Distinct evolution rate between receptor-like kinases and receptor-like proteins

Introduction

Plants are exposed constantly to the attacks from potential virulent microbes. As the sessile organisms, plants have developed innate immunity to detect and fight against the invading pathogens in order to survive.

Three components are involved in plant resistance and susceptibility to disease. Firstly, microbes have pathogen-associated molecular patterns (PAMPs) – the conserved components from pathogens, such as bacterial flagellin and fungal chitin, which plants have evolved the capacity to recognize and respond to, and this results in PAMP-triggered immunity (PTI) (Silke et al. 2002). Secondly, to achieve successful invasion, pathogens make and deliver effector proteins into plant cells to suppress PTI (Thomas et al. 2009). Thirdly, plants have employed a repertoire of resistance (R) genes that encode guarding proteins that sense and recognize specific pathogen effectors, either directly or indirectly via effector action on host cellular components. R gene-dependent recognition results in effector-triggered immunity (ETI) (Thomas et al. 2009), resulting in restored resistance. To understand resistance, we need to better understand PTI, not only because of its intrinsic interest and important role in crop protection, but also because host components involved in PTI are targets of pathogen effectors.

To trigger PTI, PAMPs from microbial pathogens need to be perceived by cell surface receptors (Silke et al. 2002), known as pattern recognition receptors (PRRs). PRRs include receptor-like kinases (RLKs) and receptor like proteins (RLPs). RLKs and RLPs both contain a single transmembrane domain, an extracellular ligand binding domain. Detection or binding of PAMPs will trigger intracellular responses transduced by the RLKs cytoplasmic Ser/Thr kinase domain (Christiaan et al. 2012) which is absent in RLPs. Instead, RLPs are likely to transduce signals by recruiting RLKs partners upon ligand binding. Being the most frontiers to detect the existence of potential invaders by binding PAMPs, RLKs and RLPs are supposed to be facing similar selective pressure. On the other hand, the intracellular domain of RLKs is involved in signal transduction and interactions with different partners upon different stimuli, as well as becoming the targets of effectors of pathogens, all of could render RLKs to a higher selective pressure (Mariana et al. 2005).

In this study, all the available validated RLKs and RLPs protein sequences in different plant species are collected from Plant Resistance Gene database (PRGdb). The sequences are then analyzed to determine the evolution rate of RLKs and RLPs with regards to the similarities and dissimilarities in their roles in recognizing PAMPs and downstream signaling initiation.

Methods

Sequence Alignment

All the protein sequences downloaded from PRGdb were aligned using Mafft installed in HPC-class (Iowa State University) with "auto" strategy and default setting of BLOSUM62 model and gap penalty -1.53, +0.00, +0.00.

Construction of Phylogenetic Tree

Neighbor-joining tree was constructed using online FastME program available at the ATGC bioinformatics platform website. RLKs and RLPs amino acid sequence alignment file was uploaded for FastME with substitution model LG and CpRev, respectively, Gamma distribution rate across the sites and tree refinement with SPR were chosen to run the program. Random sampling using bootstrap was used to evaluate the support of clade in inferred neighbor-joining tree.

Result

Phylogenetic analyses reveal different evolution rates between RLKs and RLPs

A total of 23 protein sequences of the receptor-like kinases (RLKs) and receptor-like proteins (RLPs) are downloaded from PRGda. Genes encoding the 23 RLKs and RLPs have been investigated with published data supporting their roles in recognizing PAMPs and therefore the participation in PTI response. Detailed information about the corresponding interactive pathogens and the related diseases is provided from PRGda website. The inferred neighbor-joining (NJ) tree by FastMe suggests distinct evolution rates between RLKs and RLPs (Fig.1). With LG model, the overall amino acid substitution rate indicated by branch length shows that RLKs have a significantly higher substitution rate than that of the RLPs, ranging from 0.93 of Xa26 RLK in *Oryza sativa* to 2.43 of RPG1 RLK in *Hordeum vulgare*. In contrast, the branch length of most RLPs members are from 0.00 to 0.15. There are exceptions from both groups: RLKs BAK1 from *Arabidopsis thaliana* and related SERKs from *Nicotiana benthamiana* show very low level of substitution rate, similar to that of the RLPs members; meanwhile, two proteins of RLPs - PGIP and Hp-1 have much longer branch lengths compared to the other RLPs. Similar result was observed by NJ tree with CpRev substitution model (Fig.2).

