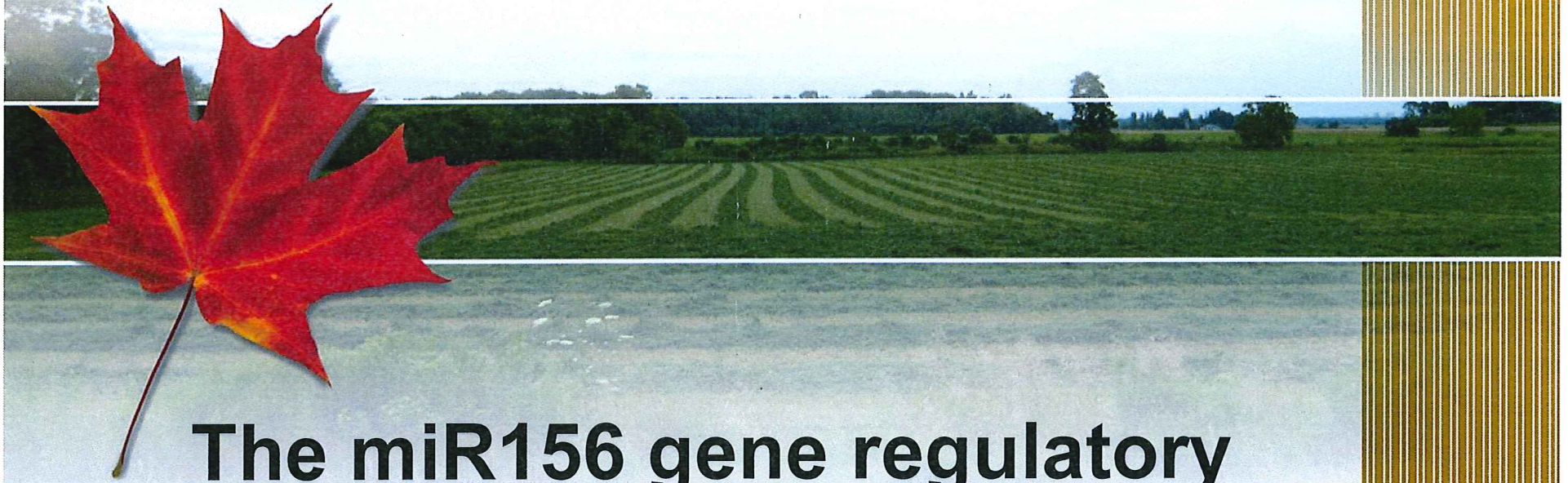




Agriculture and
Agri-Food Canada

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The miR156 gene regulatory network controls plant architecture and other traits in alfalfa

