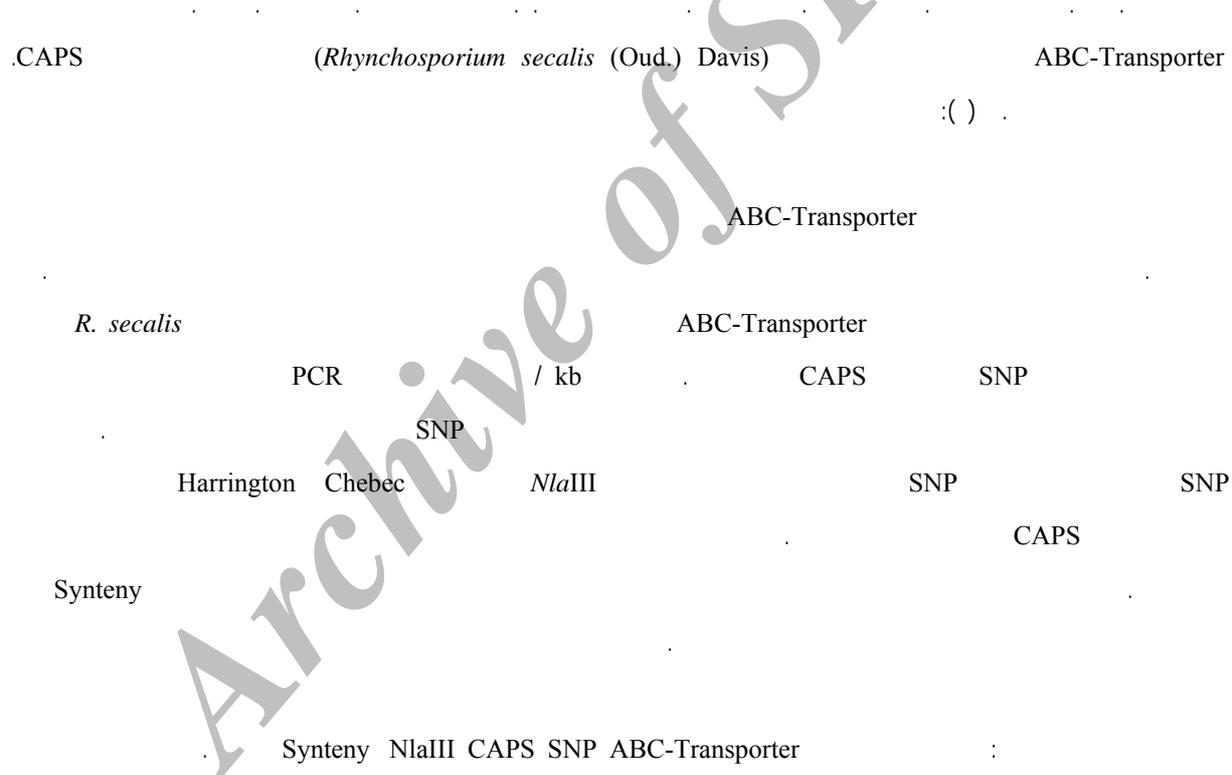


ABC-Transporter
CAPS (*Rhynchosporium secalis* (Oud.) Davis)
Mapping of an ABC-Transporter gene associated with barley scald disease
(Rhynchosporium secalis (Oud.) Davis) **using a CAPS marker**



// :

()

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

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

(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 SNP BAC 7-1 BAC 7-3

SNP (Kota *et al.*, 2001)

EST

SNP (Rostoks *et al.*, 2005)

(Van *et al.*, 2004)

SNP

()

SNP ABC-T bp

SNP (Van *et al.*, 2004)

SNP SNP

SNP (Non-Coding Regions)

SNP (Coding Regions)

SNP

5'-UTR

ABC-T NBD EST 3' UTR

(Rafalski, 2002)

SNP

SNP

(Jasinski *et al.*, 2003)

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

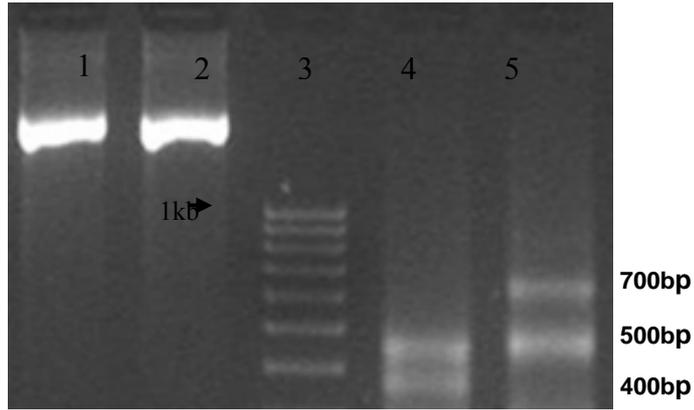
FatI, *NlaIII*

()