RLKs intracellular domain is not responsible for the higher substitution rate

The extracellular domain of RLKs and RLPs contain multiple leucine rich repeat (LRRs) units for ligand recognition and perception (Shin-Han et al. 2001). Besides the extracellular domain, both RLKs and RLPs contain a similar single transmembrane domain. However, the cytoplasmic kinase domain for initiating downstream signal transduction in RLKs is absent in RLPs. RLPs contain a short cytoplasmic tail without any identified functional domain. To investigate whether the intracellular kinase domain of RLKs, which might be under positive selection, contribute to the higher substitution rate in RLKs, the kinase domain and the adjacent N terminal amino acids are removed from all RLKs protein sequences. The trimmed RLKs amino acid sequences containing extracellular domain, transmembrane domain and a short

intracellular tail are re-aligned with RLPs sequences to infer the NJ tree using exactly the same parameters as the one used for full sequence alignment and NJ clustering. Surprisingly, although the topology looks different between the "new" and the "old" NJ tree, the branch length of most RLKs remain significant higher than RLPs (Fig. 3 and Fig.4), indicating that kinase domain is not responsible for the higher substitution rate among RLKs receptors.

Discussion

The neighbor-joining tree using protein sequences of RLKs and RLPs involved in plant PTI response reveals distinct evolution rate between RLKs and RLPs. More than half RLKs sequences tested in this analysis have longer branch lengths. In contrast, the branch lengths of all the RLP protein sequences are significantly shorter, indicating a much lower substitution rate among RLP members. 3 sequences in RLKs show extremely short branch length are BAK1 from *Arabidopsis thaliana* and Serk3A andSerk3B from *Nicotiana benthamiana*. The branch lengths of BAK1, SERK3A and SERK3B are similar to those of the RLPs. Compared to other RLKs, BAK1 has less LRR repeats and may not be involved in direct ligand perception. Growing evidence shows that BAK1 can be instantaneously recruited by other RLKs, FLS2 for example. It acts as a co-receptor of FLS2 and is required for the full activation of FLS2 triggered immunity (Antje et al. 2007, Yadong et al. 2013). Notably, the closely related SERK proteins have gain increasing support to associate with RLKs and regulate their function, suggesting their role as co-receptors upon ligand binding. RLPs are capable of binding PAMPs directly, however, they also act as co-receptors in a way that additional RLKs are required to transduce the signals upon ligand perception. The role of RLPs, RLK BAK1 and SERKs acting as co-receptors may somehow distinguish them from other RLKs facing distinct selective pressure. NJ tree constructed using RLKs partial amino acid sequences after removing the intracellular kinase domain sequences indicate the kinase domain alone doesn't contribute to the relatively fast evolution rate of RLKs. The distinct substitution rate may be a result of dominant and ancillary roles in signal transduction and PTI initiation between different receptor-like kinases and receptor-like proteins. Further investigations need to be done, and meanwhile, more RLKs and RLPs to be identified and included for further analysis will help address this question.