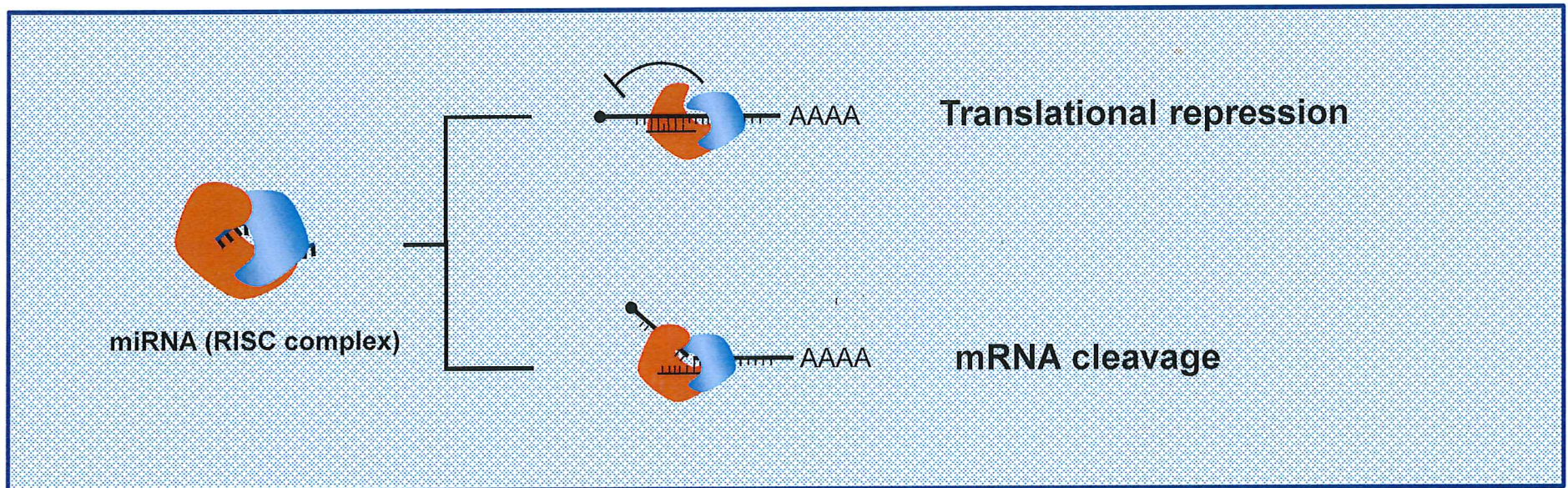
Abdelali Hannoufa

Agriculture and Agri-Food Canada, London

Canada 

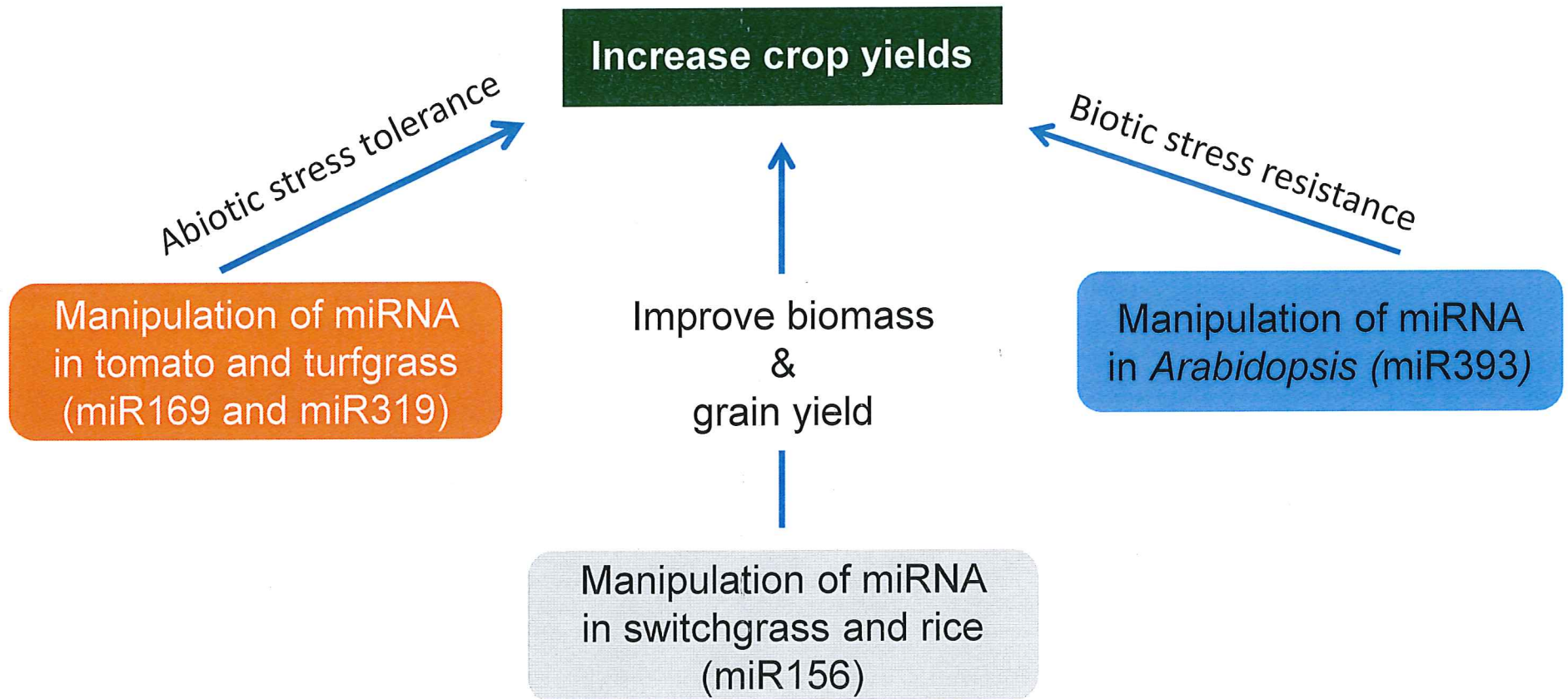
Background: Plant microRNAs

- Small RNA molecules; ~ 21-26 nt in length
- Sequence-specific regulators of posttranscriptional gene expression

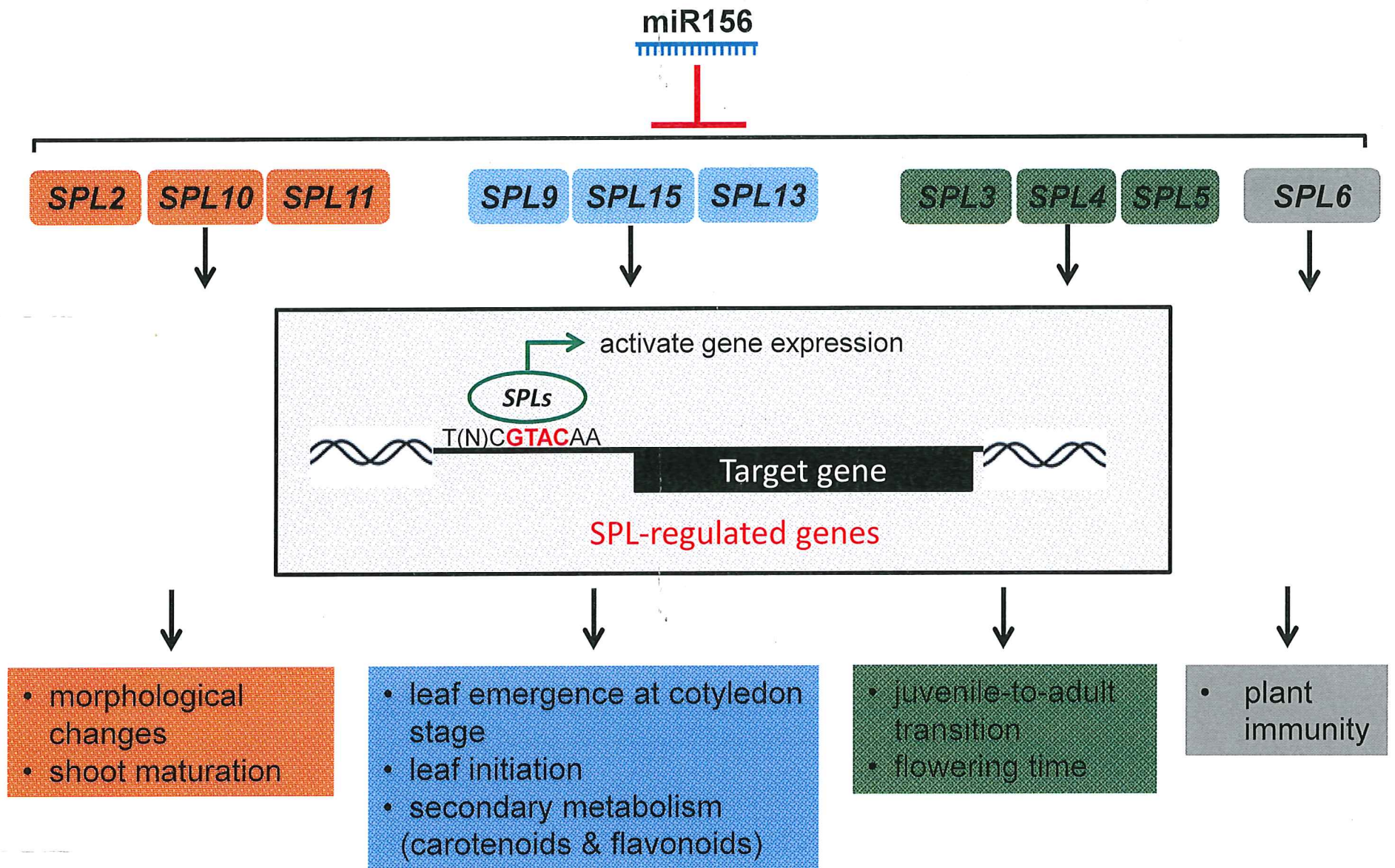


MicroRNA – Future Opportunity

MicroRNAs provide a potential new tool for crop improvement.



