

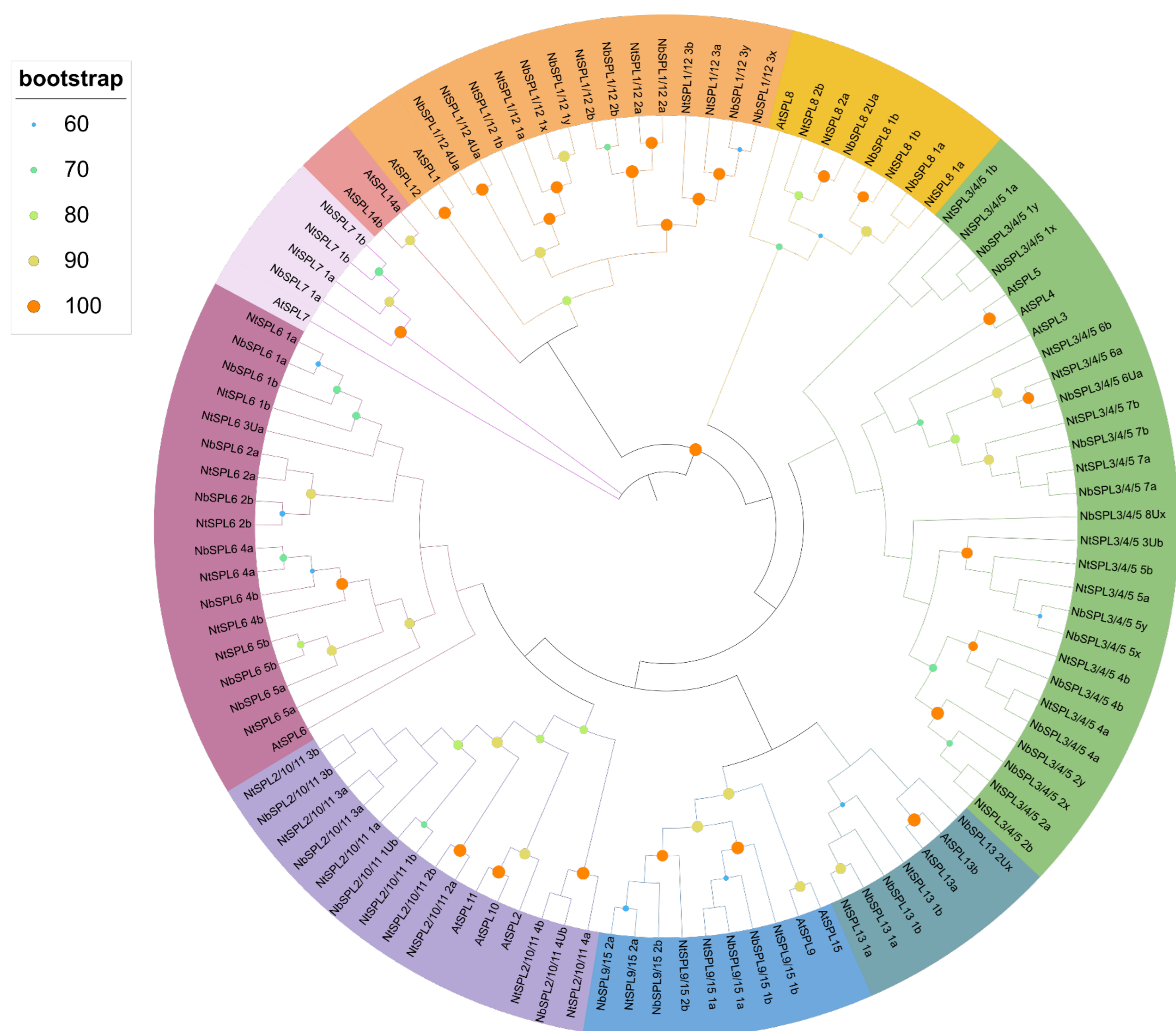
# Comparative analysis of the Squamosa Promoter Binding-Like (SPL) gene family in *Nicotiana benthamiana* and *Nicotiana tabacum*

