Expression profiling of receptor-like cytoplasmic protein kinases (class VI) of Arabidopsis

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INTRODUCTION

Receptor protein kinases are represented by a large gene family in Arabidopsis. Recent investigations have provided experimental evidence that these proteins play important roles in signal transduction during a variety of plant processes including plant growth and development.

In plants, there are two different types of receptor kinases, including receptor-like serine/threonine (Ser/Thr) kinases (RLKs) and receptor histidine (His) kinases. Plant Ser/Thr receptor-like kinases are coded by a large, monophyletic RLK/Pelle gene family with more than 610 members. The family includes genes coding for receptor kinases and non-receptor kinases such as receptor-like cytoplasmic kinases (RLCKs) as well (Figure 1, for further details see References).

Cytoplasmic RLKs represent approximately 25% of the RLK family and contain only a kinase domain, with no apparent signal sequence or transmembrane domains, and thus were collectively named receptor-like cytoplasmic kinases (RLCKs) (**Figure 2**). RLCKs form 10 families (I-X) with 193 protein coding genes.

Concerning the function of plant receptor-like cytoplasmic kinases, at the present only few members have been characterized and it is very likely that they play major role in the transmission of external signals perceived by RLKs. Moreover, based on our current experimental results we suppose that at least some of these cytoplasmic kinases (belonging to RLCK class VI) are RhoGTPase-dependent considering their signal transduction activities. Plant specific RhoGTPases are versatile molecular switches in many processes during plant growth, development and responses to the environment and thus a possible implication of RLCKs in these signaling transduction pathways is of central importance. RhoGTPase-mediated activation os kinase cascades is well known from yeast and animal cells, but has not been described in plants unitl now.

As part of our investigations related to RhoGTPase-mediated signal transduction in plants, we started to characterize the whole RLCK VI protein family in *Arabidopsis*. This family consists of 14 members and our first aim was to determine the expression profile of the genes coding for these proteins in order to find possible roles for the members of this kinase family in plant development as well as in the transduction of signals resulting from various stresses and hormones.

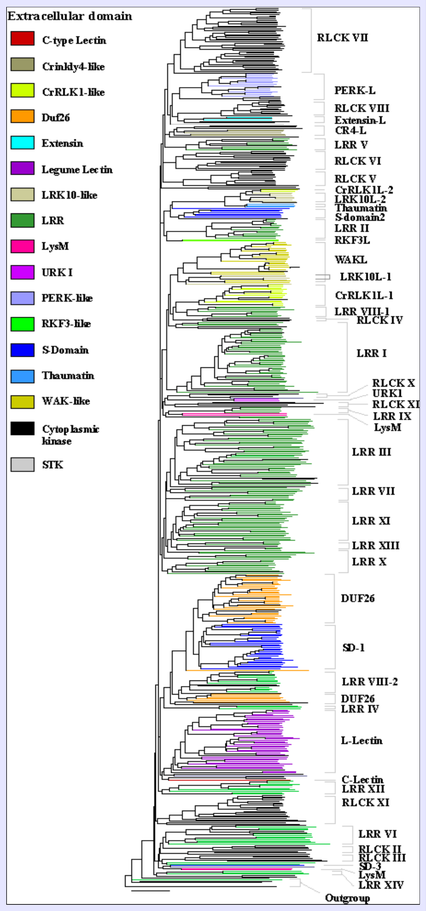

Figure 1: RLK phylogeny based on the kinase domain aminoacid sequences

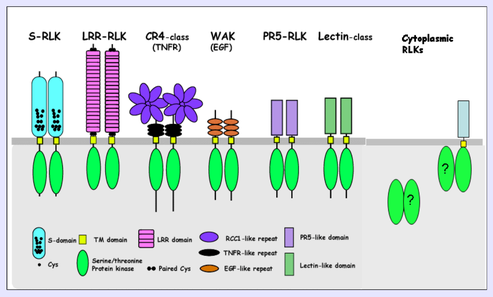

Figure 2: Classification of RLKs in plants based on domain structure

THE EXPERIMENTAL APPROACH

The technique of real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to determine the transcript levels in Arabidopsis seedlings under a series of abiotic stress/hormone treatments as well as in different organs. Quantitative gene expression data were normalized for the expression of GAPDH-2 and actin (Act2/Act8) genes.

In order to help the interpretation of the results, the expression of stress-responsive control gene, RD29A, was also determined.

