

Accessory Publication

Table S1. Candidate wheat GRX transcripts grouped in terms of similarity to known rice orthologues
TIGR transcript assembly sequences belonging to likely GRX genes are shown in bold, with the number of corresponding ESTs given in parentheses

Orthologous cluster	Wheat transcript accessions
CPYC (ι) Os06g44910	CA721020, CD866717, CB307498, CA649156, TA72960_4565 (6) , CK217883, CA610725, CA680768, CA665087, CA657450, TA72959_4565 (22) , CD866693, CA697873, CK200830, CK201161, TA72958_4565 (2)
CPYC (θ) Os02g43180	CA603679, CA653978, CA709270, CN012562, CA691557, CD926604, DN829206, TA72590_4565 (2) , AL829842, CA645309, CJ550981, CK216884, CA639780, BQ609662, BQ608765, CA682770, TA72592_4565 (17) , TA72591_4565 (8) , CA484697, CA696634, TA72589_4565 (3)
CPYC (κ) Os08g45140	CA663697, CK211538, CV766888, CJ723975, CV777681, CV772548, TA74923_4565 (4) , TA74922_4565 (20) , CV767163, CJ514760, CJ620076, CA657651, CA663674, CA683007
CPYC (η) Os04g42930 Os02g40500	CV771482, TA51747_4565 (64) , CJ551440, CK212100, CK211567, CA594851, CA661589, CJ582053, CA695609, CA718121, CA687907, TA49902_4565 (2) , CA689771, TA51226_4565 (5) , CA688473, DR735325, CK203435, CK194026, CD924078, CA666778, CA617811, BJ279648, CJ581492, CA712749, CA711402, TA51757_4565 (36) , CA630565, TA50566_4565 (36) , CA641691, CA699800, CD871550, CA717922, CA699297, CA686632, CA688005, CA637332, CJ553468, CD928809, CJ599958, CA699799, CA612139, CD912075, CA606592, CJ561754, CA653241, CA698460, CA623272, CA693641, CD876709, CA652149, CJ697790, DR739883, BQ904775, CA606085, CA631709, CA646026, CA666598, CA666691, CA668348, CA608810, CA714461, CA619705
CGFS (μ) Os01g07950 Os12g07650	TA83490_4565 (12) , BG909857, BQ172447, CA631804, CA634993, CD874050, CK161058, DR737299, BG909857, BQ172447, CA631804, CA634993, CD874050, DR737299
CGFS (ν) Os01g34620	CJ530923, BG606327, TA61483_4565 (17) , CA653480, TA61499_4565 (8) , CA644396
CGFS (ξ) Os10g35720	CF133183, CA608459, CA654573, TA69965_4565 (14) , CJ627255, TA69966_4565 (15) , CA602873, CD898894, TA69964_4565 (12) , TA69967_4565 (11) , CD490987, AJ602202, BQ236514, CA593523, CA679492, CA699896, CA724942, CD930030, CK203158, AJ602746
CGFS (λ) Os03g63420	TA78947_4565 (17) , CD919126, CA653518, CV765383, BF483681, CK152327, TA85234_4565 (3) , TA85236_4565 (5) , CA736230, CV779630, BJ249733, CA684084, CA705464, CA709344, CJ641985, TA78948_4565, TA92171_4565 (4) , TA92172_4565 (2)
CC-type (γ) Os05g10930 Os01g09830	CV762413, CK204298, TA77596_4565 (9) , CK212584, TA77595_4565 (11) , CA649509, CD883628, CK197062

CC-type (β) Os01g13950 Os05g48930 Os01g47760	TA108884_4565, BQ162737, CA645134, CK207645, TA90355_4565 (3) , CA486038, CA721726, CA688723, TA66245_4565 (6) , CD885077, CK211838, CA592978, CV781115, CA732099, TA66239_4565 (4) , CK200465, TA66243_4565 (3) , CD880395, BM138193, TA66252_4565 (2) , TA66246_4565 (2) , CA685169, CA652734, CK200181, TA108632_4565 (2)
CC-type (ε3) Os01g70990	CA610028, CA608968, CD871873, CA641396
CC-type (ε4) Os05g05730 Os01g27140	TA110687_4565 (2) , CA689352, AJ601991, CA680002, TA102057_4565 (2) , CA694798
CC-type (ε5) Os12g15330	CN011047, CJ599840, CV779001, CD874485, CA742382, CA485458, CA658370, CA608403
CC-type (δ) Os07g05630	TA102841_4565 (2)
CC-type (α) <i>OsROXY1</i> <i>OsROXY2</i>	CO348297
Probable artefacts	CD913549, TA76809_4565, CD875293, CJ662056, TA85235_4565, CV763611, CV764238, TA98938_4565, BM135259, TA95971_4565, AY072771, AL831023, AL826362, TA88795_4565, CA717037, BJ232365, CV781931