CviAII (Sjaskste and Roder, 2004)

SNP C→T SNP

"...ABC-Transporter"



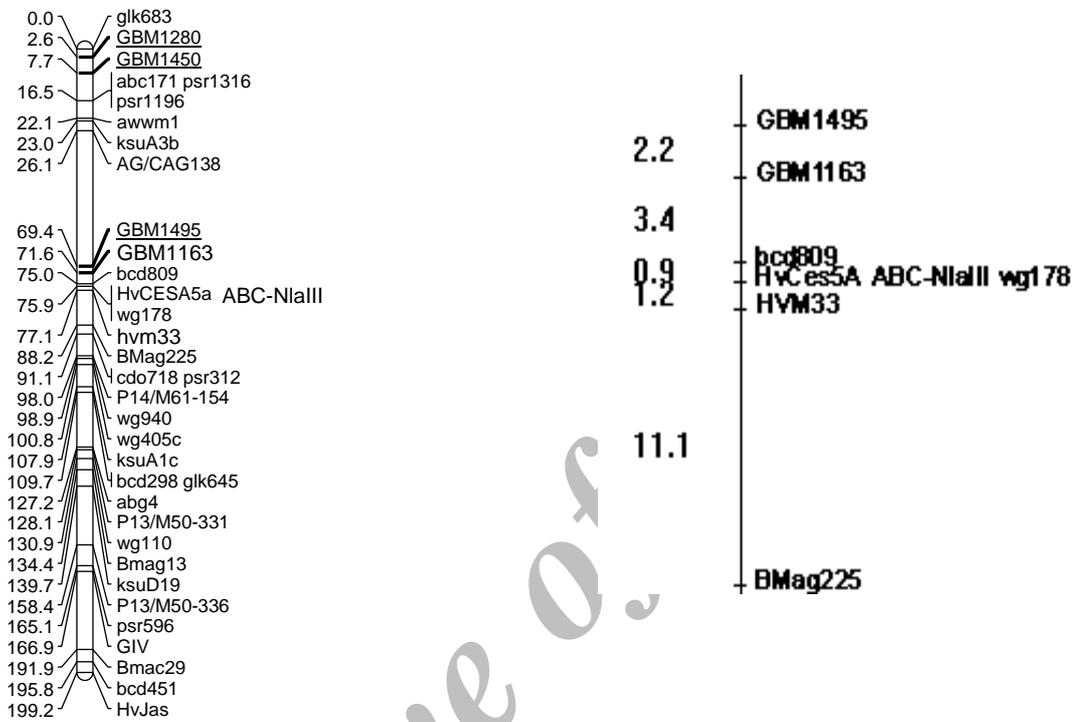
Chebec) (1kb) (PCR) NlaIII PCR
 (Harrington) (

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)
 Hv.CesA5
 ABC-T (Cellulose Synthase)
 Synteny

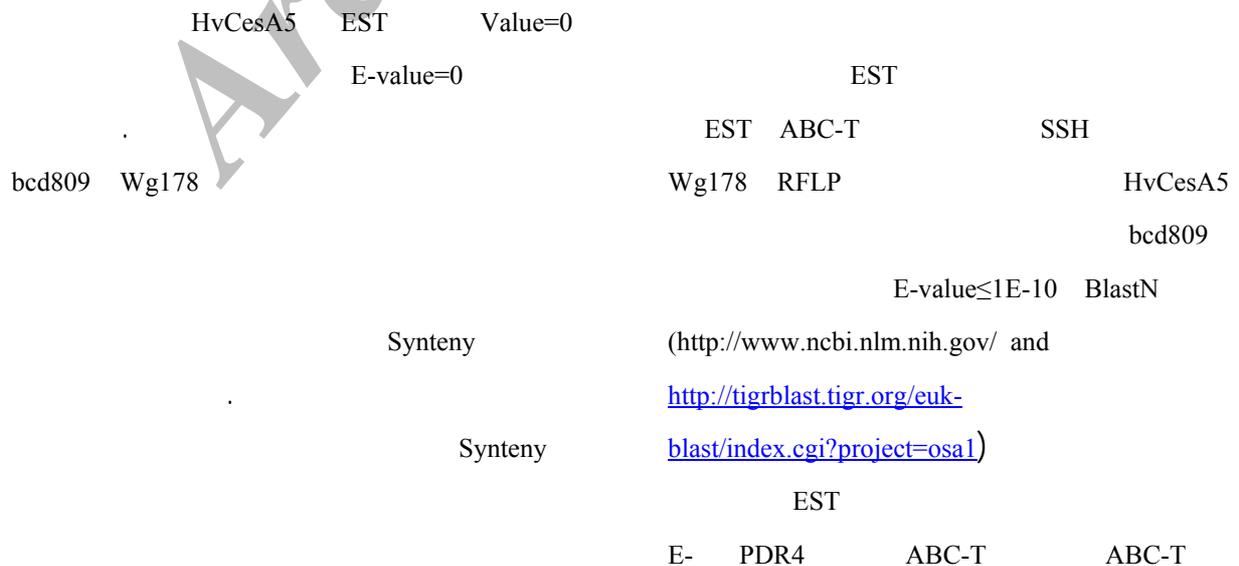
(Stein *et al.*, 2007)