MiR156-SPL gene regulatory network in *Arabidopsis*



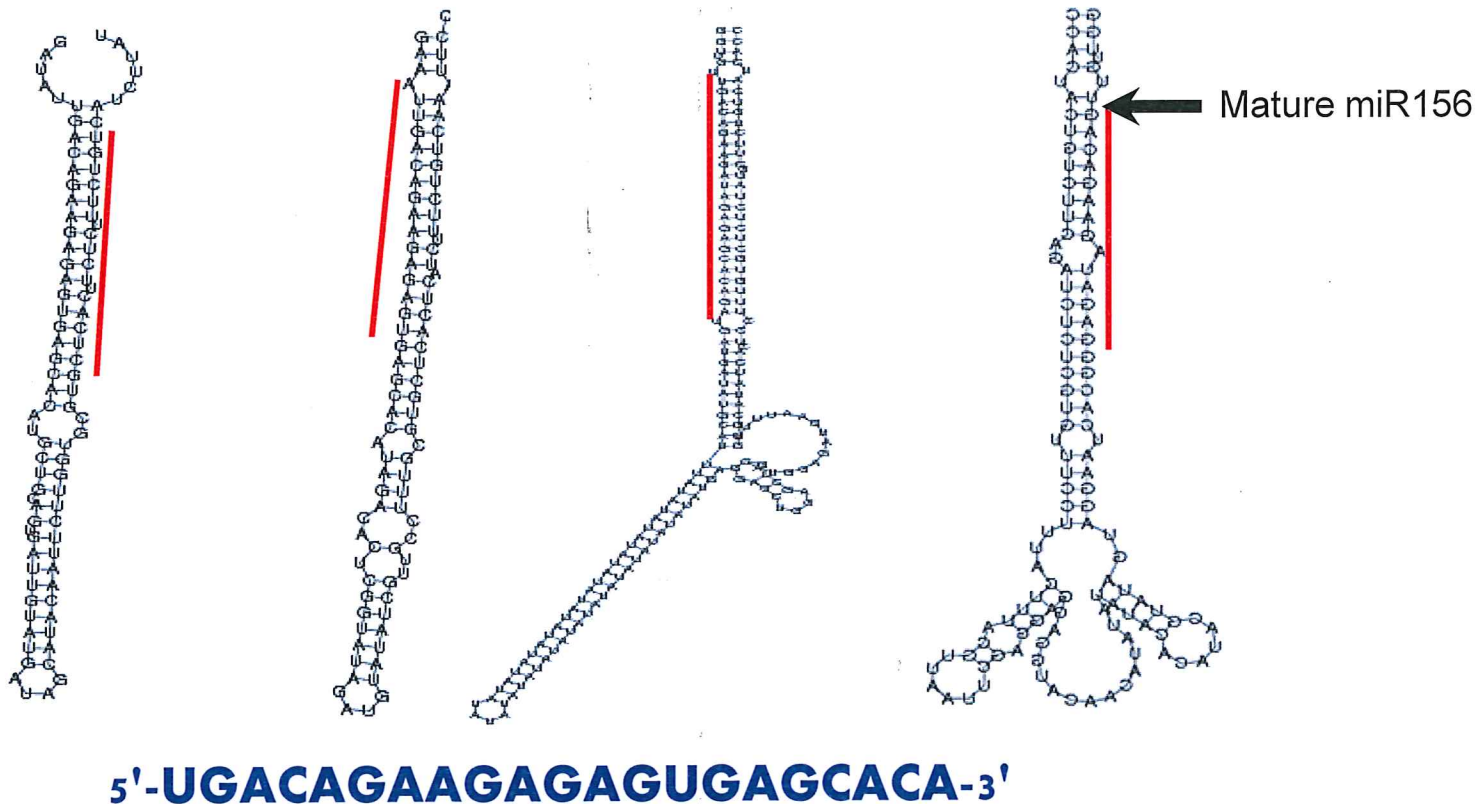
Characterization of miR156 in alfalfa

- No miR156 precursors were found in public alfalfa sequence databases.
- Short miR156-like sequences were found in alfalfa NGS database, but not long enough to predict secondary structure.

```
lcl|4178900-5      ATAGTAGACAATATGTTGATGGTTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTT-----
lcl|107417079-1    ATAGTAGACAATATGTTGATGGTTGGTGGTGTGTTTGTCTTCTGTAATCA-----
lcl|106580481-1    -----ATATGTTGATGGTTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTG-----
lcl|95354150-1     -----TATGTTGATGGTTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGCGTGACATT-TGATGTATA-----
lcl|116778904-1    -----TTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTATAGCGGGCAGTATGC-----
lcl|42739154-1     -----TGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTCTTCTCCATAAT-----
lcl|71051065-1     -----TGATGGTTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTCGTCCTCTCTGTCA-----
lcl|125841795-1    -----GTTTTGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCT-----
lcl|124825122-1    -----GGTTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGG-----
lcl|11619991-2     -----TTGGTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGGC-----
lcl|41361947-1     -----GTGGTGTGTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTG-----
lcl|73659039-1     -----TGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCT-----
lcl|106940949-1    -----TGGTTTTTGTCTTCTGTAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGAT-----
lcl|83520490-1     -----TGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGT-----
lcl|59681923-1     -----TTTTGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGATTACA----
lcl|26101993-1     -----TGTTTTGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGATTCA----
lcl|119549022-1    -----TGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGATTCA-----
lcl|64888716-1     -----TTTGTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGGACTGATTCATG--
lcl|63165137-1     -----TTTGTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGATTCATGG--
lcl|111104515-1    -----TGTTTCTTGTAAATCAGGTGGGTGTGCTTTC-TCTCTTCTGTCATCTCGGACTGATTCATGGC
```

