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# Phylogeny of *Alternaria* and Evolution of Pathogenicity on *Arabidopsis*

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## Abstracts

Genes for *Alternaria* major allergen, Alt a 1 (*alt a 1*) and glyceraldehydes-3-phosphate dehydrogenase (*gpd*), were amplified from 52 species of *Alternaria* and related genera, and sequence information was used to reconstruct phylogenetic relationships of *Alternaria*. Alt a 1 gene sequences evolved 3.8 times faster and contained 3.5 times more parsimony-informative sites than *gpd* sequences. Analyses of Alt a 1 gene and *gpd* exon sequences strongly supported grouping of *Alternaria* spp. and related taxa into several species-groups described in previous studies, especially the infectoria, alternata, porri, brassicicola, and radicina species-groups. The sonchi species-group was newly suggested in this study. Based upon the phylogeny of *Alternaria*, 15 species from five species-groups were selected for the test of pathogenicity on *Arabidopsis*. Among them, *A. arborescens, A. longipes, A. brassicicola, A. japonica,* and *A. solani* appeared to cause severe pathogenic symptom on *Arabidopsis* leaves. They were not closely related phylogenetically, in locality of collection, and in original hosts. PR gene expressions were critically different in three ecotypes of *Arabidopsis* against different *Alternaria* species, implying that differentiated mechanism of pathogenicity and defense responses.

## Backgrounds

*Alternaria* is a fungi classified in Pleosporaceae, Dothidiales of Asocomycota. *Alternaria* species are well-known plant pathogens causing diseases on a variety of plant species. The mechanism of pathogenicity of *Alternaria* on *Arabiodopsis* has been studied with *Alternaria brassicicola* as a model system. From the studies, it was revealed that defense response of *Arabidopsis* against *A. brassicicola* was majorly mediated by jasmonic acid signaling pathway and camalexin, a phytoalexin. On the other hand, studies of *Alternaria alternata* causing diseases on pear, strawberry, and tangerine revealed that *Alternaria* species produce host-specific toxins and have different host ranges among very closely related

species. To understand evolution of plant pathogenicity of *Alternaria*, it is required that pathogenic and defense response mechanisms are comparatively studied with the context of species and gene evolution.

In this study, phylogeny of *Alternaria* was reconstructed with sequence information of *alt a 1* and *gpd* genes. Disease development on *Arabidopsis thaliana* was examined for selected 15 species of *Alternaria* from 5 species-groups and PR gene expression was compared to examine differential defense response of three ecotypes of *A. thaliana* against *Alternaria* species.

## Results and Discussion

Alignment of Alt a 1 exon sequences resulted in a 419-character dataset of which 247 characters (58.9%) were variable and 190 characters (45.3%) were parsimony-informative. Alignment of *gpd* exon sequences resulted in a 409-character dataset of which 81 characters (19.8%) were variable and 54 characters (13.2%) were parsimony-informative. Parsimony analysis based upon combined data set of alt a 1 and gpd exon sequences (ILD test, $P = 0.09$) resulted in 6 most parsimonious trees (Fig. 1), which differ primarily in the position of *A. mouchaccae* and relationships among members of the infectoria species-group. Monophyletic grouping of the alternata, infectoria, porri, radicina, and sonchi species-groups and the *Embellisia* and *Nimbya* groups were supported by high bootstrap values (>89%). Bootstrap supports for brassicicola species-group and the *Ulocladium* group were low (62% and <50%, respectively). The *Ulocladium* group was divided into two monophyletic groups, one of which was composed of *A. cheiranthi, E. indefessa*, and *U. chartarum* and the other was composed of *U. cucurbitae*, *U. botrytis*, and *U. atrum*. ML analysis of the combined data set of alt a 1 and gpd exon sequences revealed a tree with similar topology as that revealed in Fig. 1 with notable exception in the position of *Ulocladium* group, which was placed as a sister taxon to the group comprising the infectoria species-group, *Embellisia* group, *Nimbya* group, *U. alternariae*, and *A. argyranthemi* (data not shown). NJ analysis of the combined data set of alt a 1 and gpd exon sequences also revealed a tree with similar topology as that revealed in Fig. 4 with notable exception in the position of brassicicola species-group, which did not cluster with radicina species-group but instead was placed as a sister taxon to the group comprising alternata, porri, radicina, and sonchi species-groups, and the *Ulocladium* group (data not shown). Branches strictly conserved in all three phylogenetic analyses are shown as bold branches in Fig 1.

