Construction of subtractive cDNA library and identification of wheat (*Triticum aestivum* L.) transcripts induced by brown rust (*Puccinia triticina*)

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Introduction

Brown rust caused by *Puccinia triticina* Erics. is one the most devastating foliar diseases of wheat (*Triticum eastivum* L.). Close to sixty leaf rust resistance genes (*Lr*) conferring various level of resistance against brown rust in seedlings and/or adult plants have been identified. Significant part of them was inbred into the cultivars. Despite the high number of known *Lr* genes, their economical importance, isolation and characterization of *Lr1*, *Lr10*, *Lr21* and *Lr34* our understanding of the mechanisms and processes involved in leaf rust resistance is still very limited.

The goal of the project is to identify wheat transcripts involved in signaling and resistance against brown rust. We focused on *Lr9* gene because it provides effective and durable resistance against this pathogen.

SSH library construction

RNA was extracted from susceptible cv. Thatcher and isogenic Tc*Lr9* line 12, 20, 26, 32 and 44 hours after inoculation with *P. triticina* urediniospores.

cDNA synthesis and construction of suppression subtractive hybridization SSH cDNA library (BD PCR-Select cDNA Subtraction Kit; Clontech) was performed in *forward* and *reverse* orientation in order to identify wheat genes induced and repressed by the pathogen in the resistant line.

The final products of two subtractive hybridizations and nested PCR were cloned in pGEM-T vector, transformed to *E. coli* JM109 and selected as the SSH *forward* (357 clones) and SSH *reverse* (436 clones) library.

Subtractive cDNA clones

The macroarray (the membranes loaded with cDNA-inserts) were differentially hybridized with DIG-labeled *forward* and *reverse* cDNA. Hybridizations of the *forward* library revealed that over 115 clones were pathogen induced or activated (Figure 1).