Reference

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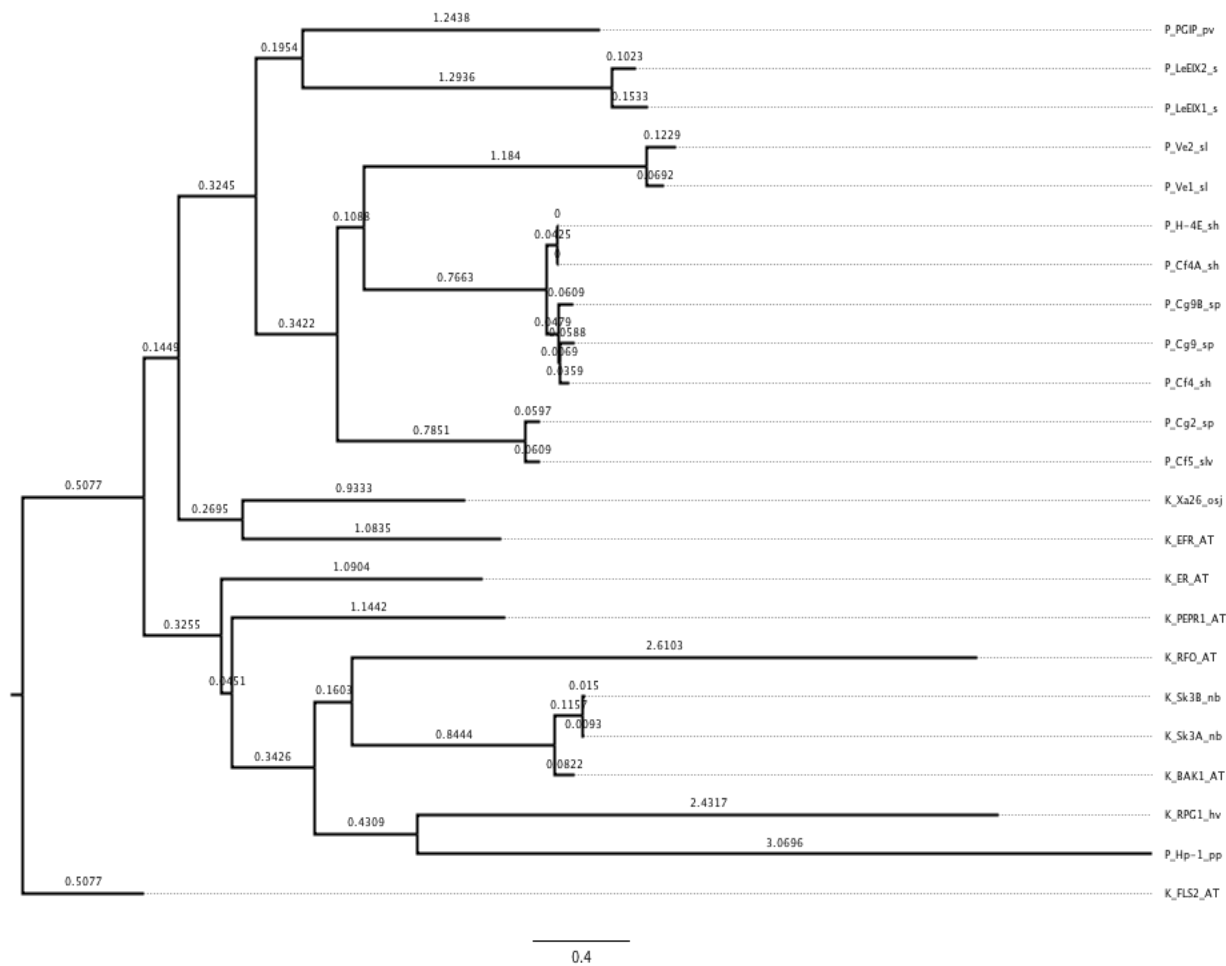
Figures

