Pollen mRNAs of *Phalaris coerulescens* and their Possible Role in Self-Incompatibility

by

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Summary

Self-incompatibility is a highly specific recognition system between pollen and pistil which acts as a barrier to self-fertilisation. In *Phalaris coerulescens*, gametophytic self-incompatibility is under the control of two unlinked genes, S and Z. An incompatible reaction occurs when both the S and Z alleles of the pollen are present in the genotype of the recipient stigma. In this very rapid reaction either pollen grains fail to germinate or pollen tube growth is arrested shortly after contact with the stigma.

This study examined the contribution of genes specifically expressed in the male gametophyte to the physiological processes during pollen tube germination and pollen-stigma interaction. Among the aims of this study was the isolation of the Z-gene.

To this end, selected cDNA clones from a pollen mRNA library were differentially screened with RNA from non-pollen tissues and pollen RNA from plants of different Z-genotypes. Clones which showed differences in hybridisation intensity with different Z-alleles and were pollen-specific were grouped depending on their behaviour in cross-hybridisation studies. Representative clones of each of the eighteen subgroups were subsequently analysed for linkage to the Z-locus in RFLP studies. However, none of the clones analysed showed linkage to Z.

To investigate the possible role of these 18 cDNA clones in pollen function, their temporal and spatial expression was analysed and their inserts were either partially or fully sequenced. The results of the transcriptional analysis revealed that the majority of the cDNAs were indeed predominantly expressed in pollen, as anticipated from the result of the differential screen. A comparison of their expression in pollen from plants with different self-incompatibility genotypes, including self-fertile mutants, revealed various degrees of variation in expression level but no correlation with the S-Z genotypes of the pollen parents was indicated.

In one case, clone B7b, the transcripts were completely absent in two self-fertile mutants. Further characterisation of B7b showed that the clone represents a gene-family with at least 15 members, which are closely linked since they were inherited as a single locus in a segregation analysis. DNA blot analyses revealed that the lack of B7b-transcripts in the two self-fertile mutants was correlated with the absence of the corresponding DNA sequences. However, examination of a large number of plants identified more, including self-incompatible wildtypes, in which the B7b family was not detectable. It was therefore suggested that the apparent absence of the gene-family and a self-fertile phenotype are independent phenomena. An open reading frame was found in 5 out of 9 cDNAs sequenced. The deduced protein sequences indicated the presence of a putative signal peptide and demonstrated the presence of a new cysteine-motif. Database searches failed to

show any homology to known sequences. Possible functions for this gene-family are discussed.

Sequence analysis of the other 17 cDNA clones revealed sequence homology of 3 clones to protein kinases, among them a MAP-kinase, a calcium-dependent calmodulin-independent protein kinase and a kinase with homology to the *Pto/Fen* family. In addition, a fructose-1,5-phosphate aldolase, a *LAT52* homolog and a putative glucose-transport protein were identified. The remaining 12 cDNAs did not show any homology to sequences in the databases and are likely to represent new genes.

The involvement of protein kinases in the self-incompatibility reaction of *Phalaris* was addressed by examining phosphorylation of pollen proteins from self-incompatible and self-fertile mutant plants to determine whether the self-compatible phenotype was correlated with failed phosphorylation of any proteins. The results showed that this was not the case with any of the self-fertile mutants of *Phalaris*. Using immunodetection and immuno-precipitation, it was demonstrated that the S-protein is not phosphorylated during pollen germination *in vitro*. Nor was any evidence obtained for the phosphorylation of the S-protein in response to stigma proteins. In addition, an experimental system was developed which allows direct monitoring of protein phosphorylation during pollen germination and tube growth.