neb.com/NEBcutter2/index.php>)

ABC-T

PCR

CAPS

SNP

DNA

(Biolabs, NEW England) *Nla*III

BAC7-3 BAC7-4

NEBuffer4)

X

BAC7-1 BAC7-3

/ (Biolabs, NEW England

/ (/) BSA

bp bp

PCR

/

"...ABC-Transporter"

PCR bp

SNP

CAPS SNP

SNP bp

SNP BAC 7-1 BAC 7-3

SNP

SNP (Kota *et al.*, 2001)

EST

SNP (Rostoks *et al.*, 2005)

(Van *et al.*, 2004)

SNP

()

SNP ABC-T bp

SNP

SNP (Van *et al.*, 2004)

SNP

SNP (Non-Coding Regions)

SNP (Coding Regions)

SNP

5-UTR

ABC-T NBD EST 3' UTR

(Rafalski, 2002)

(Jasinski *et al.*, 2003) SNP

SNP

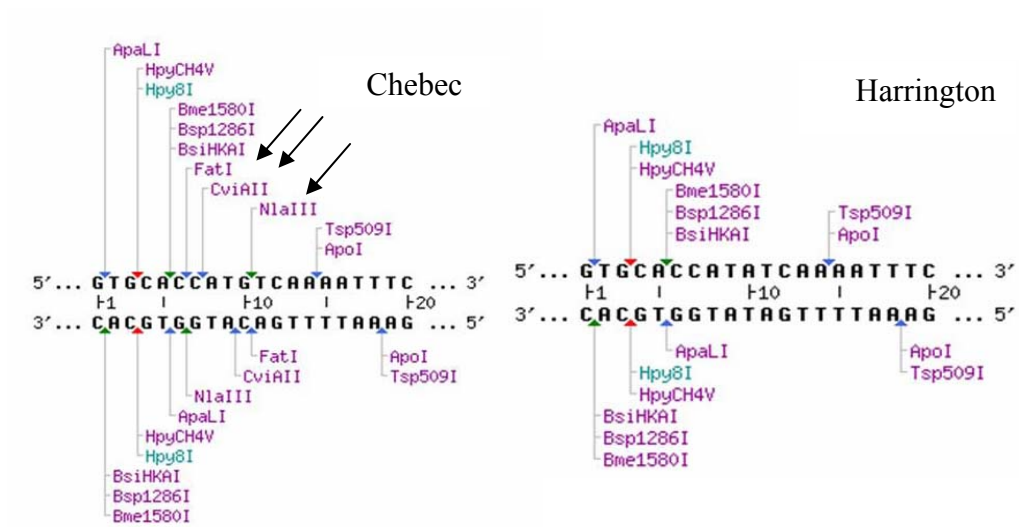
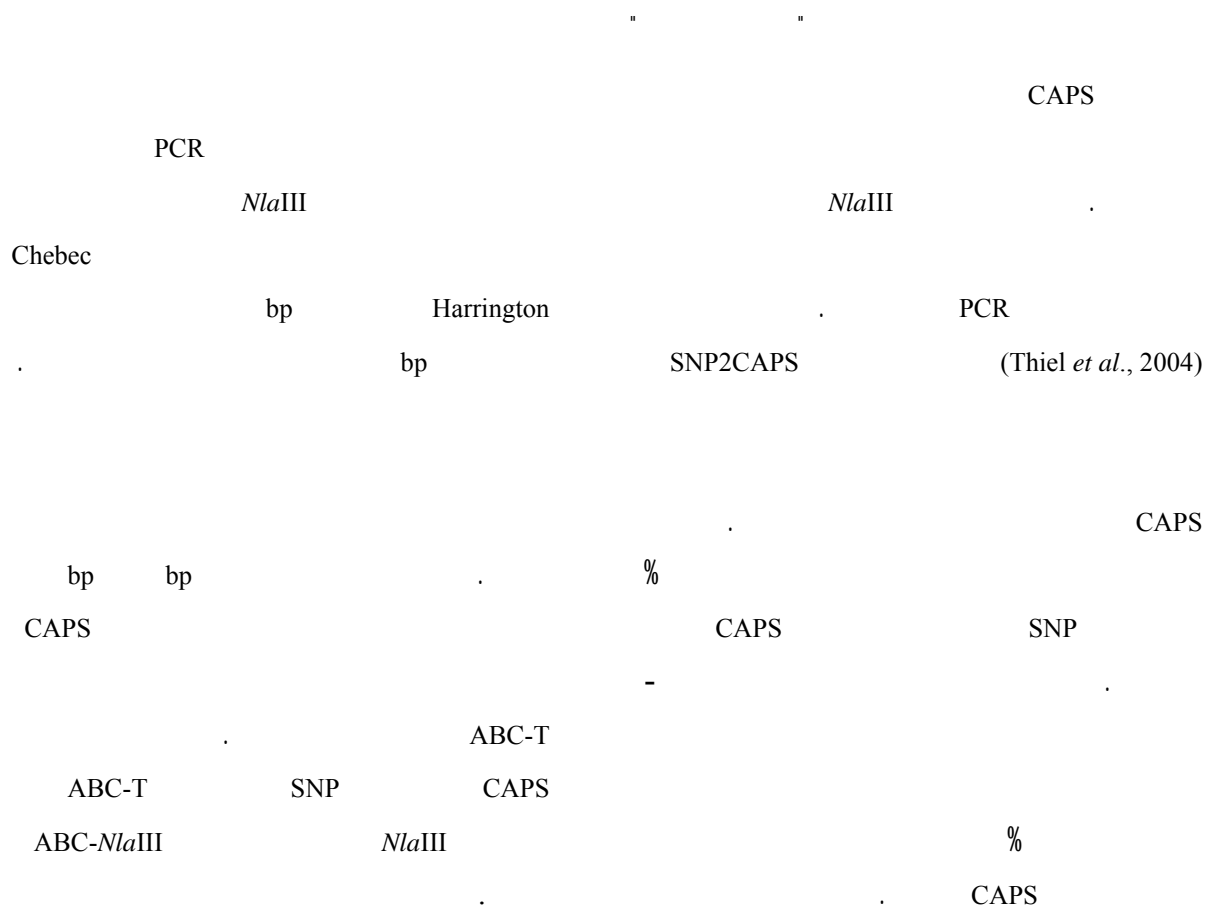
Harrington Chebec (Van *et al.*, 2004)