Table S2. Clustering of potential wheat GRX ESTs into CDS models
CAP3 (Huang and Madan 1999) was used in the clustering with manual curation

Transcript assembly	Clustered ESTs
CPYC <i>TaGRX-η1</i>	AF542185, AL810179, AL811584, AL814666, AL820569, AL820679, AL820680, AL822234, AL823319, AL825626, AL828833, BE444264, BF484892, BJ279648, BJ284634, CA603281, CA608810, CA617811, CA627762, CA630565, CA631709, CA643679, CA653241, CA661589, CA695061, CA716788, CA718121, CD452645, CD864937, CD864938, CD916730, CD928809, CD928810, CJ525035, CJ532384, CJ560298, CJ560533, CJ561275, CJ561494, CJ561754, CJ563760, CJ565041, CJ570277, CJ580956, CJ580957, CJ582053, CJ583609, CJ583610, CJ585283, CJ591765, CJ591773, CJ599681, CJ615567, CJ622141, CJ633534, CJ667538, CJ667763, CJ668706, CJ668961, CJ670865, CJ672118, CJ677179, CJ687438, CJ690043, CJ690044, CJ691621, CJ697790, CJ697796, CJ705194, CJ719649, CJ726004, CN008643, CN012058, CO346662, CV771482, CV775905, CV776311
CPYC <i>TaGRX-η2</i>	CA695609, CA711402, CA637332, CJ599958, CD912075, BE414367, CA604600, BJ289856, BE500153, BQ606860, CJ644994, AL808570, CJ522926, CJ631488, CA618963, CJ690998, CJ565106, CJ554836, CJ581267, BE517947, CA685423, CJ687730, CJ584642, CJ594123, CJ700005, CJ662330, CJ672185, CJ549398, BE398640, CJ572234, CJ679058, CJ590375, CJ518321, CJ536737, CJ627160, CJ657116, CJ696453, BJ294284, BJ287957, CA618033, CA618028, BJ296185, CA602255, CJ551440, CK212100, DR735325, CK203435, CA686632, CD876709, DR739883, CD883744, CD913541, CJ604256, CD863577, CA485555, CD885507, CD885528, CD913542, CJ709515, CD863578, CJ705461, CD912076, CK203787, BQ807119, CD885529, CA594851, CA612139, CA606592, CD924078, CA699297, CA666778, CA666691
CPYC <i>TaGRX-η3</i>	CK211567, CA712749, CA641691, CJ553468, BQ904775, CJ577328, AL821973, AJ603581, BJ291415, CO348483, CJ585949, CJ588135, CJ585875, CJ544638, CJ605515, CJ591261, AL819310, AL830438, CD897226, BJ298037, CD918052, CJ652551, CD920829, CD906519, BQ904743, CV066112, CJ692266, CJ692197, CD917852, CJ661004, CJ654928, CJ694321, CJ697312, CJ695002, CJ695010, CD911613, CD918919, CA593745, CA646026, CK194026, CA698460, CA687907, CA714461, CA623272, CA652149, CA717922, CA688005, CA699800, CA666598
CPYC η singletons	CA668348, CA693641, CA699799, CA619705, CD871550, CA606085, CJ581492
CPYC <i>TaGRX-κ1</i>	CJ509760, BJ274985, CK161389, CN010507, BJ269998, CV766888, CD931574, CJ514760
CPYC <i>TaGRX-κ2</i>	CJ497482, CA714760, CA744811, CJ549250, CJ549258, CJ604833, CJ656974, CJ656981, CJ735410, CJ710031, CD869960
CPYC <i>TaGRX-κ3</i>	CA663697, CJ601333, CA657651, CA683007, CD892159, CV767163, CV777681, CJ558931, CJ666204, CV772548, CA663674, CJ706758
κ singletons	CK211538 CD892160, CJ620076, CJ723975,
CPYC <i>TaGRX-θ1</i>	CD866717, CB307498, CA649156, CA610725
CPYC <i>TaGRX-θ2</i>	CA665087, CA635893, CA680768, CA719500, BE498318, CJ527351, CJ662964, CJ635801, CJ555537, CJ579335, BE490426, BE444415, CJ568998, CJ644903, CJ536646, CJ579306, CJ601302, BE443475, BE443040, CJ675925, CJ685858, CJ685881, CJ706728, CJ686023, BE443589, CJ582214, BE444618, BE442597
CPYC <i>TaGRX-θ3</i>	CK217883, CA721020, CA657450