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, CAPS (Bundock *et al.*, 2003)
RAPD 2003) EST SNP SSR
P450

CAPS
(Graner *et al.*, 1999) CAPS
RFLP (Rostoks *et al.*, 2005) SNP

CAPS

CAPS SNP

SSR

CAPS SNP

(SARDI)

References

- Brookes, A. J. 1998.** The essence of SNPs. *Genet.* 234: 177-186.
- Bundock, C., T. Christopher, P. Egger, G. Ablett, J. Henry and A. Holton. 2003.** Single nucleotide polymorphisms in cytochrome P450 genes from barley. *Theor Appl Genet.* 106: 676–682.

- Eichhorn, H., M. Klinghammer, P. Becht and R. Tenhaken. 2006. Isolation of a novel ABC-transporter gene from soybean induced by salicylic acid. *Expt. Bot.* 57: 2193–2201.
- Fernandez, R. S., T. G. E. Davies, J. O. D. Coleman and P. A. Rea. 2001. The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. *J. Biol. Chem.* 276: 30231-30244.
- Giordano, M., P. J. Oefner, P. A. Underhill, L. L. Cavellisforza, , R. Tosi and P. M. Richiardi. 1999. Identification by denaturing high-performance liquid chromatography of numerous polymorphisms in a candidate region for multiple sclerosis susceptibility. *Genomics.* 56: 247-253.
- Graner, A., S. Streng, A. Kellermann, A. Schiemann, E. Bauer, R. Waugh, B. Pello and F. Ordon. 1999. Molecular mapping and genetic fine-structure of the rym5 locus encoding resistance to different strains of the barley yellow mosaic virus complex. *Theor. Appl. Genet.* 98: 285-290.
- Gut, I. G. 2001. Automation in genotyping of single nucleotide polymorphisms. *Hum. Mutat.* 17: 475-492.
- Jasinski, M., Y. Stukkens, H. Degand, B. Purnelle, J. M. Brynaert and M. Boutry. 2001. A plant plasma membrane ATP Binding Cassette–Type Transporter is involved in antifungal terpenoid secretion. *The Plant Cell.* 13: 1095–1107.
- Jasinski, M., E. Ducos, E. Martinoia and M. Boutry. 2003. The ATP-Binding Cassette Transporters: structure, function and gene family comparison between rice and arabidopsis. *Plant Physiol.* 131: 1169–1177.
- Kobae, Y., T. Sekino., H. Yoshioka., T. Nakagawa., E. Martinoia and M. Maeshima. 2006. Loss of AtPDR8, a plasma membrane ABC Transporter of arabidopsis thaliana, causes hypersensitive cell death upon pathogen infection. *Plant Cell Physiol.* 47: 309–318.
- Kota, R., R. K. Varshney, T. Thiel, K. J. Dehmer and A. Graner. 2001. Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.). *Hereditas.* 135: 145-151.
- Kwok, P. Y. 2000. High-throughput genotyping assays approaches. *Pharmacogenomics.* 1: 95-100.
- Manly K. F., R.H. Cudmore and J.M. Meer. 2001. Map Manager QTX, crossplatform software for genetic mapping. *Mammalian Genome.* 12: 930–932.
- Pighin, J. A., H. Zheng, L.J. Balakshin, I.P. Goodman, T.L. Western, R. Jetter, L. Kunst and A.L. Samuels. 2004. Plant cuticular lipid export requires an ABC transporter. *Science.* 306: 702–704.
- Rafalski, A. 2002. Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biol.* 5:94–100.
- Rostoks, N., S. Mudie, L. Cardle, J. Russell, L. Ramsay, A. Booth, J. T. Svensson, S. I. Wanamaker, H. Walia, E. M. Rodriguez, P. E. Hedley, H. Liu, J. Morris, T. J. Close, D. F. Marshall and R. Waugh. 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Mol Gen Genomics.* 274: 515-527.
- Sasabe, M., K. Toyoda, T. Shiraishi, Y. Inagaki and Y. Ichinose. 2002. cDNA cloning and characterization of tobacco ABC transporter: NtPDR1 is a novel elicitor-responsive gene. *FEBS Letters.* 518: 164-168.
- Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond, S. C. Somerville and J. M. Manners.