FatI, NlaIII

()

CviAII (Sjaskste and Roder, 2004)

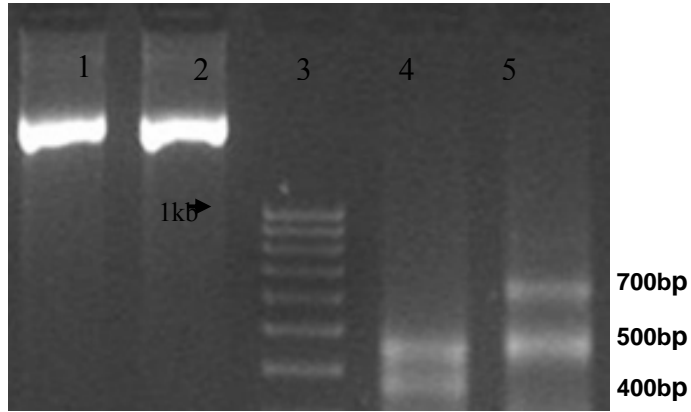
SNP C→T SNP



Harrington Chebec () SNP

Fig.1 Restriction enzyme sites in flank of SNP (Position 10) for Chebec and Harrington

"...ABC-Transporter"



Chebec) (1kb) (PCR) NlaIII PCR
 (Harrington) (

Fig2. Digestion of PCR product by NlaIII,(1 and 2 Parents PCR products), (3- Ladder 1kb), (4- Chebec after digestion), (5- Harrington after digestion)