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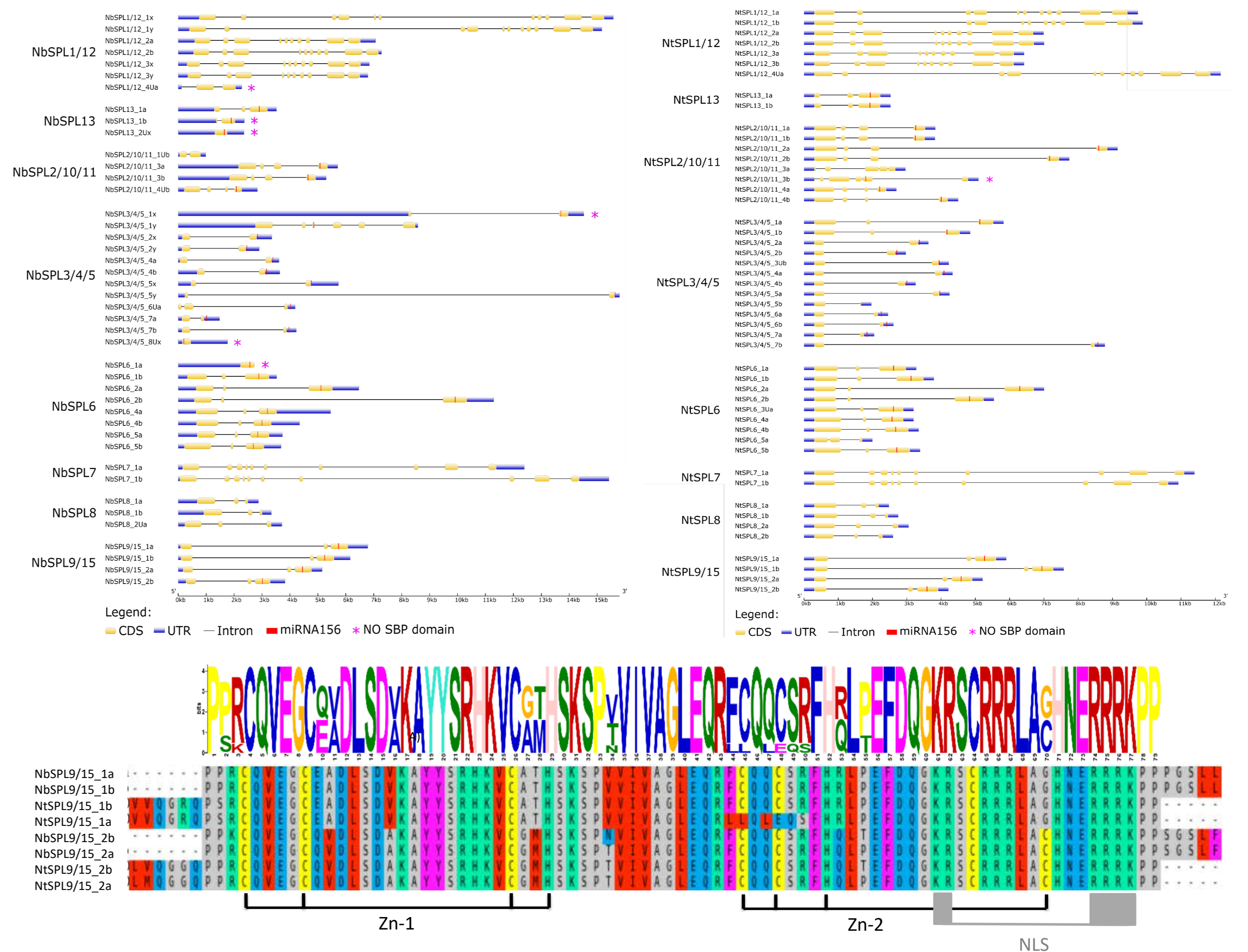
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SQUAMOSA promoter binding-like (SPL) proteins are transcription factors known to play key roles in **growth and developmental processes**, including leaf development, plant architecture and juvenile-to-adult and vegetative-to-reproductive phase transitions. In this study, we report the identification of **49 SPL genes** in *Nicotiana tabacum* cv. K326 and **43 SPL genes** in *N. benthamiana* lab strain. The SPL genes of the two species were classified into 8 phylogenetic groups according to the SPL classification in Arabidopsis. The exon-intron gene structure and SBP domains are highly conserved between homeologues and orthologues. 30 of the NbSPL genes and 33 of the NtSPL genes are targets of **miR156**. These results served as basis for candidate gene selection for a knockout experiment using CRISPR/Cas9 in *N. benthamiana* that revealed some clues about the biological function of NbSPL13 genes. The knockout of these genes brought to a significant **delay of 4-5 days in flowering time**, which could be the basis for the increase of available infiltration time for recombinant protein production. Additionally, the expression of SPL genes was analysed by RNA-seq at different growth stages for both tobacco and *N. benthamiana*, revealing that genes not under miR156 control were constitutively expressed throughout development and the other miR156-regulated genes varied their expression at different developmental stages.