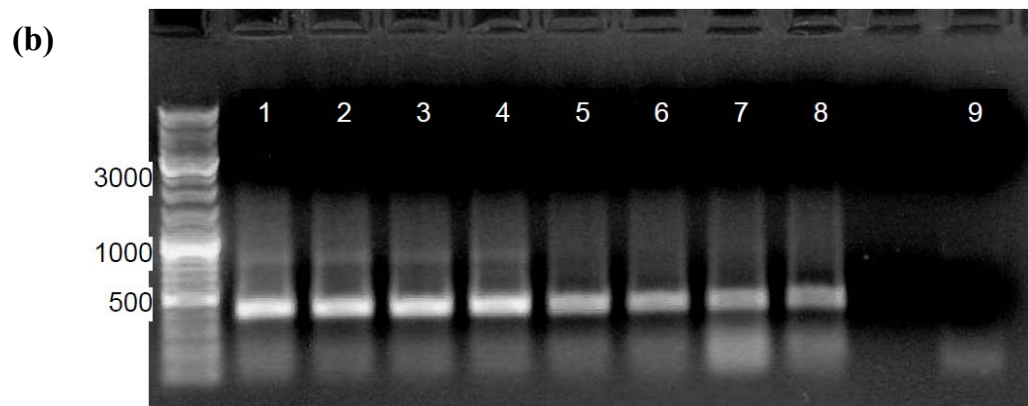
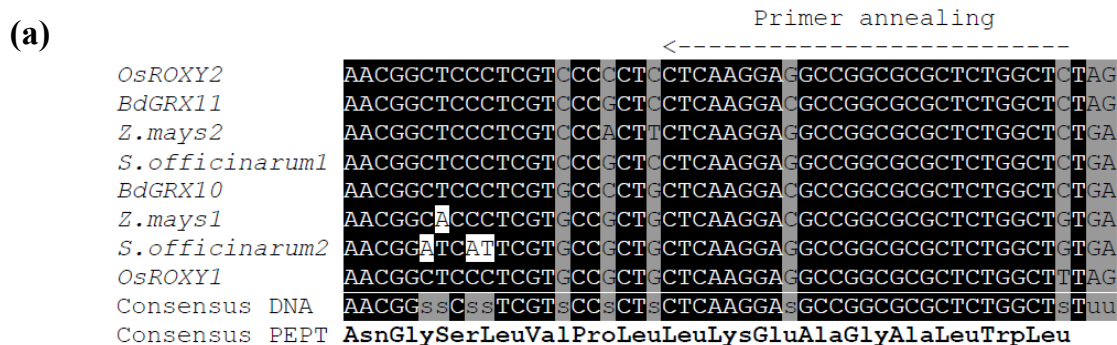
CPYC θ singletons	CA697873, CK200830, CD866693, CK201161 CA645309, CA696634, BJ308931, BJ303082
CPYC <i>TaGRX-i1</i>	DN829206, CD926604, CA713569, CA706361, BE402642, BQ608415, CA707972, BE419524, AL830700, CA735601, CA653978, CA682770, BQ608765
CPYC <i>TaGRX-i2</i>	AL829842, CJ550981, BQ609662, CA639445, BE418744, BJ237808, BQ620497, CJ536850, BG907391, BJ282422, BG907392, BQ752855, BQ804219, CN010929, BQ170056, BJ232165, BQ608595, CJ645103, CJ658602, BQ838644, BE402049, BQ607401
CPYC <i>TaGRX-i3</i>	AL813916, CA615360, AL827161, CA603679, CA691557
CPYC ι singletons	CK216884, CN012562, CA709270, CA639780, CA484697
Unclassified CPYC class cluster	CA689771, CA688473, CA685169
CGFS <i>TaGRX-ξ1</i>	CJ727245, BE606364, BE606352, BE404657, BE415157, CF133183, CD930030
CGFS <i>TaGRX-ξ2</i>	CA608459, BE515808, CJ714354, CD887668, AL825650, BQ239468, BJ313032, AJ602746, AJ602202
CGFS <i>TaGRX-ξ3</i>	CA657676, CD915061, CD898894, CA699896, CD915060, CD920622, CA695235, CA695239, CD923668, CA602873, BG909989
CGFS <i>TaGRX-ξ4</i>	CD900123, CD903052, BJ318769, BJ211167, CJ518427, CJ585501, CJ691836, CJ609797, CJ573358, CJ680159, CJ650512, CJ542431, BJ218538, CA634058
CGFS <i>TaGRX-ξ5</i>	CA654573, CA707953, CA692542, CJ703706, BQ838945, CJ597998, CJ623410, BE442687, BJ316801, BE444621, BE444071, BE443536
CGFS <i>TaGRX-ξ6</i>	CJ677622, BF428798, BQ161226, CJ570742, BE490824, BJ269630
CGFS ξ singletons	CD490987, CK203501, CK203158, BQ236514, CA593523, CA724942, CJ627255
CGFS <i>TaGRX-λ1</i>	BJ243681, BJ249733, CA608007, CA653518, CA684084, CA705464, CA725220, CA735537, CJ495593, CJ495806, CJ499191, CJ500346, CJ501241, CJ501771, CJ503237, CJ507799, CJ511478, CJ512731, CJ513661, CJ515677, CO348423, CV779630
CGFS <i>TaGRX-λ2</i>	CJ639952, CJ639954, CJ531533, CJ531535, CD919126, BQ166704, CD901119, CD919127
CGFS <i>TaGRX-λ3</i>	CA737640, CA737675, CA739400
CGFS λ singletons	CA709344, CK152327, BE426090, BF483681, CV765383, CJ533277, CJ641985, BQ160797, CA736230, BE488845, BE489212, BE488751, CJ533671
CGFS <i>TaGRX-v1</i>	CJ530923, BG606327, BF478480, BQ579630, BQ743885
CGFS <i>TaGRX-v2</i>	BJ254531, CD939921, BE498140, CA642761, BJ248381, CF134324, CA497528, BU100703, CA661179, BG606259, CA644396
CGFS <i>TaGRX-v3</i>	BJ225769, BJ255067, CJ537146, BJ230672, CD902178, CJ539793, CJ648027, CA667255, CD927619, CJ645404, BJ248806, CN008256, CA653480
CGFS v singletons	-
CGFS <i>TaGRX-μ1</i>	BG909861, CJ607809, CJ609347, CA735949, BJ252598, CJ610932, BE445109, CJ722534, BQ172447
CGFS <i>TaGRX-μ2</i>	CA631804, CD874050, CJ713933, BG909828, BG909857, BJ246658, BG313888
CGFS μ singleton	CA634993, CK161058, DR737299
CC-type <i>TaGRX-γ1</i>	BE442717, CD872078, CV762413, BQ294664, CD937099, BG263054, CD937154, CD872079, BQ743581