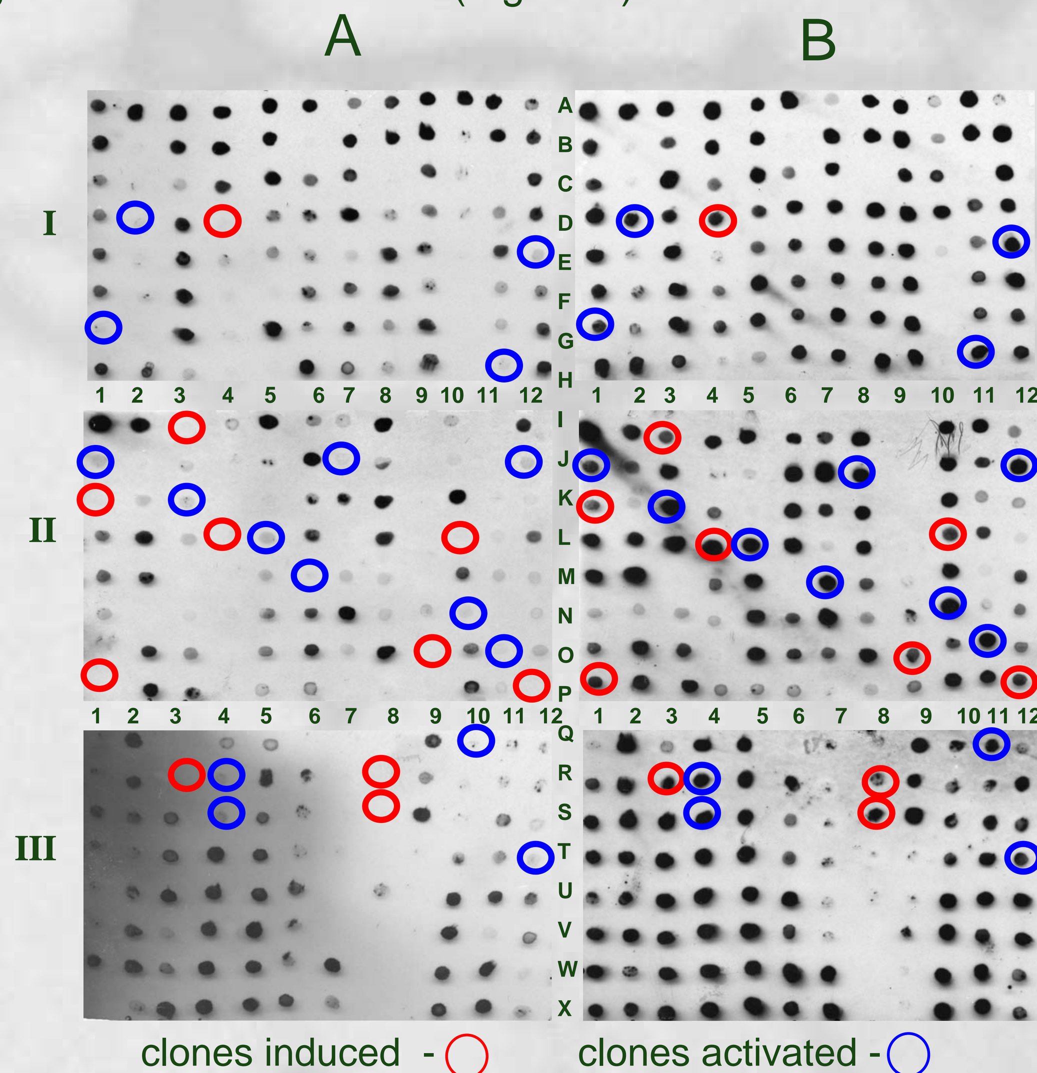

Figure 1. Differential macroarray screening of cDNA clones from SSH *forward* library

Homology to known genes

The 115 clones from SSH forward library were sequenced. Their nucleotide sequences were used for a search in ExPASy Proteomics Server. The 77 clones were assigned to proteins from Swiss-Prot or EMBL GenBank. The analysis indicated that the significant part of the clones (nucleotide as well as amino acid sequences) revealed high homology to transcripts / proteins of known or putative function in plant response during the pathogen infection (Table 1).

Functional analysis

For wheat functional analysis we are currently adopting the BSMV-based VIGS system. The BSMV vectors were obtained from Dr. Merete Albrechtsen, Univeristy of Aarhus, Denmark (M. Bruun-Rasmussen *et al.*, 2007) (Figure 3).

Silencing of phytoene desaturase (PDS) resulted in photobleaching of leaves what confirmed that the system can be used for functional analysis of selected genes in Tc*Lr9* line (Figure 4).

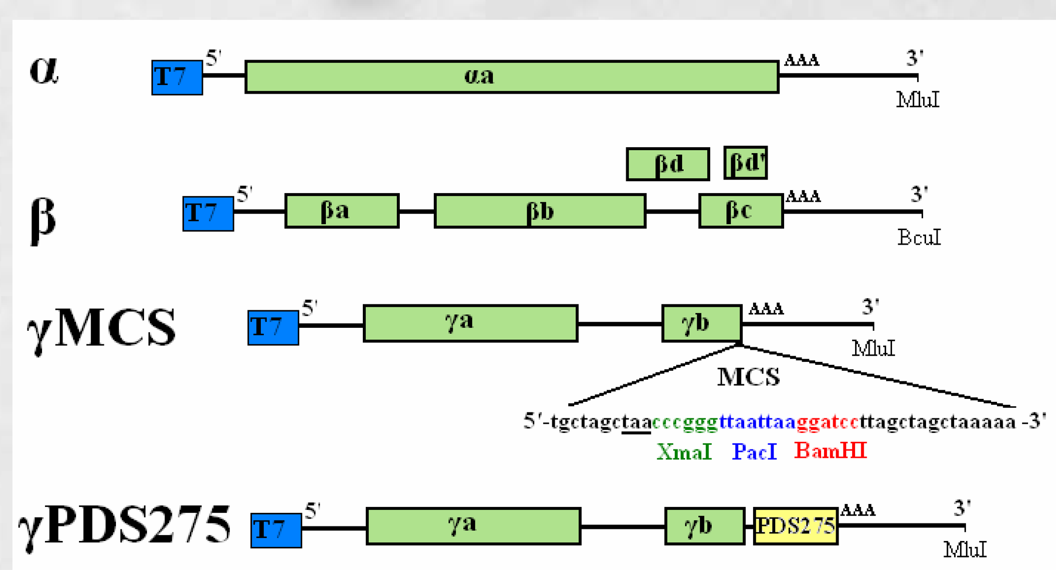

Figure 3. Components of the BSMV VIGS system (M. Bruun-Rasmussen *et al.*, 2007).