In order to evaluate the evolutionary rates of the Alt a 1 and *gpd* genes, pairwise distance values of exon and intron regions for each gene were calculated and compared to each other. By comparing distance values from exon regions, it was revealed that the evolutionary rate of the Alt a 1 gene was 3.8 times faster than the *gpd* gene. When distance values of the intron regions were compared to the exon regions, it was apparent that intron sequences evolved much faster. However, the difference in

evolutionary rate between intron and exon regions was not the same for the Alt a 1 gene the *gpd* gene. The intron of the Alt a 1 gene evolved 2.7 times faster than the exon regions and introns of the *gpd* gene evolved 6.1 times faster than the exon regions. When the intron of the Alt a 1 gene and introns of the *gpd* gene were compared, it was revealed that the Alt a 1 intron evolved 1.7 times faster than *gpd* introns. To examine the distribution of variable sites of each gene, parsimony step numbers over five base windows were depicted. From the diagram, it is evident that sequence variation is distributed evenly throughout both genes except in intermittent conserved sites.The high evolutionary rate of intron domains is apparent from the diagram.

All of the 15 species selected to examine disease development on *Arabidopsis* leaves caused certain level of disease symptoms with a varying degree of lesion size, discoloration, and hyphal growth on the leaves. Among them, *A. arborescens, A. longipes, A. brassicicola, A. japonica*, and *A. solani* caused most severe disease symptoms. The severity of pathogenicity of *Alternaria* was not closely related to the phylogenetic relationships, locality of strain collection, or original host species. Three accessions of *Arabidopsis* showed different defense responses. Col-0 accession usually developed small spot

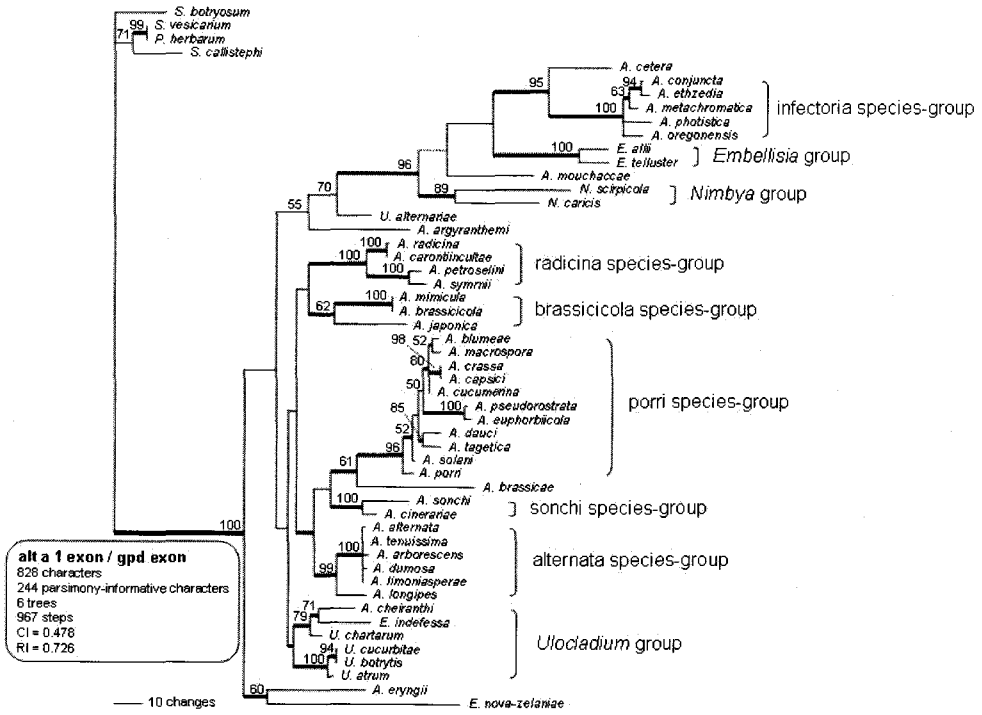


Fig. 1. One of six equally parsimonious trees of combined data set based upon Alt a 1 exon and *gpd* exon sequences. Broken lines represent branches that were not conserved in a strict consensus tree of six equally parsimonious trees and thick lines represent branches conserved in in analyses using distance, parsimony, and ML methods. Numbers represent parsimony bootstrap values from 1000 replicates. The scale bar indicates the number of nucleotide substitutions. (From Fungal Genetics and Biology (2005) 42: 119-129)

lesions, Ler accession usually developed larger transparent lesions, and WS-2 accession developed mix of small spots, moderate-sized black lesions, and larger transparent lesions. Difference of defense responses among three accessions was related to the different expression of PR genes. Accession Ler expressed PR1 gene in high level, which is involved in salicylic acid signal transduction pathway and PR3 gene in low level, which is involved in jasmonic acid pathway, except in *A. brassicicola* and *A. japonica*. In contrast, accession col-0 expressed PR3 highly but PR1 in low level. Considering the independency disease development by *Alternaria* species from phylogenetic relationships and species origin, and different induction of PR genes in *Arabidopsis*, it is proposed that different mechanisms are involved in disease development, which might be acquired independently.