- 2000.** Coordinated plant defense responses in arabidopsis revealed by microarray analysis. *PNAS*. 97: 11655–11660.
- Shi, M. M. 2001.** Enabling large-scale pharmacogenetic studies by highthroughput mutation detection and genotyping technologies. *Clin. Chem.* 47: 164-172.
- Sjakste, T and M. Roder. 2004.** Distribution and inheritance of b-amylase alleles in north european barley varieties. *Hereditas*. 141: 39- 45.
- Stein, N., M. Prasad, U. Scholz, T. Thiel, H. Zhang, M. Wolf, R. Kota, R. K. Varshney, D. Perovic, I. Grosse and A. Graner. 2007.** A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. *Theor Appl Genet*. 114:823–839.
- Stukkens, Y., A. Bultreys, S. Grec, T. Trombik, D. Vanham and M. Boutry. 2005.** NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from *Nicotiana plumbaginifolia*, plays a major role in plant pathogen defense. *Plant Physiol*. 139: 341–352.
- Taconi, G., L. Cattivelli, N. Faccini, N. Pecchioni, A. M. Stanca and G. Vale. 2001.** Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare L.*). *Theor Appl. Genet*. 102:1286–1291.
- Thiel, T., R. Kota, I. Grosse, N. Stein and A. Graner. 2004.** SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. *Nucleic Acids Research*. 32 e5.
- Van, K., E. Y. Hwang, M. Y. Kim, Y. H. Kim, Y. Cho, P.B. Cregan and S. H. Lee. 2004.** Discovery of single nucleotide polymorphisms in soybean using primers designed from ESTs. *Euphytica*. 139: 147–157.
- Varshney, R. K., T. C. Marcel, L. Ramsay, J. Russell, M. S. Roder, N. Stein, R. Waugh, P. Langridge, R. E. Niks and A. Graner. 2007.** A high density barley microsatellite consensus map with 775 SSR loci. *Theor. Appl. Genet*. 114:1091–1103.
- Voorrips, R. E. 2002.** MapChart: software for the graphical presentation of linkage maps and QTLs. *J. of Hered.* 93:77–78.
- Weiland, J. J. and M. H. Yu. 2003.** A Cleaved Amplified Polymorphic Sequence (CAPS) marker associated with root-knot nematode resistance in sugarbeet. *Crop Sci*. 43:1814–1818.
- Willmore, K. L., P. Eckermann, R. K. Varshney, A. Graner, P. Langridge, M. Pallotta, J. Cheong and K. J. Williams. 2006.** New eSSR and gSSR markers added to Australian barley maps. *Aust. J. of Agric. Res.* 57: 953-959.
- Yazaki, K. 2006.** ABC transporters involved in the transport of plant secondary metabolites. *FEBS Letters*. 580:1183–1191.
- Zwiegelaar, M. and I. A. Dubery. 2006.** Early activation of cell wall strengthening-related gene transcription in cotton by a *Verticillium dahliae* elicitor. *South African J. of Bot.* 72: 467–472.

" "

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

Aalami, A¹., C. Oldech², H. Alizadeh³, M. Omid⁴, M. R. Bihamta⁵,
A. A. Boushehri⁶, and K. Willmore⁷

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

Received: July, 2007.

1- Ph.D. Student, The University of Tehran, Karaj, Iran (Corresponding author).

2- Senior Scientist, South Australian Research and Development Institute, Urrbrae. SA.

3- Assistan Prof., Faculty of Crops and Animal Sciences, The University of Tehran, Karaj, Iran.

4 and 6- Associate Prof., Faculty of Crops and Animal Sciences, The University of Tehran, Karaj, Iran.

5- Prof., Faculty of Crop and Animal Sciences, The University of Tehran, Karaj, Iran.

7- Research Officer, South Australian Research and Development Institute, Urrbrae, SA.