(Schenk *et al.*, 2000; Zwiigelaar and Dubery
 QTL .2006) ABC-T

Net blotch
 (Willsmore *et al.*, 2006) SSR / bcd809 RFLP
 / GBM1163

(Tacconi *et al.*, 2001) Wg178 RFLP
 / Hvm33 SSR

si

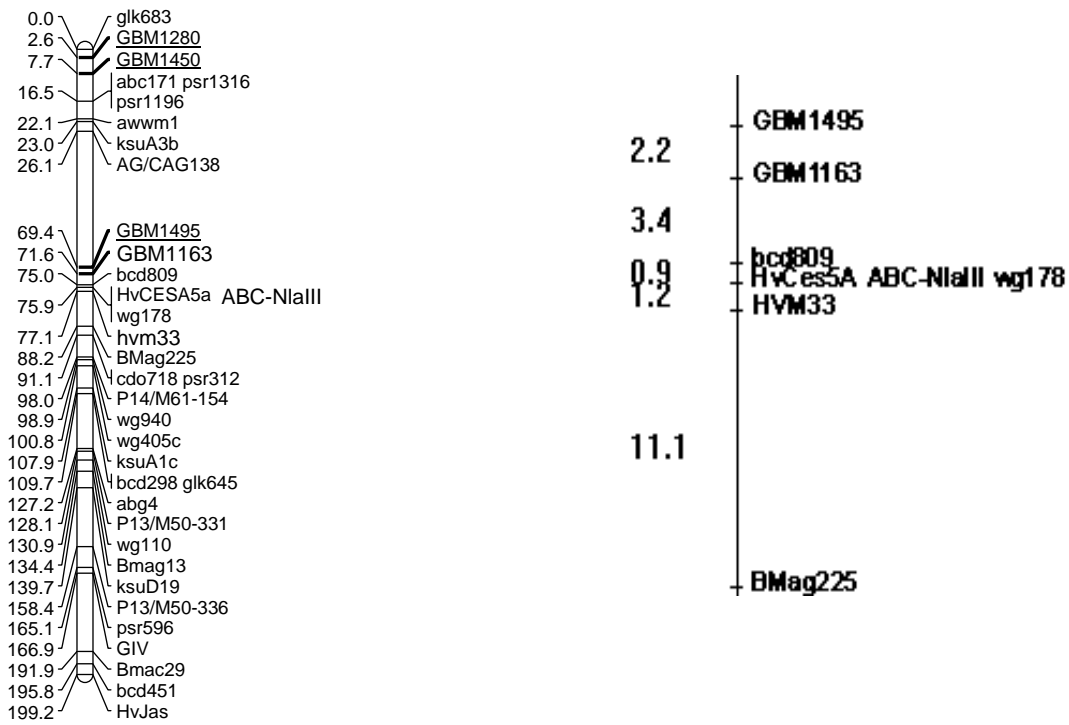
Graingenes2
 (<http://wheat.pw.usda.gov/GG2/index.shtml>)

Synteny (Comparative Mapping) (Vershney *et al.*, 2007)

Synteny ABC-T Hv.CesA5
 (Cellulose Synthase)

