DBS
DIPARTIMENTO
DI BIOSCIENZE

Modifying phyllotaxis in *Brassica* seed crop species for yield improvement

UNIVERSITÀ DEGLI
STUDI DI MILANO

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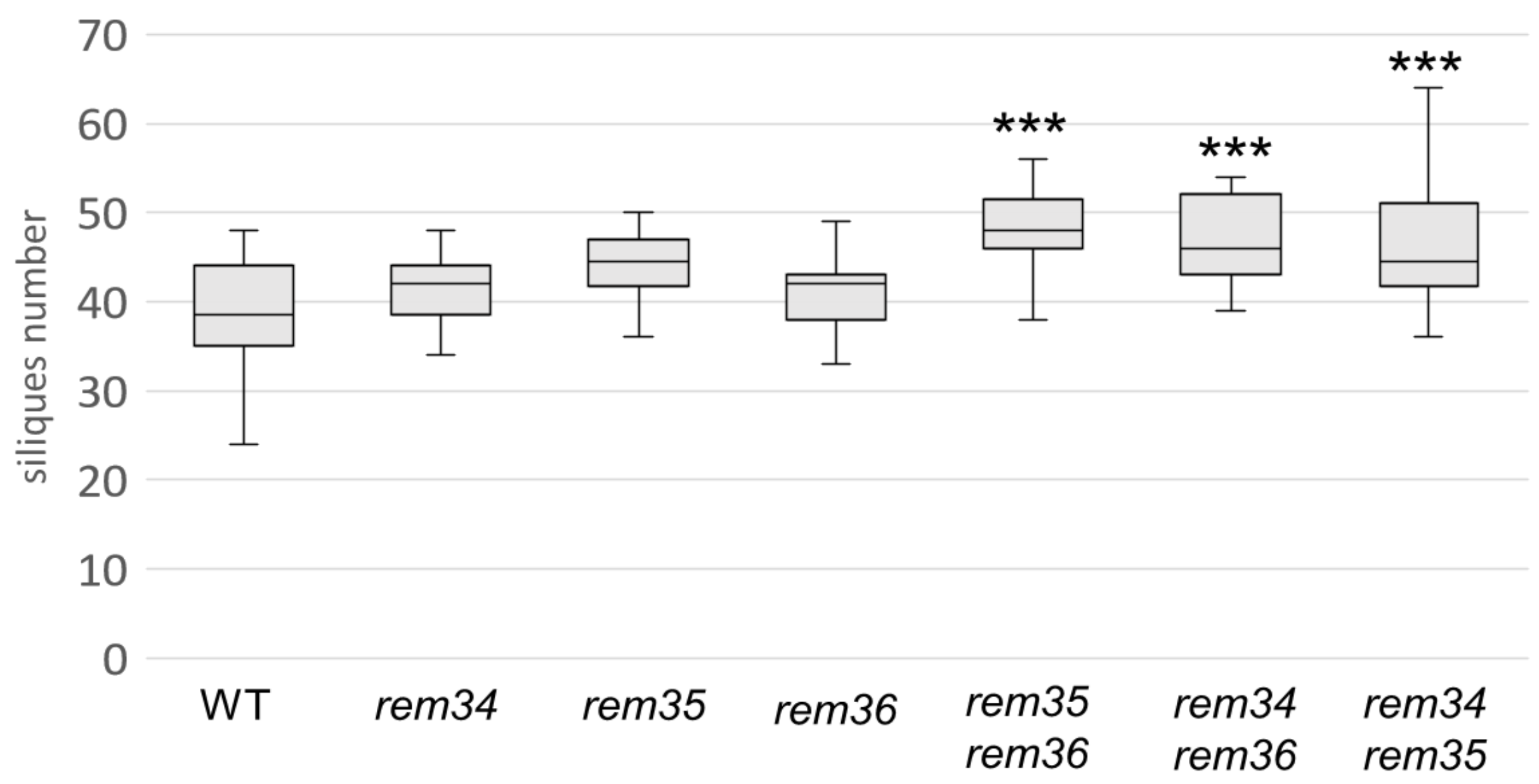
INTRODUCTION