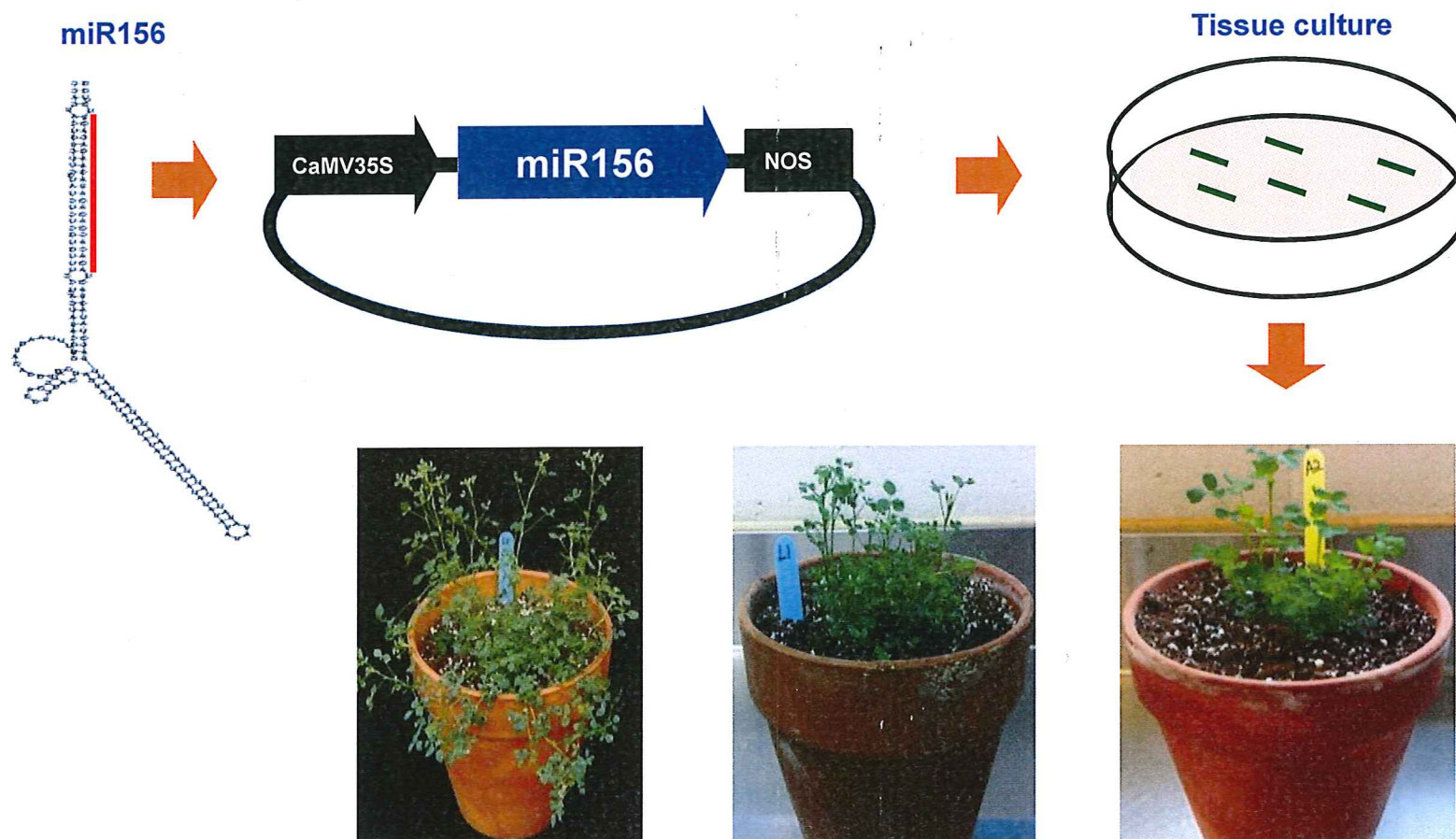
Characterization of miR156 in alfalfa

- *In silico* analysis identified 10 miR156 precursors in *Medicago truncatula* sequence databases.
- *M. truncatula* sequences were used to clone alfalfa miR156.



Cloning and overexpression of alfalfa miR156

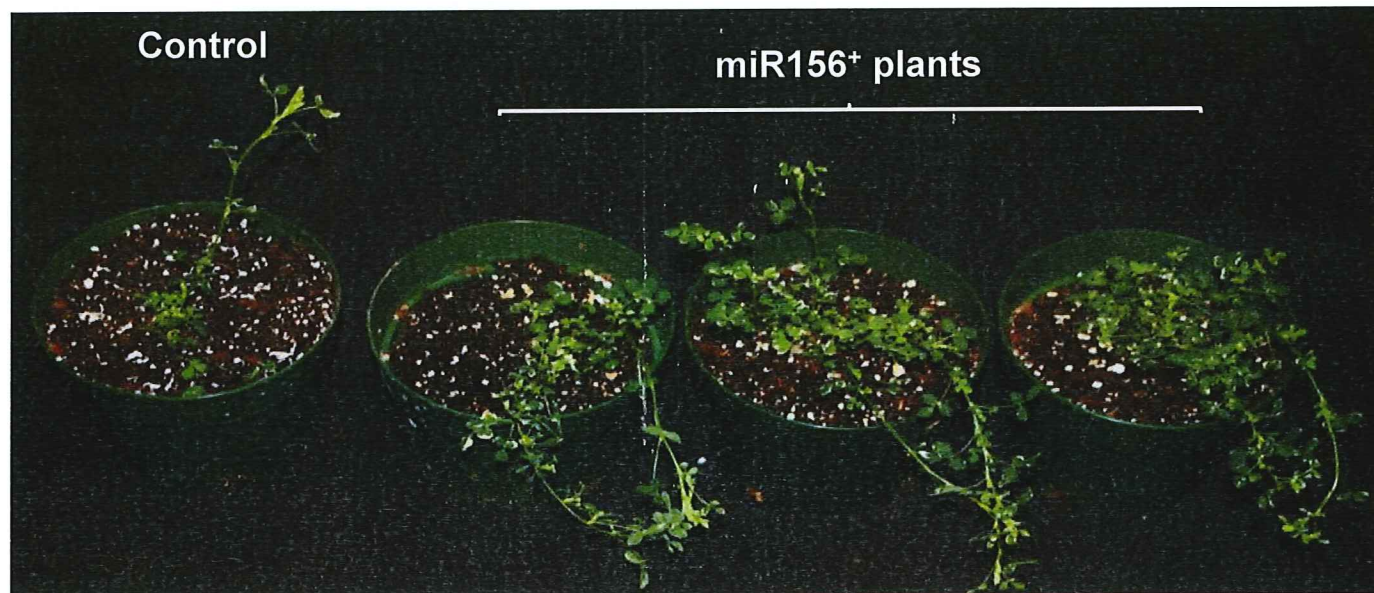
M. truncatula sequences were used to clone alfalfa miR156 by PCR-based method.