CC-type TaGRX-γ2	CK204298, CK197418, BQ743171, CA730225
CC-type TaGRX-γ3	CA649437, CA608354, CA651675, CA613199, BE517713
CC-type γ singletons	CK197062, CA649509, CD883628, CK212584 CD927581, CA615423, BE405498, CA601590
CC-type TaGRX-β1	CJ734195, CA721726, CD927265, CJ731098
CC-type TaGRX-β2	CA688723, BQ161946, BQ162564, CK211838
CC-type TaGRX-β3	CK200465, CD930221, CD930222, AL830620, CK162039
CC-type TaGRX-β4	BE406860, CA732099
CC-type TaGRX-β5	BE444299, CJ581614, CJ688071
CC-type TaGRX-β6	BQ162737, CD869831, CD869021, BE405654, CK207645
CC-type TaGRX-β7	CD911592, CD911591
CC-type TaGRX-β8	CA647347, CA642465
CC-type β singletons	CD885077, CK200181, CD880395, DY543241, CV781115, BM138193, CA592978, CA486038, CA645134, CA652734
CC-type ϵ3 singletons	CA610028, CA608968, CA641396, CD871873
CC-type TaGRX-ϵ4-1	AJ601899, CV774915
CC-type TaGRX-ϵ4-2	CA689352, AJ601991
CC-type TaGRX-ϵ4-3	CJ703655, CJ597943
CC-type ϵ4 singletons	CA694798, CA680002
CC-type TaGRX-ϵ5-1	CN011047, CJ599840
CC-type TaGRX-ϵ5-2	CV779001, CD874485
CC-type ϵ5 singletons	CA742382, CA485458, CA658370, CA608403
CC-type δ1 singletons	BJ305759, BJ311346
CC-type α singleton	CO348297