Fig. 1 Neighbor-joining tree using full length protein sequence with substitution model LG
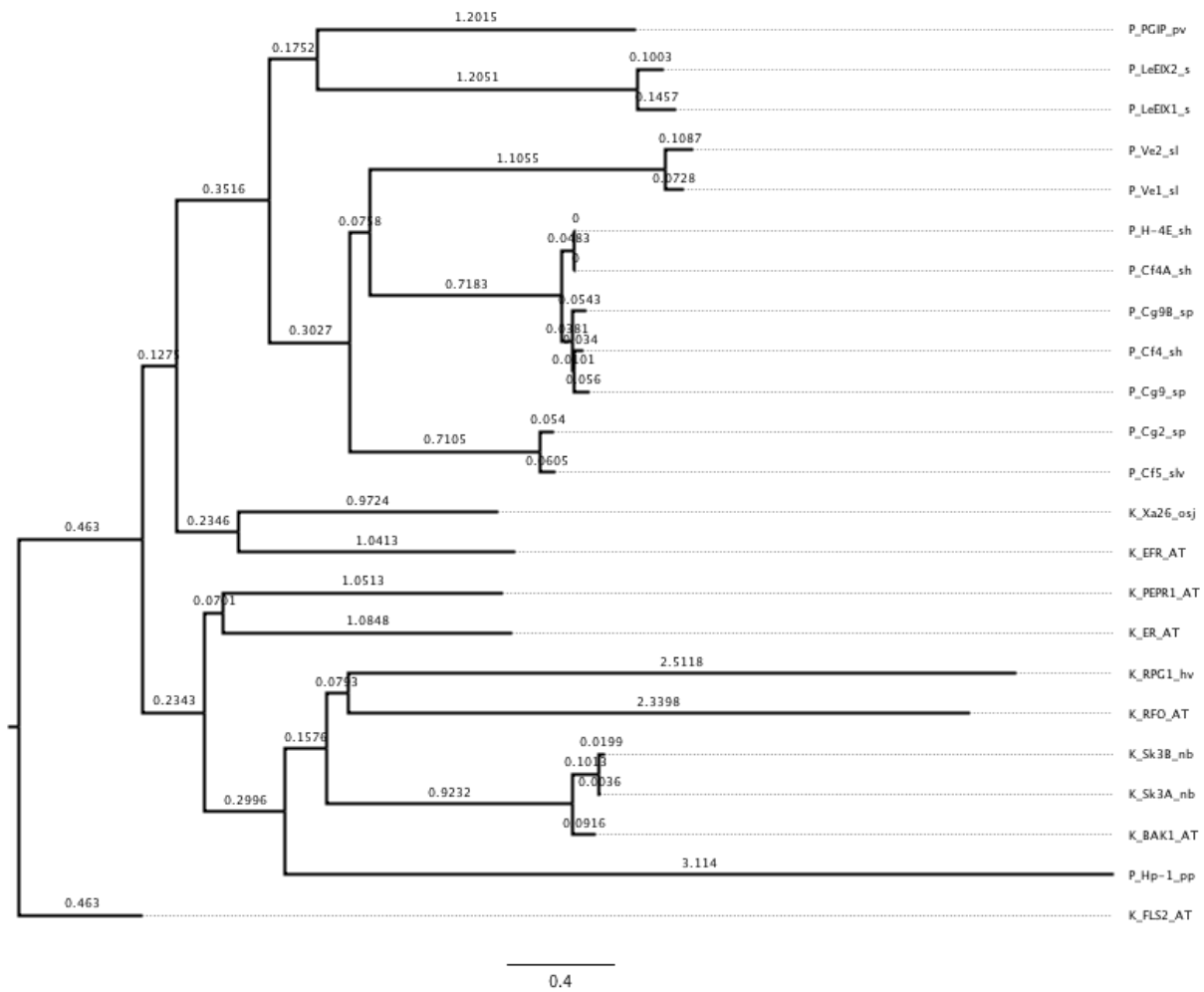
Fig. 2 Neighbor-joining tree using full length protein sequence with substitution model CpRev