CONCLUSIONS

Class VI of receptor like cytoplasmic kinases in *Arabidopsis* displayed significant variations in their transcript levels in specific organs and in response to different treatments applied. We conclude that there are several members of the kinase family (RLCK class VI) which may have primary roles during plant development (e.g. flowering, root development etc.) and a few member can have roles in the transduction of signals resulting from various stresses and hormones. Furthermore, huge differences could be observed among the genes considering their expression in continuously dividing suspension cultured cells.

Based on the expression data it can be concluded that the biological functions of RLCK proteins, despite their structural similarity, might be highly divergent and is regulated, at least partly, at the transcriptional level.

Our further studies aim to reveal functional significance of this high gene expression variabilty and its implications for RhoGTPase mediated signal transduction pathways in plants.

RESULTS

Variations of relative transcript abundance among the RLCK class VI family genes in *Arabidopsis* organs

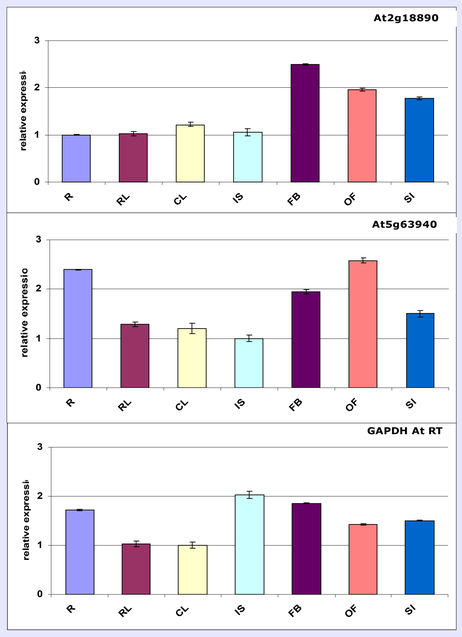

Figure 3: Relatively constant level of transcripts for all the organs tested

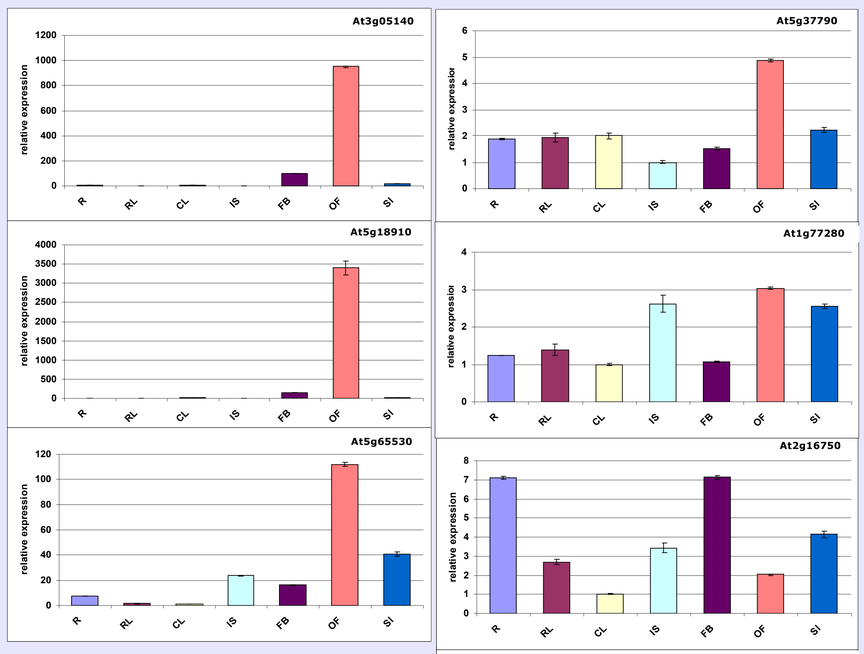

Figure 4: High and moderate level of transcripts in reproductive organs

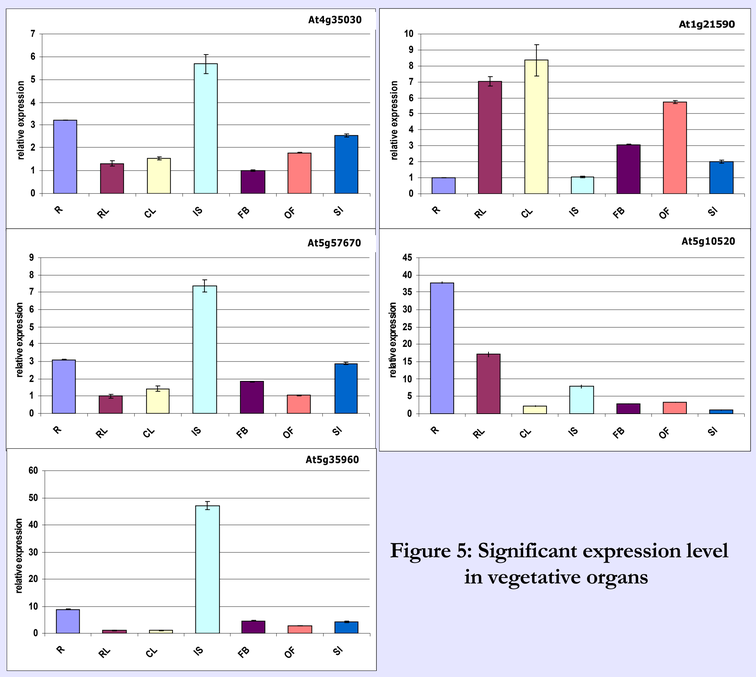

Figure 5: Significant expression level in vegetative organs

The transcript levels have been tested on RNA extracted from roots (R), rosette leaves (RL), cauline leaves (CL), inflorescence stems (IS), flower buds (FB), open flowers (OF), siliques (SI), and seedlings (S).

The relative transcript levels are shown as compared to the lovest expression level (value 1).

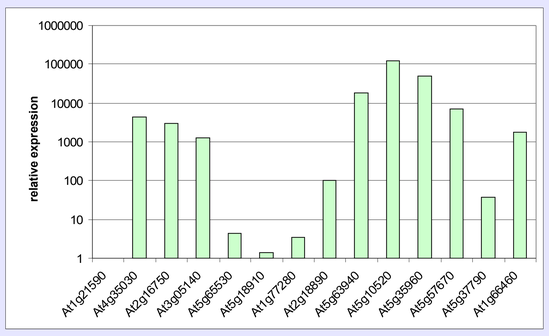