Arabidopsis thaliana double mutants in *REM34*, *REM35* and *REM36* show interesting architectural phenotypes related to yield improvement. Indeed, they have an aberrant phyllotactic pattern associated to an increased number of siliques on the main stem. Since the world population is expected to rise in the next years and is preferable to conserve wild environments rather than dedicate more land to agriculture, it is meaningful to get crop genotypes of increased yield. Rapeseed is the second oil crop for global production (M. Shahbandeh, 2022, statista.com) and is phylogenetically near to *Arabidopsis.* Therefore, it was selected as crop where to try to get similar mutants, targeting the orthologs of *REM34*, *REM35* and *REM36.*

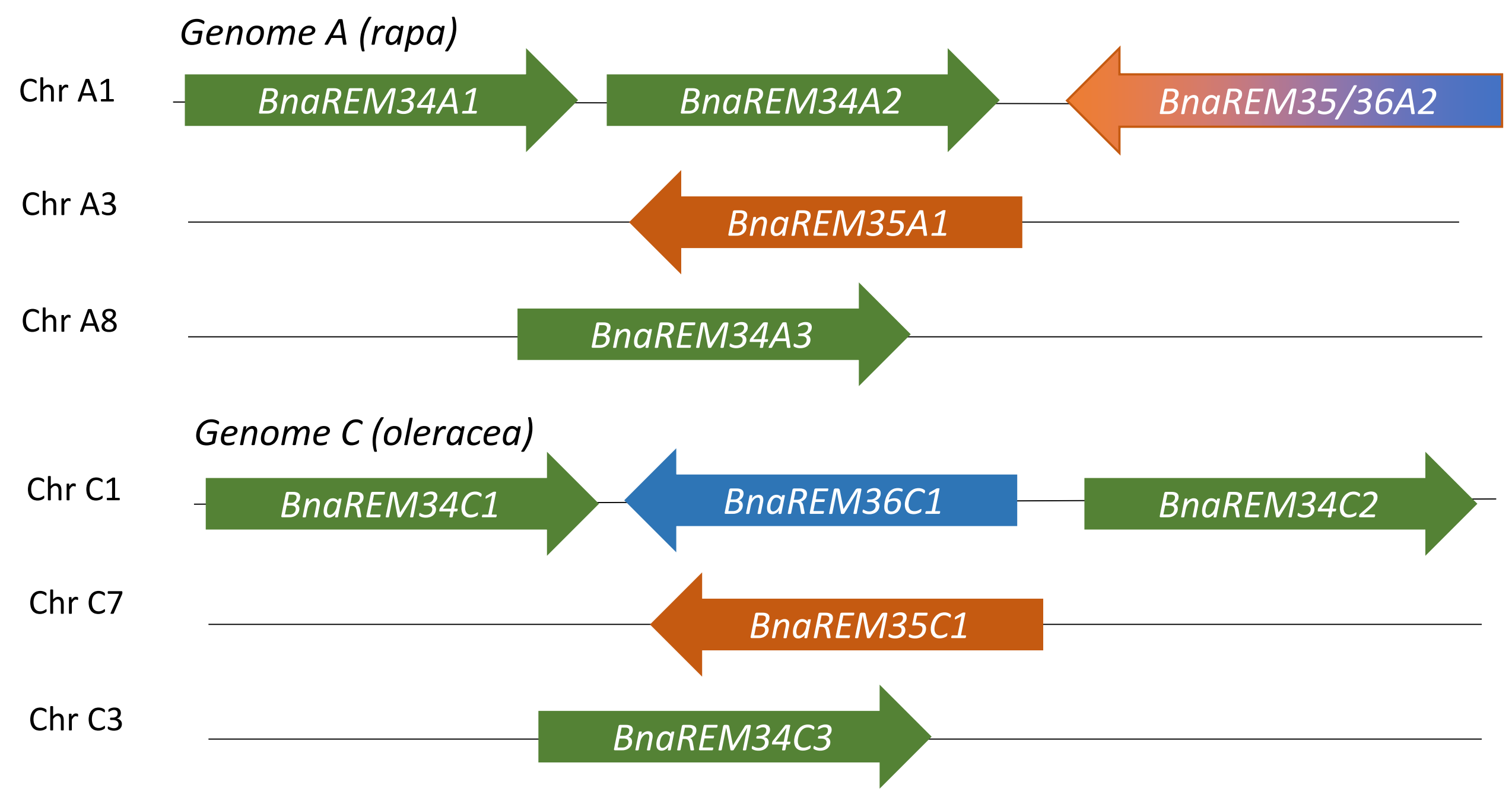


AIMS OF THE PROJECT

- Study and characterize the cluster of *REM34*, *REM35* and *REM36* orthologs in *B. napus*;
- Develop *B. napus* mutants in the orthologs of *REM34*, *REM35* and *REM36*

CLUSTER IDENTIFICATION

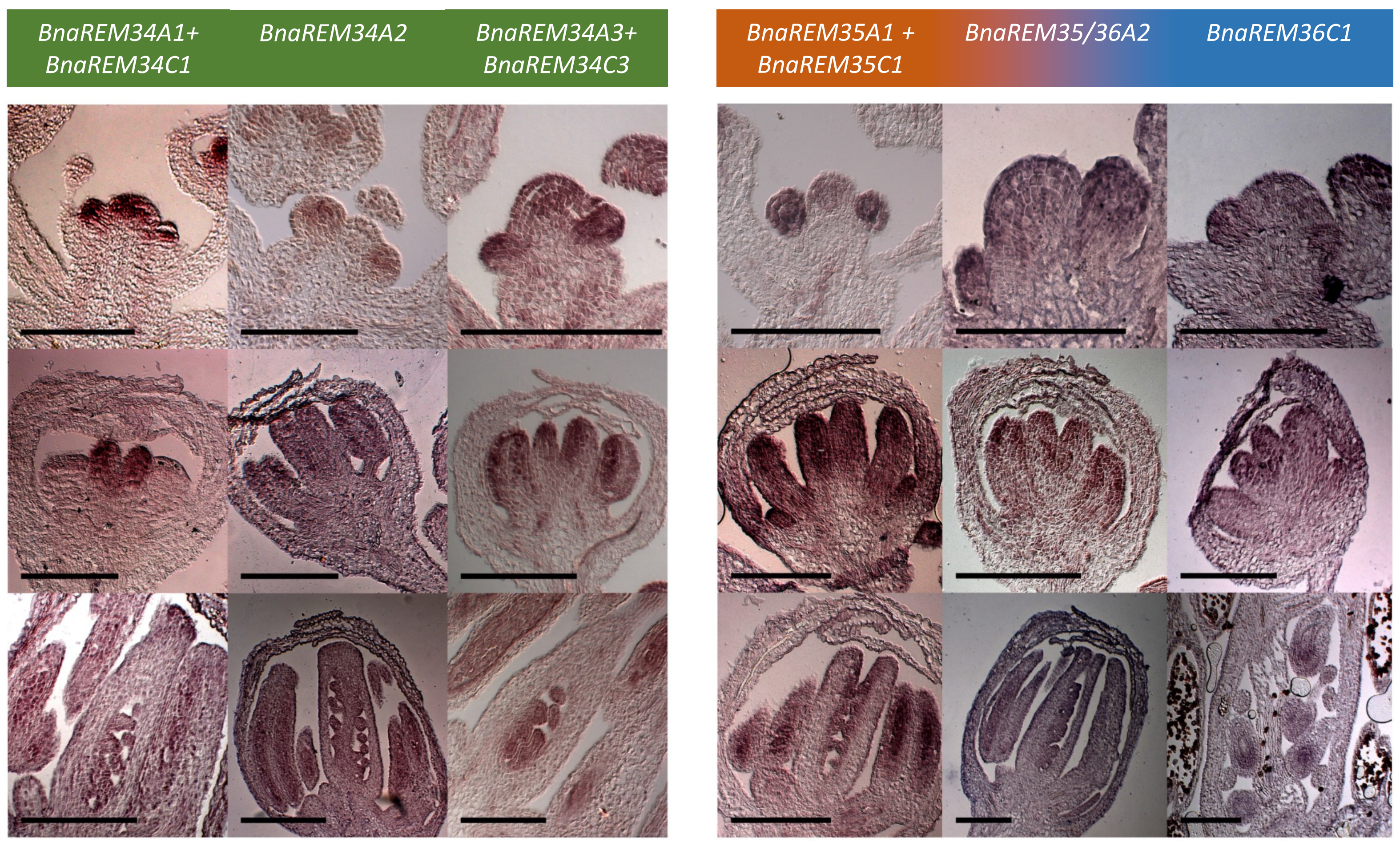
The candidate *Brassica napus* cluster was identified by bioinformatic tools, resulting in a pool of 10 genes of which some placed in linkage. 6 are homologs of *REM34*, 2 are homologs of *REM35*, 1 is homologous of *REM36* and 1 is equally related to *REM35* and *REM36*.