1.2356

2.9778

0.5708

K_EFR_AT

P_Hp-1_pp

0

K_Sk3B_nb

1.2138

0.0538

K_Sk3A_nb

0.1375

1.0958

P_PGIP_pv

0.0797

P_LeEIX2_s

0.194

1.4369

0.1775

P_LeEIX1_s

0.2052

0.0913

P_Ve2_sl

1.228

0.1021

P_Ve1_sl

0.5545

0.1548

0.0554

P_Cg2_sp

0.7082

0.0652

P_Cf5_slv

0.0942

0.196

0

P_H-4E_sh

0.0552

P_Cf4A_sh

0.8154

0.0457

P_Cg9B_sp

0.0551

0.0633

P_Cg9_sp

0.0122

0.0314

P_Cf-4_sh

0.0692

1.2755

K_Xa26_osj

0.196

0

K_BAK1_AT

1.286

0.0014

K_ER_AT

1.1366

K_PEPR1_AT

0.222

1.888

K_RPG1_hv

1.2395
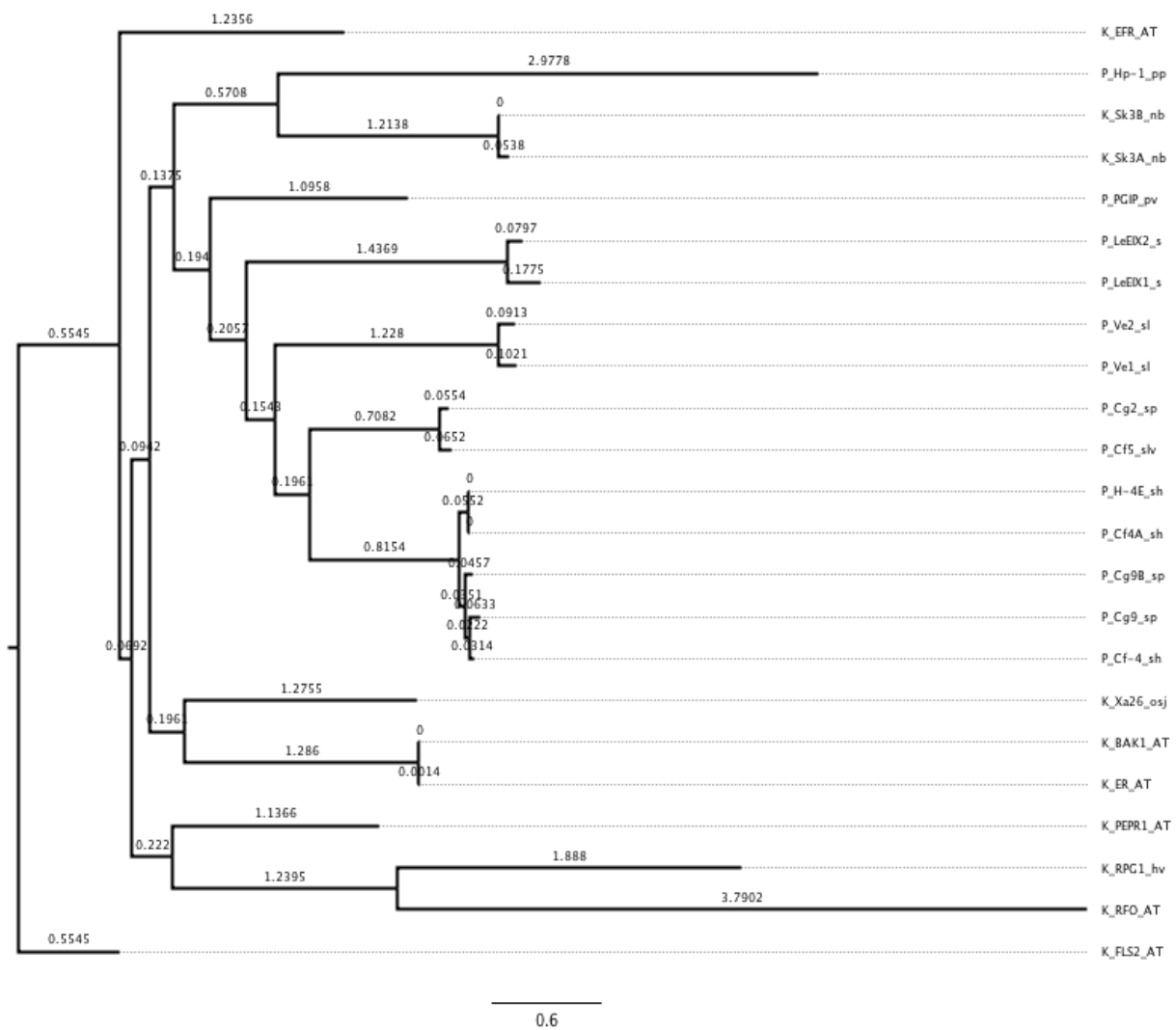
3.7902

K_RFO_AT

0.5545

K_FLS2_AT

0.6

Fig. 3 Neighbor-joining tree using truncated RLKs protein sequence and full length RLPs protein sequence with substitution model LG
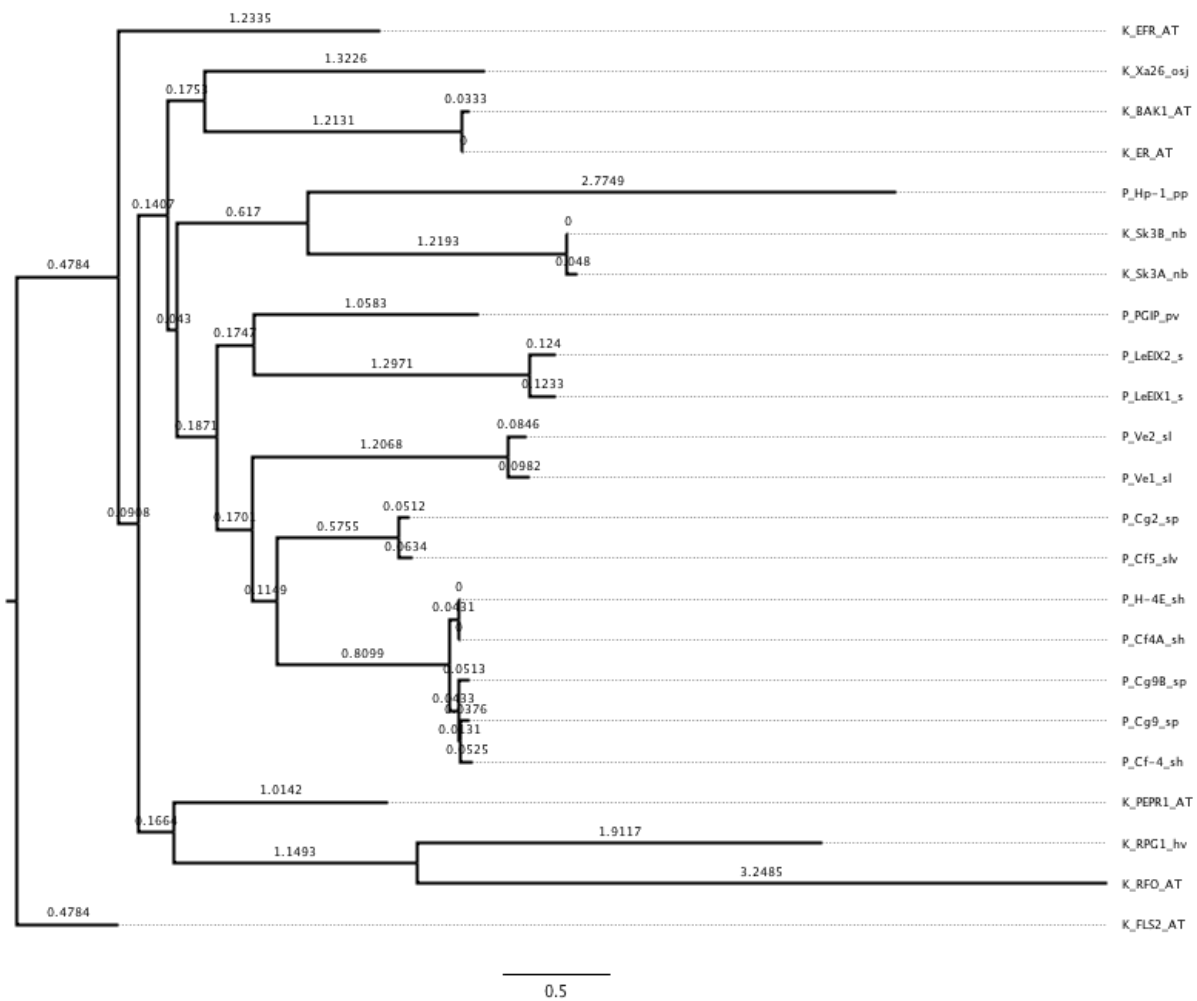
Fig. 4 Neighbor-joining tree using truncated RLKs protein sequence and full length RLPs protein sequence with substitution model CpRev