Clone number	Transcripts / proteins assigned to clones of SSH cDNA *forward* library	Species	Gene ID SwissProt EMBL	E-value
F_I_5	Putative annexin	*O. sativa*	Q6H450	2e-56
F_I_7	Peptidyl-prolyl cis-trans isomerase	*T. aestivum*	FKB70	7e-47
F_I_8	Putative anexin	*O. sativa*	Q6H450	9e-60
F_I_14	Putative 60S ribosomal protein L30	*O. sativa*	Q6F331	2e-21
F_I_16	Phosphoethanolamine cytidylyltransferase	*H. vulgare*	AY198340	3e-42
F_I_22	NAD-dependent epimerase/dehydratase Putative 3-beta hydroxysteroid dehydrogenase/isomerase protein	*Zea mays* *O. sativa*	B4FH62 Q94HJ5	2e-41 3e-40
F_I_24	Secretory carrier-associated membrane protein	*O. sativa*	Q6Z8F5	8e-40
F_I_31	Putative calcium-transporting ATPase 8, P-type ATPase (Fragment) [CA9]	*O. sativa* *H. vulgare*	Q7EZ84 Q94IM4	4e-34 2e-20
F_I_32 F_I_33	Vacuolar H+-pyrophosphatase	*T. aestivum*	A9LRZ1 A9LRZ1	1e-27 1e-38
F_II_2 F_II_6 F_II_121	Putative aldehyde oxidase	*O. sativa*	Q8LHR9	3e-49 1e-48 4e-47
F_II_10	Putative callose synthase	*H. vulgare*	Q7Y1B7	5e-53
F_II_11	Choline-phosphate cytidylyltransferase B	*Zea mays*	B4F7T8	9e-21
F_II_25	Cytochrome P450	*Zea mays*	B6UA52	3e-44
F_II_29	Putative RAN binding protein	*O. sativa*	Q6AV40	2e-39
F_II_33 F_II_76	4- nitrophenylphosphatase	*Zea mays*	B6TQ54	3e-52 3e-52
F_II_35	Eukaryotic translation initiation factor 5A3	*T. aestivum*	Q3S4I1	6e-49
F_II_74	Putative lecithin diacylglycerol cholesterol acyltransferase	*O. sativa*	Q67U60	1e-31
F_II_75 F_III_107	Putative heat-and-acid stable phosphoprotein	*Zea mays*	B6SNE2	7e-37 5e-37
F_II_77	CTP synthase, putative	*O. sativa*	Q2QNR9	2e-54
F_II_81	cDNA similar to glutamyl-tRNA reductase Pre-mRNA-splicing factor cwc15	*H. vulgare* *Zea mays*	EV253805B 6SU82	0.08e-30
F_II_92	ATP sulfurylase fragment [APS1]	*Camellia sinensis*	Q1HL02	4e-29
F_II_97	Putative RAN binding protein Importin-beta N-terminal domain containing protein	*O. sativa* *O. sativa*	Q6AV40 Q10GR1	8e-41 8e-41
F_II_98	ATPase-like protein	*O. sativa*	Q7XI12	1e-55
F_II_99	Triose phosphate translocator	*T. aestivum*	AF314182	0.0
F_II_102	Nuclear transportin [TRN-SR] mRNA,	*T. aestivum*	DQ019632	3e-40
F_II_122	Triosephosphate isomerase	*O. sativa*	Q69K00	3e-56