Table S3. Oligonucleotide sequences (5'-3') used in this study

Primer name or target sequence	Restriction site	Primer sequence (5' to 3')	Amino acid coordinates
T7	N/A	TAATACGACTCACTATAGGG	N/A
Amplification of TaROXY1 for cloning into binary vector	<i>XbaI</i> <i>BspHI</i>	AAATCTAGAAATGCAGTACGGCGCGGCCGCGCCGAGCAG AAATCATGACTAGAGCCAGAGCGCGCCGGCCTCCTTGAG	Full length
Amplification of TaROXY1 for cloning into yeast 2-hybrid vector	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCATGCAGTACGGCGCGGCCGCGC AAAGGATCCCTAGAGCCAGAGCGCGCCG	Full length
BOP1	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCATGAGCAATACTTTCAAGAATCAC / AAAGGATCCCTAGAAATGGTGGTGGTGGTGATG	1-467 (Full length)
BOP2	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCATGAGCAATCTTGAAGAATCTTTGAG / AAAGGATCCCTAGAAGTGATGTTGATGATGGTGATGATAC	1-491 (Full length)
PAN	<i>MfeI</i> <i>BglII</i>	AAACAATTGATGCAGAGCAGCTTCAAAAC / AAAAGATCTCTAGAAGTGATGTTGATGATGGTGATGATAC	1-452 (Full length)
TGA3	<i>EcoRI</i> <i>SalI</i>	AAAGAATTCATGGAGATGATGAGCTCTTCTTCTTC / AAAGTCGACTTTACGTTGGTTCACGTTGCCTAGCC	1-385 (Full length)
TGA3Δ1	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCATGGAGATGATG / AAAGGATCCCTACTGAATTGCTA	1-305
TGA3Δ2	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCGCTCGCAAAAGTC / AAAGGATCCCTCAAGTGTGTTCTCG	110-385
TGA3Δ3	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCGCTCGCAAAAGTC / AAAGGATCCCTACTGAATTGCTA	110-305
TGA3Δ4	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCGCATTTGAGATG / AAAGGATCCCTCAAGTGTGTTCTCG	169-385
TGA3Δ5	<i>EcoRI</i> <i>BamHI</i>	AAAGAATTCATGGAGATGATG / AAAGGATCCCTATCCCTGCTG	1-146

Fig. S1. (a) A comparison of 3' ends of *ROXY-like* gene sequences from monocots. Sequences from *Brachypodium*, *Zea mays* (1; NM_001157291, 2; NM_001153687) and *Saccharum officinarum* (1; CA245702, 2; CA245703) used for the design of a reverse primer for amplification of *ROXY*-genes from wheat. The primer annealing position is also shown. (b) Amplification products after PCR for *ROXY*-like genes on wheat (cv. Cranbrook) gDNA using a range of annealing temperatures; 65 (lane 1), 66, 67, 68, 69, 70, 71 and 72°C (lane 8). A reaction was run (lane 9) with the absence of wheat gDNA as a control. Sizes of major bands of the molecular weight marker (GeneRuler) are given. (c) An informative site summary of ten *TaROXY1-α* clones. The highlighted base “G” at position 55 of B-7 is expected to be a sequencing-introduced error because of support by 11 other sites for grouping with A-4, A-8 and A-6. The star indicates that this insert sequence was used in complementation assays.