Propagation of alfalfa by stem cuttings



MiR156 enhances branching in alfalfa



↓ Cut-back



Control

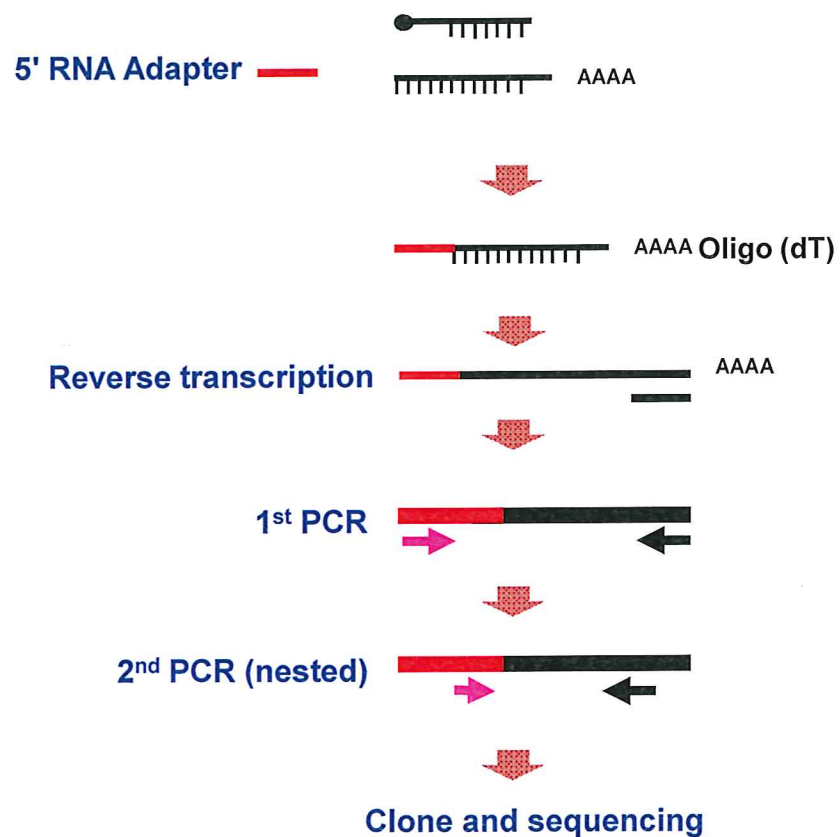
A8

A11a

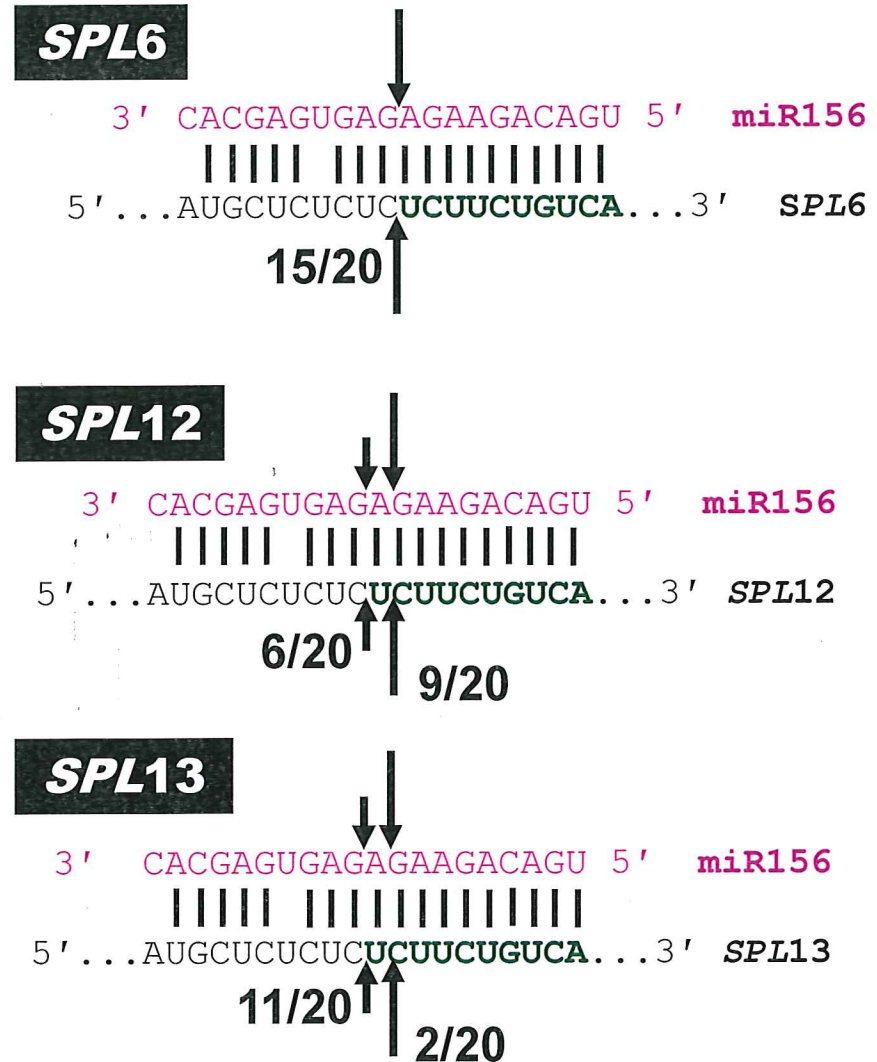
A17

Identification of miR156 target genes in alfalfa

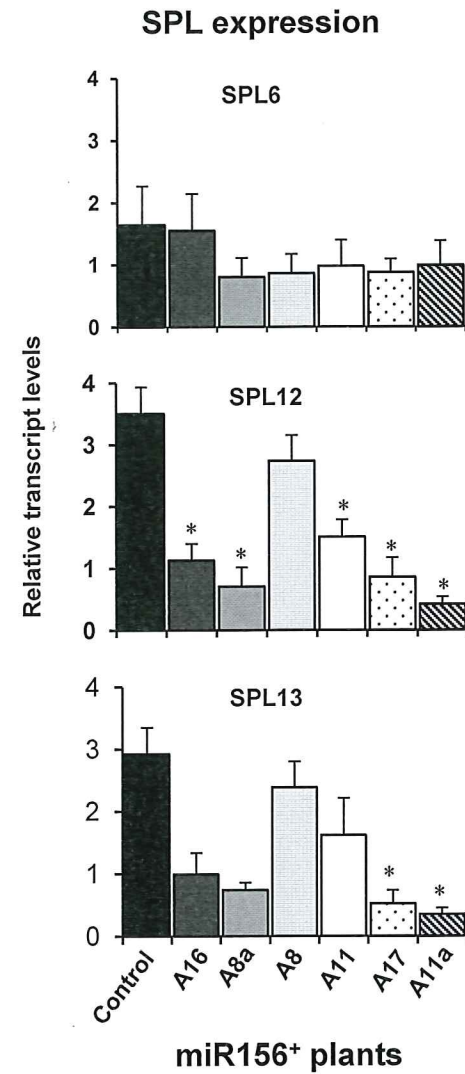
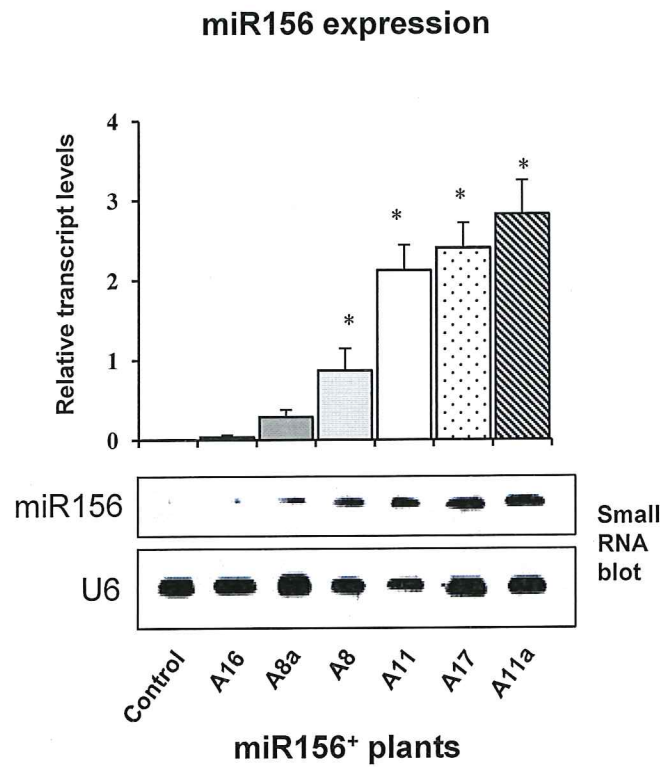
5'-RACE



Target genes



MiR156 silences 3 *SPL* genes in alfalfa