F_II_126	TaWin1 (TaWIN1) protein 14-3-3E (14-3-3E) protein	*T. aestivum* *H. vulgare*	Q9FXQ9 A1X810	1e-34 1e-34
F_II_164	Transmembrane protein 56	*Zea mays*	B6TI82	1e-50
F_III_13	Folylpolyglutamate synthetase	*T. aestivum*	A4UIZ0	e-113
F_III_14 F_III_118	Calmodulin-binding family protein, putative	*O. sativa*	Q2QXN6	2e-89 e-104
F_III_15 F_III_112 F_III_114	eIF4-gamma/eIF5/eIF2-epsilon domain containing protein	*O. sativa*	Q2R678	1e-80 2e-79 3e-63
F_III_27 F_III_158	Eukaryotic initiation factor 5C isoform F	*Zea mays* *Zea mays*	BT070066 B6TRV8	5e-158 2e-75
F_III_31	Protein kinase APK1B	*Zea mays*	B6T9Q6	2e-18
F_III_43	Aspartate aminotransferase	*Zea mays*	B6TK79	4e-74
F_III_47	AA-type ATPase family protein	*O. sativa*	Q2R029	5e-42
F_III_54 F_III_73 F_III_131 F_III_133	Chaperone protein dnaJ	*Ricinus communis*	B9RNG7	5e-96 6e-96 e-106 1e-64
F_III_56 F_III_138	26S protease regulatory subunit 4 homolog 26S proteasome regulatory particle triple-A ATPase	*O. sativa* *O. sativa* *O. sativa* *O. sativa*	P46466 P46466 Q8W422 Q8W422	2e-78 6e-82 3e-78 1e-81
F_III_64	Calmodulin-binding protein	*Zea mays*	B6SL12	7e-79
F_III_103	Stromal 70 kDa heat shock-related protein	*Zea mays*	B6UFB3	5e-68
F_III_104 F_III_145	Putative long chain acyl-CoA synthetase	*O. sativa*	Q5W6W7	6e-86 9e-88
F_III_117	Lipase class 3-like	*O. sativa*	Q6K2K7	7e-33
F_III_121	N-acetyl-gamma-glutamyl-phosphate reductase	*O. sativa*	Q10GQ5	9e-95
F_III_124	Putative system A transporter isoform 2	*O. sativa*	Q67VL0	9e-74
F_III_128	Proteasome activator subunit 4-like	*O. sativa*	Q5Z876	3e-82
F_III_130	Putative aspartate aminotransferase	*O. sativa*	Q7F2K1	2e-49
F_III_132	Phospholipase D alpha 1 precursor	*O. sativa*	Q43007	1e-108
F_III_136 F_III_137 F_III_150	GAMYB-binding protein	*H. vulgare*	Q6J6M8	3e-66 e-118 e-116
F_III_139	Diphosphomevalonate decarboxylase	*Zea mays*	B6TCA9	7e-80
F_III_143	Protein translocase subunit secA	*O. sativa*	Q657P0	e-103
F_III_146 F_III_153	Putative root border cell-specific protein	*O. sativa*	Q6ZL16	1e-41 1e-46
F_III_147	Zeaxanthin epoxidase	*T. aestivum*	Q9AVE7	6e-75
F_III_149	GDP dissociation inhibitor protein	*O. sativa*	O22471	1e-65
F_III_155	GTP-binding protein	*T. aestivum*	Q6YKA9	e-105