(c)

Gene	Clone	48	55	66	69	90	115	151	193	204	266	271	294
$\alpha 3$	A-4	G	A	A	A	G	G	A	G	G	T	A	C
	A-8*	G	A	A	A	G	G	A	G	G	T	A	C
	A-6	G	A	A	A	G	G	A	G	G	T	A	C
	B-7	G	G	A	A	G	G	A	G	G	T	A	C
$\alpha 1$	A-2*	G	A	G	G	A	C	G	G	A	G	T	G
	B-5	G	A	G	G	A	C	G	G	A	G	T	G
	B-2	G	A	G	G	A	C	G	G	A	G	T	G
	B-8	G	A	G	G	A	C	G	G	A	G	T	G
$\alpha 2$	A-1*	A	G	G	G	G	G	G	T	G	G	A	C
	B-9	A	G	G	G	G	G	G	T	G	G	A	C

Fig. S2. ROXY-like protein sequences from *Arabidopsis*, rice and *Brachypodium* aligned to a partial wheat sequence (CO348297, labelled TaROXY- α).

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TaROXY1- $\alpha$   MQYGA-AAEQAWYMPAM----PVTLAAETAARVERLASESAVVVFIVSS
BdGRX10    MQYGA-AAEQAWYMPSAIA--AVAAAPETAARVERLASESAVVVFSVSS
BdGRX11    MQY---AAEQAWYMPMGPA--AAASSMESAVARVERLASESAVVVFSVSS
OsROXY2    MQYGA-AAEQAWYMP--AA--APAPMVESAVARVERLASESAVVVFSVSS
OsROXY1    MQYGAAAAEQAWYMPAAAM--VAAAAETAARVERLASESAVVVFSVSS
ROXY1     MQYQTESWGS--YKMSLGFGLGMVADTGLLRIESLASESAVVVFSVST
ROXY2     MQYKTETRGSLSYNNNSKVMNMMNVFPSETLAKIESMAENAVVIFSVST
consensus  MQY...shtp..Y.....s.s...pps..+lephAUESAVVIFVSo

TaROXY1- $\alpha$   CCMCHAVKRLFQGMGVHPTVHELDLDPRGLELERALALDLCAGPG-GAA
BdGRX10    CCMCHAVKRLFQGMGVHPTVHELDLDPRGLELERALALDGGSGGAGAG
BdGRX11    CCMCHAVKRLFQGMGVHPTVHELDLDPRGRELERALALVAGFGAFGSAA
OsROXY2    CCMCHAVKRLFQGMGVHPTVHELDLDPRGRELERALALVGYGGPAAASP
OsROXY1    CCMCHAVKRLFQGMGVHPTVHELDLDPGRDLERALALVAGGGAA---A
ROXY1     CCMCHAVKRLFQGMGVSPAVHELDLHPYGGDIQRALIFLLGCS--GSSSP
ROXY2     CCMCHAVKRLFQGMGVSPAVHELDLHPYGVETHRALRLLLGSSGGATSP
consensus  CCMCHAVKRLFQGMGVSPAVHELDLHPYGVETHRALRLLLGSSGGATSP

TaROXY1- $\alpha$   PVV PVVFIGGKLVGAMDRVMAAHINGS-----
BdGRX10    GVV PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
BdGRX11    PVV PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
OsROXY2    PVV PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
OsROXY1    AAV PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
ROXY1     GSL PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
ROXY2     GAL PVVFIGGKLVGAMDRVMAAHINGSLVPLLKTAGALWL
consensus  ssl PVVFIGGKLVGAMDRVMAAHINGS.....

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Fig. S3. *Arabidopsis* CC-type GRX gene expression measured by microarray. **(a)** *ROXY19* is strongly up-regulated by cycloheximide, salicylic acid, methyl jasmonate exposure, while *ROXY18* is up-regulated in response to salicylic acid. **(b)** *ROXY19* is salt responsive specifically in roots.