MiR156 affects plant architecture in alfalfa

Low

Abundance of miR156

High



Control



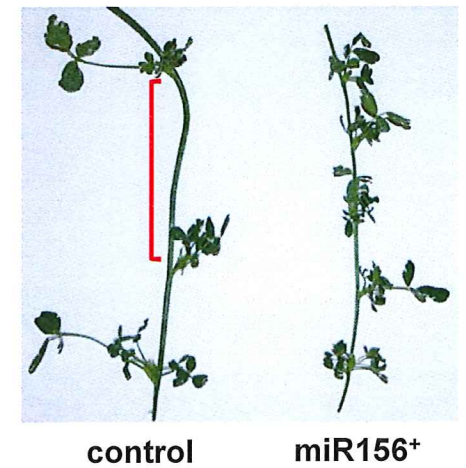
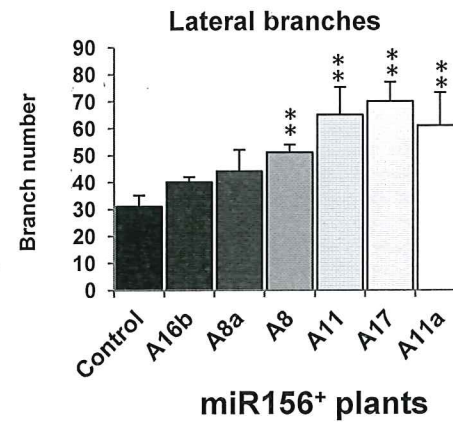
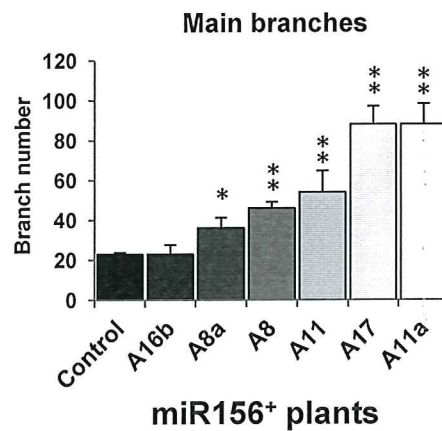
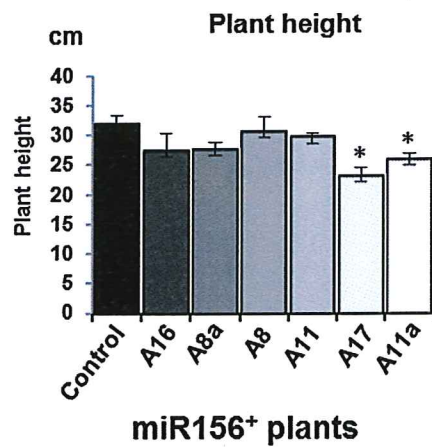
A8



A11a



A17

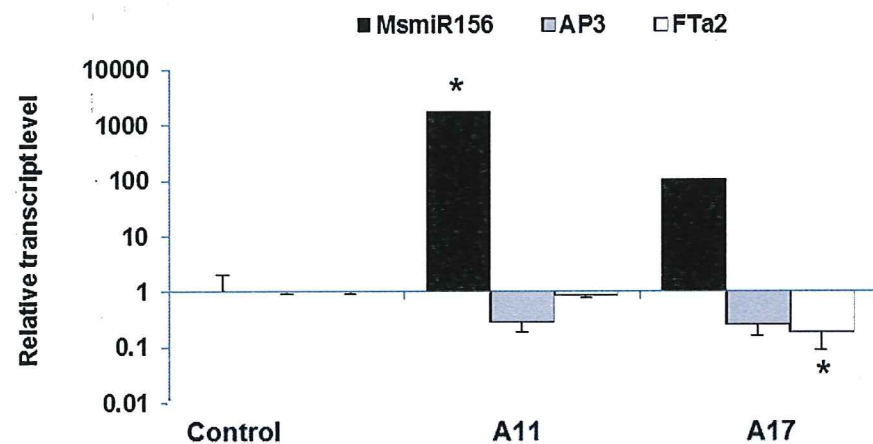


MiR156 affects flowering time in alfalfa

- Depending on the levels of miR156 expression, flowering time is delayed by 5-60 days.



