SUMMARY

The *NGATHA* (*NGA*) gene family is composed of four highly related genes: *NGA1*, *NGA2*, *NGA3*, *NGA4*, which encode transcription factors with a B3 DNA-binding domain. The *NGA* genes form a subgroup within the *RAV* clade in the *B3* gene family, characterized by the lack of the AP2 domain which is present in the other *RAV* genes (Alvarez et al., 2006).

The *NGA* genes are functionally redundant in the development of the style and the stigma in a dose-dependent manner. While the single mutants in the *NGA* genes only show very subtle or no defects in carpel morphology, the multiple mutants exhibit greater defects in the development of the apical part of the gynoecium, which is completely altered in the quadruple mutant. This phenotype is related with the lack of activation, in the apical part of the gynoecium, of the *YUCCA2* and *YUCCA4* (*YUC2/4*) genes, which encode enzymes involved in auxin synthesis. The phenotype of the *nga* mutants is very similar to that of the mutants in the *SHORT INTERNODES/STYLISH* (*SHI/STY*) genes, which encode RING-like zinc-finger type transcription factors. It has been shown that NGA and STY work together to promote style specification, directing the YUC-mediated auxin synthesis in the apical part of the gynoecium (Trigueros et al., 2009). In addition, the phenotypes of the *nga* mutants and of the plants over-expressing *NGA*, resemble those observed in genotypes with altered expression of genes with relevant functions in gynoecium morphogenesis such as *FRUITFULL* (*FUL*), *HECATE* (*HEC*), INDEHISC*E*NT (*IND*), *SPATULA* (*SPT*), etc. Thus, it can be inferred that the *NGA* genes have a key role in gynoecium morphogenesis, though their genetic interaction with the network of genes controlling this process is still unclear; therefore, in this thesis we have carried out a detailed genetic analysis to determine the position and role of *NGA* genes in this network.

With this aim, our first approach has been to identify and characterize regulators of the expression of *NGA* genes. As the four *NGA* genes show almost identical expression patterns, it seemed likely that they shared common regulators. For that reason, we made a bioinformatic analysis of the promoter regions of the *NGA* genes looking for conserved regions. In this analysis we identified a 270 bp region conserved in the four promoters; moreover, a 50 bp subdomain with very high similarity among the four promoters was found in this region. Interestingly, this region also shows similarity with a fragment in the promoter of the *STY1* gene, whose expression pattern is very similar to that of the *NGA* genes and that, therefore, could also share regulators with those genes. We have used these conserved domains to carry out yeast one-hybrid screenings of Arabidopsis cDNA libraries. These screenings led to the identification of a transcription

factor from the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) family of and of three transcription factors from the *TEOSINTE BRANCHED1, CYCLOIDEA, AND PROLIFERATING CELL FACTORS* (*TCP*) family as candidates to regulate *NGA* expression. After the identification of these candidates, different molecular analysis have been carried out to confirm the binding of these transcription factors to the promoters of the *NGA* genes. To validate their genetic interaction we have also characterized mutants and over-expression lines of these candidates, as well as genetic combinations of these mutants with reporter and loss- and gain-of-function lines of the *NGA* genes.

In addition to the identification of regulators, in this thesis we were interested in analyzing in detail the genetic interactions of *NGA* genes with other genes with a key role in the development of the gynoecium apical tissues. We had observed that over-expression lines of *HEC1*, *HEC3* and *NGA3*, showed similar phenotypes in the fruit: reduced ovaries and enlarged apical regions and gynophores, similar phenotypes to those of mutants affected in auxin signaling. For that reason, a great part of our analysis has been focused to elucidate the functional relationship between *NGA* and *HEC*. To study the regulatory hierarchy between both factors we have carried out genetic analysis combining reporter lines of these genes with mutants in *NGA* or *HEC* or with their over-expression lines. We have also carried out several molecular assays which had allowed us to conclude that both factors act at the same level, forming part of a transcriptional complex with cooperative activity.

Finally, this thesis shows how, though similar genetic and molecular analysis in which other genes of the bHLH transcription factor family have been included, we have inferred the participation of the NGA proteins in a high-order complex, possibly composed by NGA, HEC and the IND and SPT proteins, which would be required for correct auxin signaling during gynoecium morphogenesis and stigma development in *Arabidopsis thaliana*.