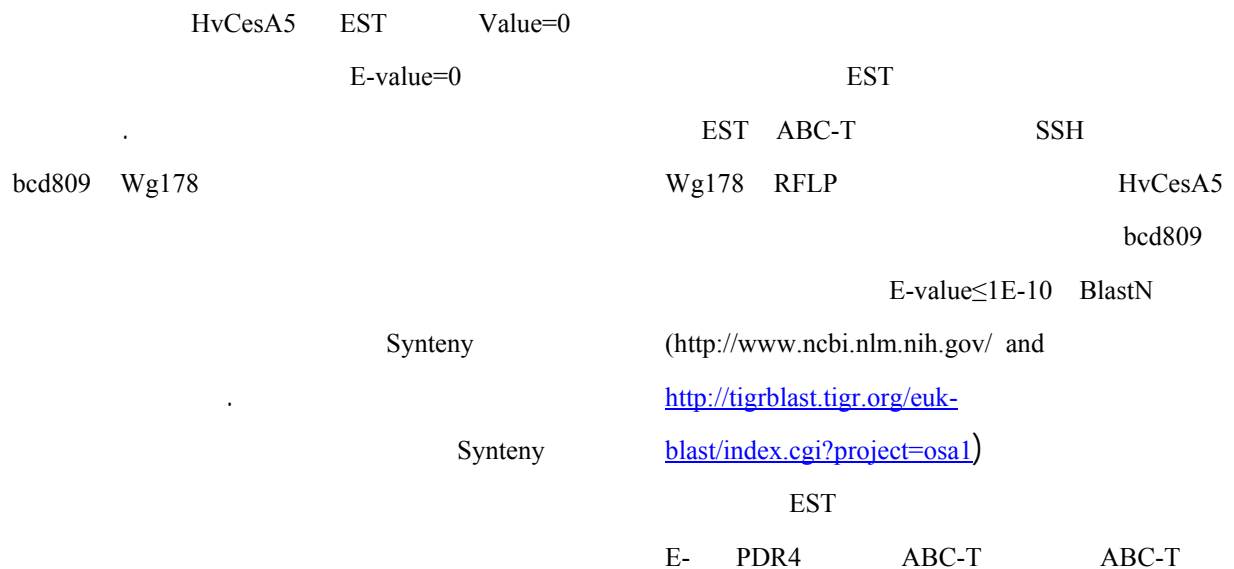
(Stein *et al.*, 2007)

3H_CxH



ABC-T

Fig. 3. Genetic map of ABC-T gene on barley chromosome 3, Genetic distances are in CentiMorgan



"...ABC-Transporter"

(Stein *et al.*, 2007)

-

(Rostoks *et al.*, 2005, Thiel *et al.*, 2004) - EST

CAPS

RAPD EST SNP SSR

RFLP EST

EST

SNP

(Weiland and Yu, 2003) CAPS (Bundock *et al.*, 2003)

RAPD EST SNP SSR

P450

CAPS CAPS

(Graner *et al.*, 1999) (Rostoks *et al.*, 2005)

RFLP SNP

CAPS

CAPS SNP

SSR

CAPS SNP

(SARDI)

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Mapping of an ABC-Transporter gene associated with barley scald disease (*Rhynchosporium secalis* (Oud.) Davis) - using a CAPS marker

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ABSTRACT

Aalami, A., C. Oldech, H. Alizadeh, M. Omid, Bihamta, M. R., Boushehri, A. A. and K. Willmore. 2007. Mapping of an ABC-Transporter gene associated with barley scald disease (*Rhynchosporium secalis* (Oud.) Davis)- using a CAPS marker. Iranian Journal of Crop Sciences. 9 (2):157-168

ABC-Transporter proteins superfamily are found in all alive organisms as connection bridge in cellular membranes. These proteins are responsible for transportation of variant substrates such as metabolites that involved in plant defense mechanisms. In this study SNPs and CAPS markers were used for mapping of an ABC-Transporter which is specific for compatible and incompatible interaction against *R. secalis* fungus. A 2.2kb fragment derived from PCR product with specific primers for mentioned gene was sequenced in 10 barley parents. Among observed SNPs, one SNP showed different restriction enzymes sites in Chebec and Harrington parents and converted to CAPS marker. Results mapping revealed position of the gene on long arm of barley chromosome 3. Also, a homology evaluation for this region of chromosome was in accordance with previous studies about high synteny for barley chromosome 3 with rice chromosome 1.

Key words: Barley, Gene Mapping, ABC-Transporter, SNP, CAPS, NlaIII, Synteny, Scald

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