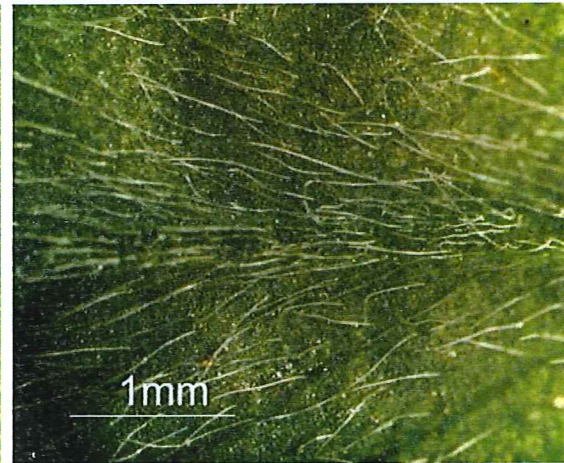
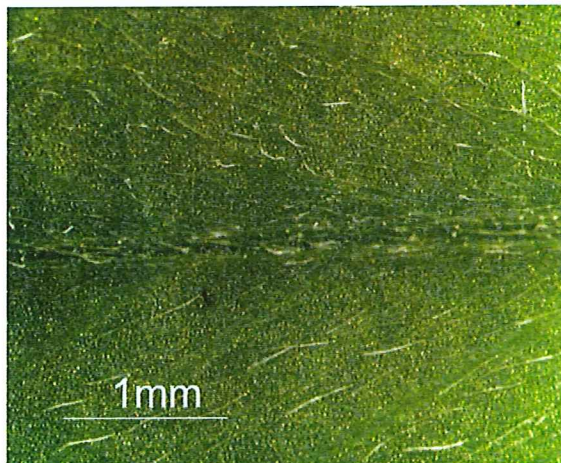
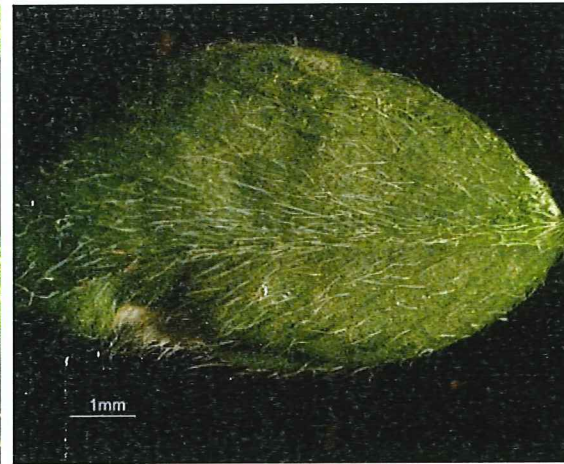
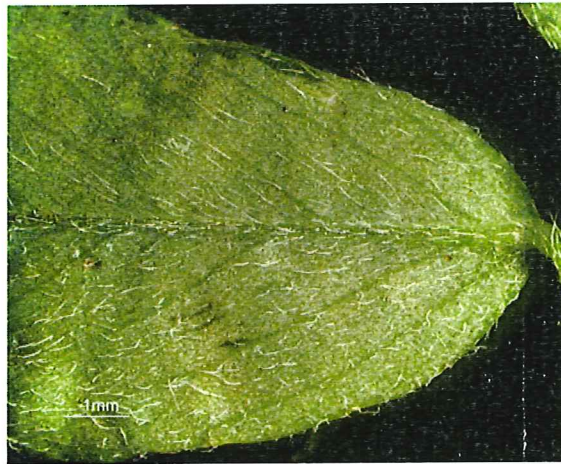
- Regulators of flowering (FTa2 and AP3 genes) are downregulated in miR156⁺ alfalfa.



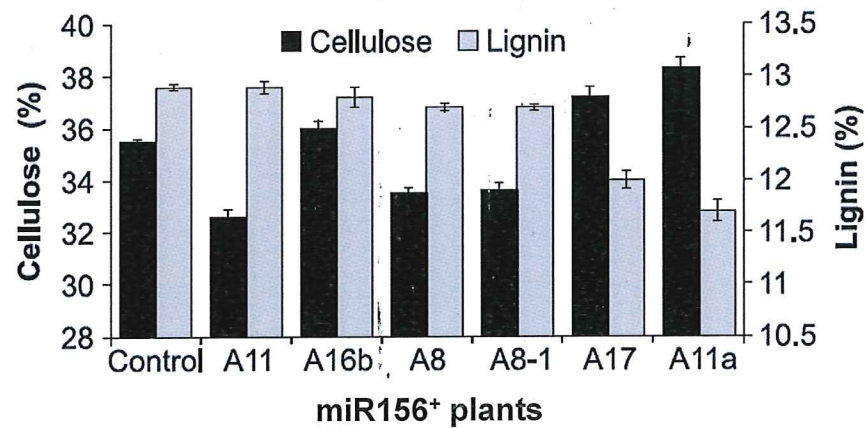
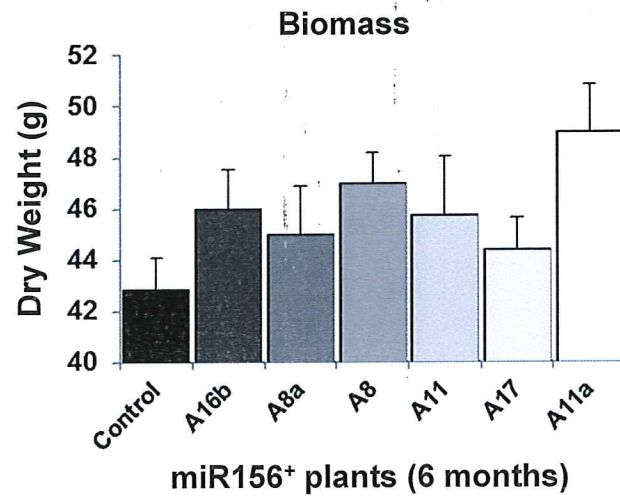
MiR156 enhances trichome density in alfalfa