To provide soundness to the bioinformatic results and test the functional conservation of the BnaREMs identified, their expression pattern was analysed, and a complementation test and a Y2H assay were performed.

EXPRESSION PATTERN

The expression pattern of the genes resembles the *Arabidopsis* one[1,2], as the genes are expressed in the inflorescence and flower meristems and in floral organs at different stages.



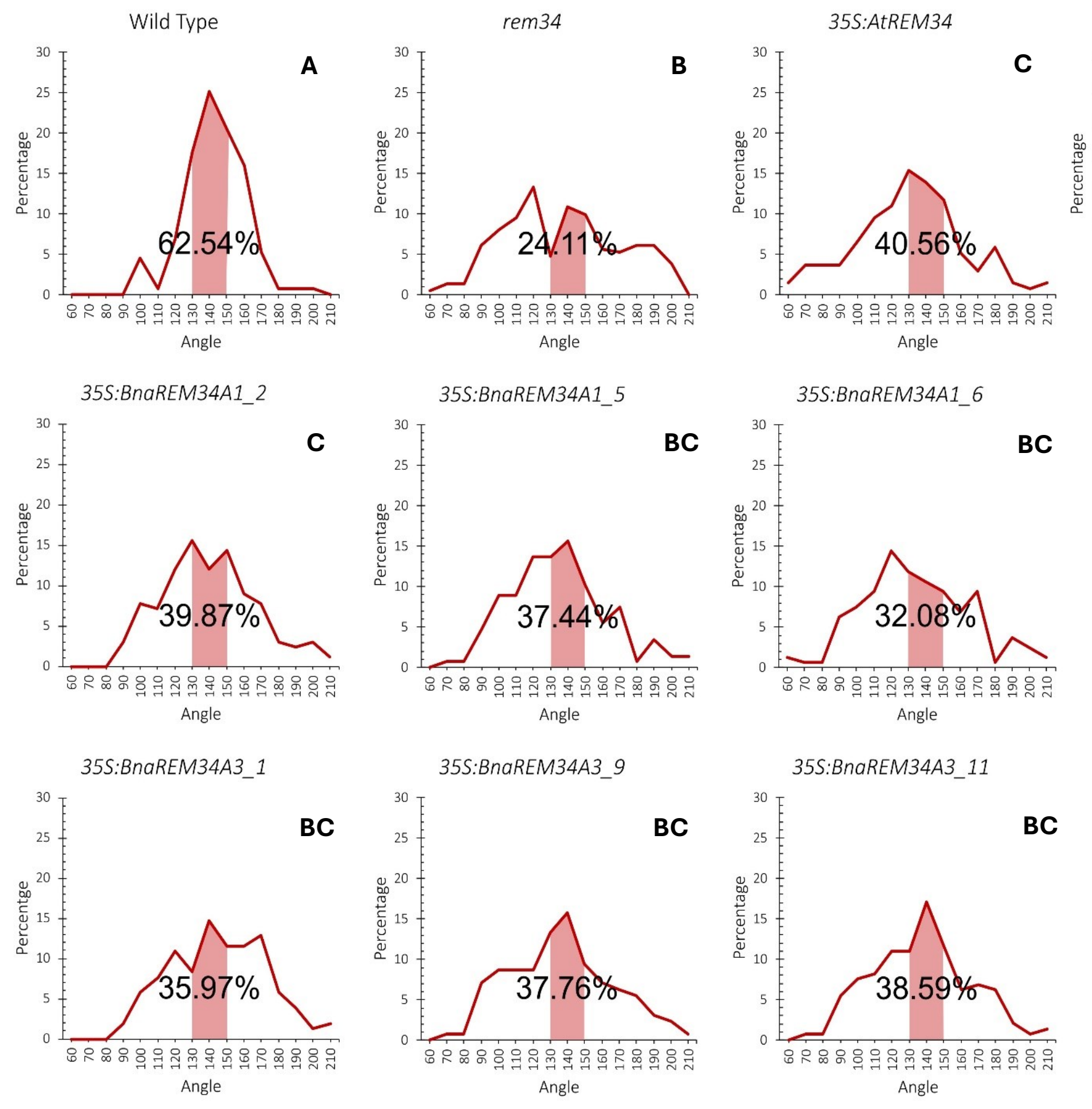
Scale bar 200 µm

To reduce the complexity, and accounting for the very high sequence identity, 4 *Bna* genes were selected for further analysis.