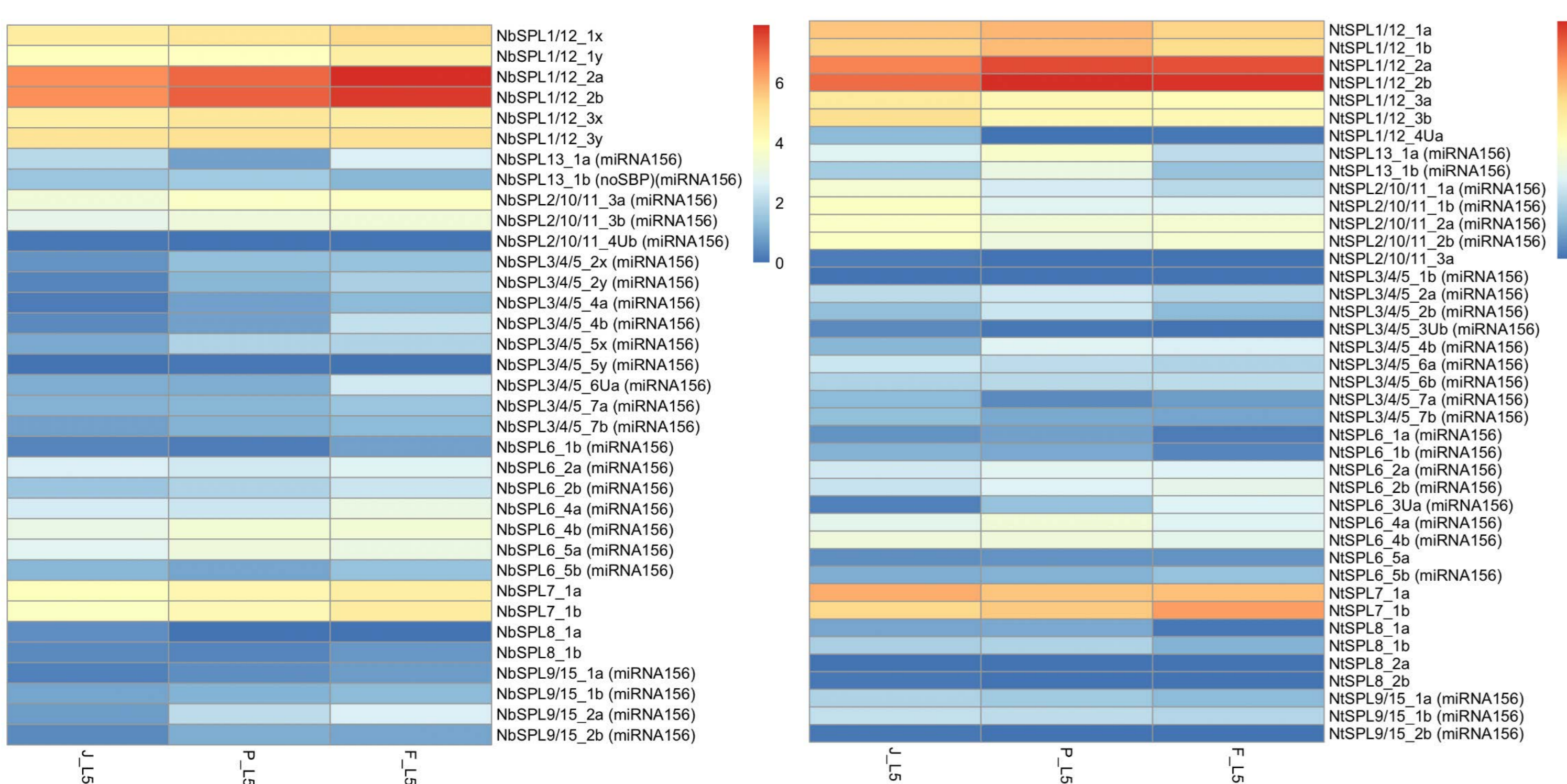
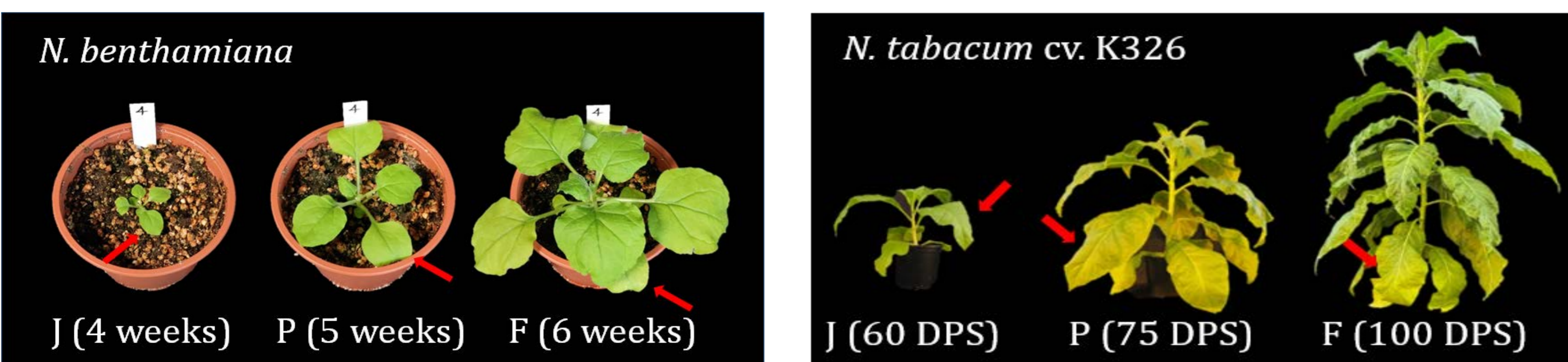
## 1 Phylogenetic tree with SPL genes from *N. tabacum*, *N. benthamiana* and *Arabidopsis thaliana*



## 2 SPL gene subfamilies of *N. benthamiana* and tobacco exhibit similar exon-intron structure and a conserved SBP domain



## 3 Expression profiles of NbSPL and NtSPL genes in leaf at juvenile (J), pre-flowering (P) and flowering (F) stages



## 4 Gene editing of NbSPL13 gene subfamily results in delayed flowering in *N. benthamiana*