Control

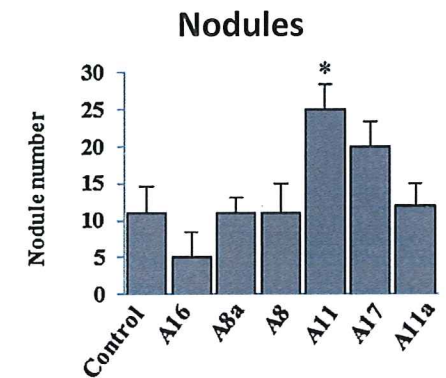
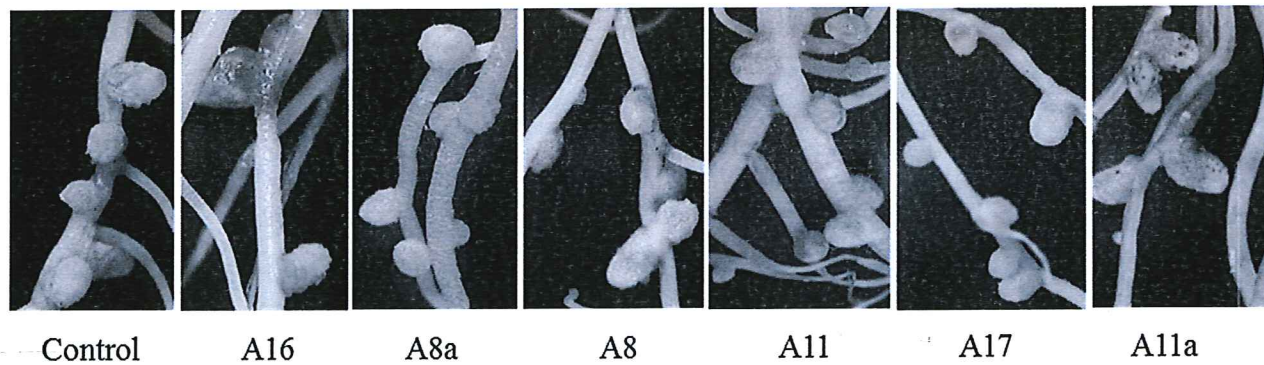
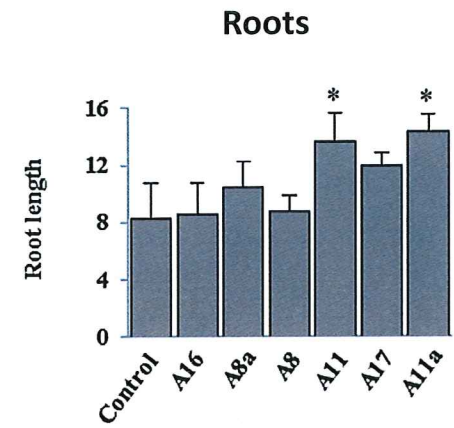
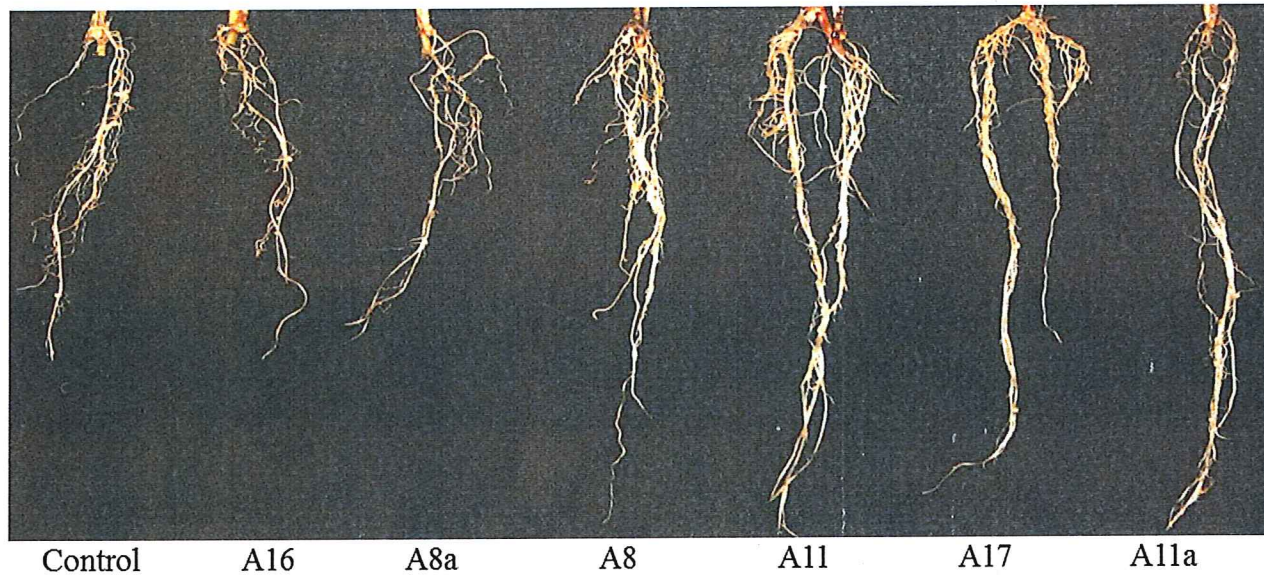
miR156⁺



Effect of miR156 on forage biomass



MiR156 increases root length and nodulation in alfalfa



Summary

- **Overexpression of miR156 improves important traits in alfalfa:**
 - Increased shoot branching → increased forage yield
 - Delayed flowering → extended vegetative growth, harvest time flexibility
 - Increased root length and nodulation → improved drought tolerance and N₂ fixation



- **Also some negative effects, especially at high expression levels; e.g.** reduced plant height, reduced stem diameter, excessive flowering delay.

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