Table 1. Protein annotation and the similarity with known genes / proteins of selected cDNA clones from wheat SSH *forward* library

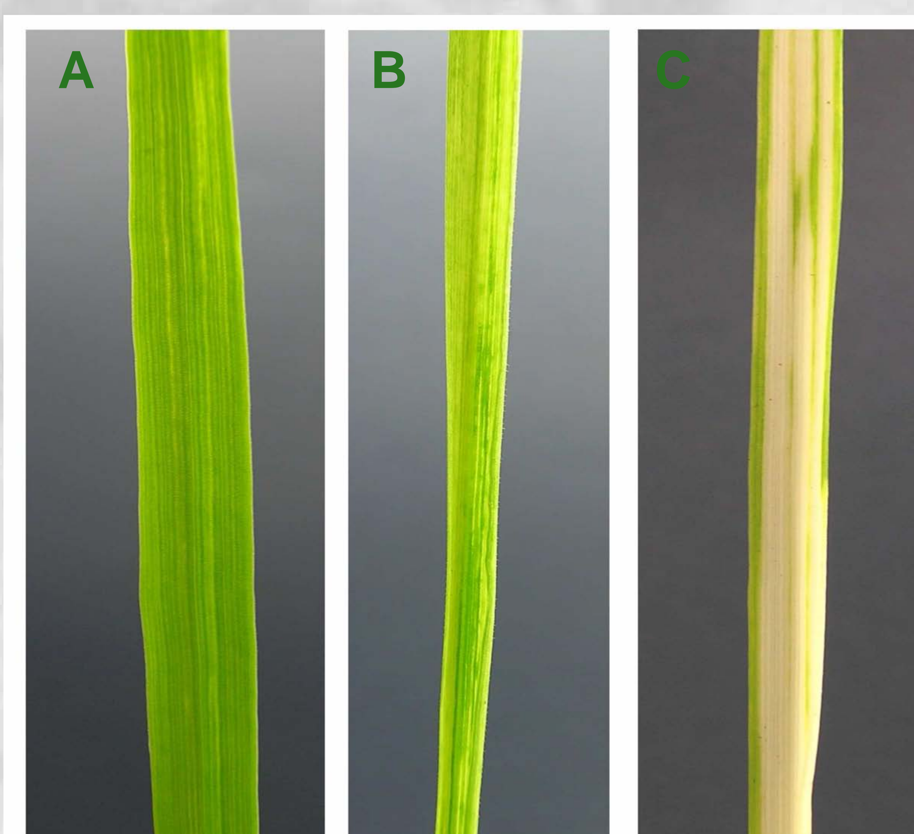

Figure 4. Tc*Lr9* leaves 10 days post inoculation with unmodified BSMV vector (B), BSMV containing 275bp PDS fragment (C). Noninoculated control leaf (A).

The 275bp fragment of barley PDS, cloned into the MCS of γBSMV induced silencing of PDS in Tc*Lr9* leaves.

Expression profiles

Expression of selected SSH clones in inoculated leaves was shown as densitometric reading of gel separated RT-PCR products. Expression in Thatcher leaves before inoculation was used as a reference.

Analysis of selected SSH clones in inoculated leaves of susceptible cv. Thatcher and resistant Tc*Lr9* indicated significant induction / changes in expression of selected SSH clones during compatible and incomaptible wheat-brown rust interaction (Figure 2).

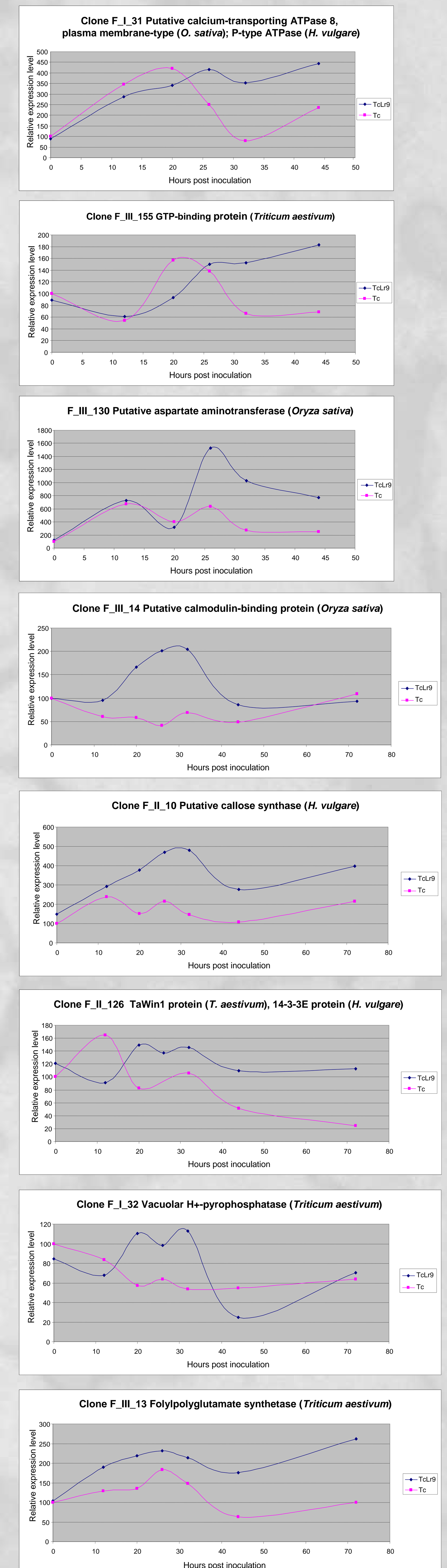

Figure 2. Expression profiles of selected SSH clones in leaves of susceptible cv. Thatcher and resistant Tc*Lr9* line after inocualtion with *P. triticina* urediospores.

References

Bruun-Rasmussen M, Madsen CT, Jessing S, Albrechtsen M, 2007. Stability of Barley stripe mosaic virus-induced gene silencing in barley. *Mol Plant Microbe Interact* 20: 1323–1331.