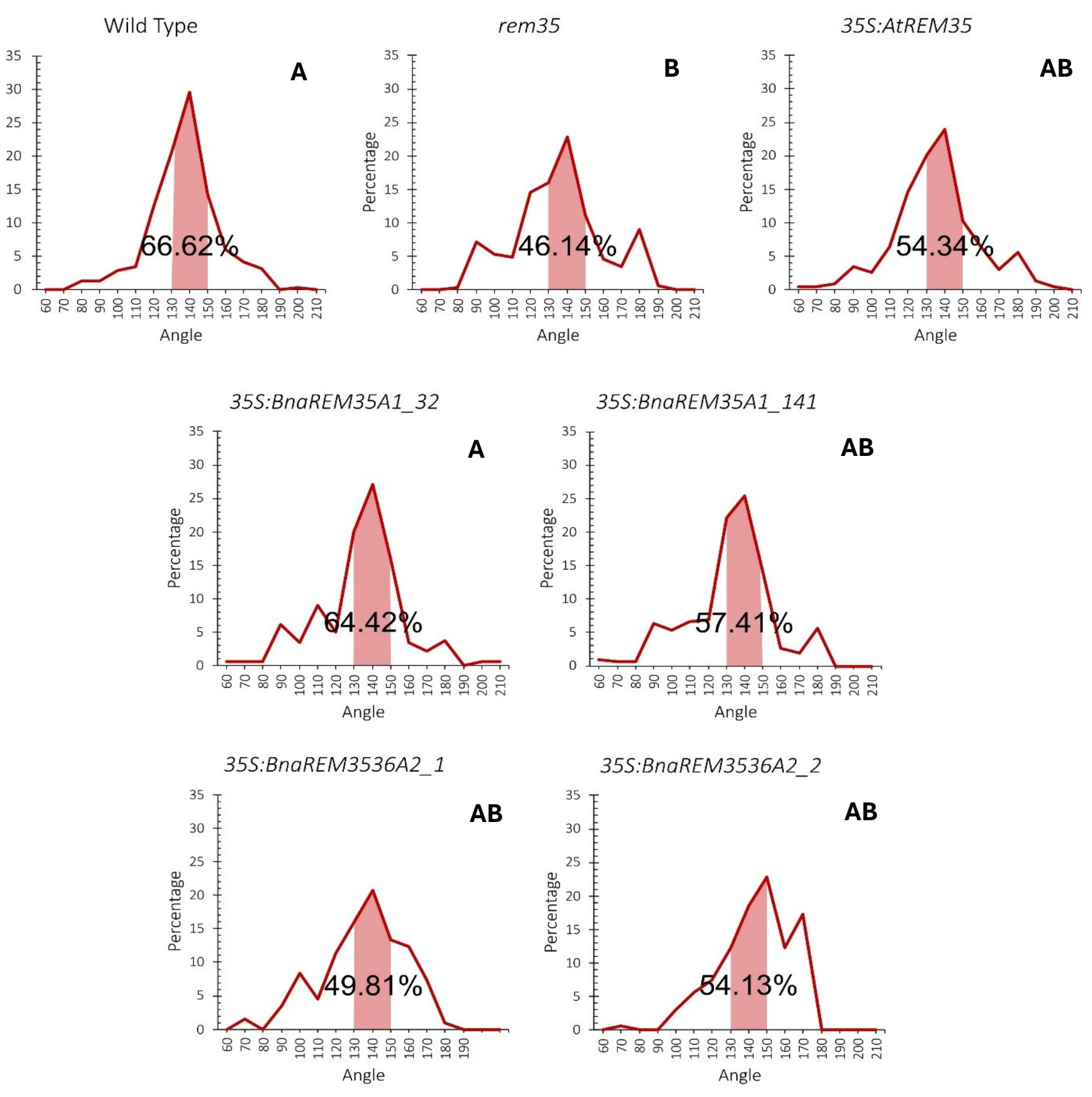
COMPLEMENTATION TEST

The overexpression of the *Bna* genes in the corresponding *At* mutants phenocopy the overexpression of the *At* gene itself in the phyllotactical pattern, witnessing a functional conservation.