Figure 6: High differences of the transcript level among the RLCK VI class in dividing suspension cultured cells (related to lovest expression of the At1g21590 gene as value 1)

The transcriptional response of the RLCK class VI family genes in seedlings undergoing abiotic/chemical treatments

Gene ID	Treatment - 6h								
	Natrium Chloride (NaCl)	Glucose	Sucrose	Polyethilene glicol (PEG)	Etephon	Paraquat	Salicilic acid (SA)	Abscisic acid (ABA)	Cold (4°C)
At5g57670	+(11,19)	+(4,68)	+(16,65)	+(14,42)	+(4,43)	+(6,16)	+(3,80)	+(4,29)	0
At1g66460	+(9,75)	+(7,00)	+(19,13)	+(18,19)	+(7,08)	0	+(11,04)	+(4,16)	0
At5g65530	+(14,32)	+(4,91)	+(7,71)	+(7,89)	+(6,18)	+(7,46)	+(4,21)	+(5,36)	0
At4g35030	+(3,98)	+(2,79)	+(4,75)	+(5,12)	+(2,51)	+(3,04)	0	+(6,07)	0
At2g16750	0	0	+(2,69)	+(2,85)	0	+(2,27)	0	0	0
At2g18890	0	0	0	+(2,18)	0	0	0	+(8,44)	+(2,87)
At5g37790	0	0	+(2,32)	0	0	0	0	+(2,69)	0
At1g77280	0	0	0	0	0	0	0	0	0
At3g05140	0	0	0	0	0	0	0	0	0
At1g21590	0	0	0	0	0	0	0	0	+(2,13)
At5g63940	0	0	0	0	0	0	+(2,10)	0	0
At5g10520	0	0	0	0	0	0	0	-	-
At5g18910	-	-	-	0	-	-	-		-
At5g52310 (RD29A)	+(941,63)	+(38,04)	+(111,78)	+(119,39)	+(11,31)	+(29,85)	+(137,14)	+(1127,42)	+(167,43)

Table 1: Variations in the transcript levels of the investigated genes 6 hours after the treatments as compared with the non-treated control

(no detectable signals could be obtained in this experiments for the gene At5g35960)

Legend: 0, variation between 0,5 to 2,0 fold (no significant change); +, more than 2.0 fold increase; -, more than 2 fold decrease; (n), n-fold increase; The experimental conditions were validated through the stress marker gene *RD29A*.

Treatments applied in the experiments

* natrium chloride (NaCl, 300 mM)
* glucose (300 mM)
* sucrose (8%)
* polyethilene glycol (PEG, 30%)
* etephon (Et, 10 mg/l)
* paraquat (PQ, 25µM)
* salicylic acid (SA, 10 mM)
* abscisic acid (ABA, 100µM)
* cold treatment (4°C)

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