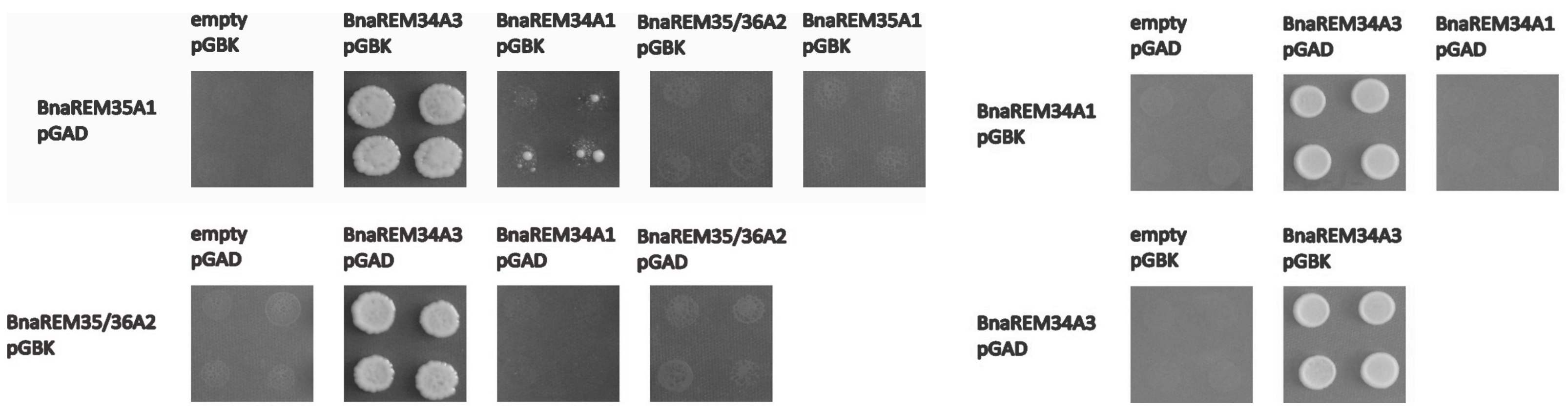
BnaREM34



BnaREM35 – BnaREM36



PROTEIN INTERACTIONS



As AtREM35 can homodimerize and heterodimerize with AtREM34[1], the interactions between the *Bna* proteins were tested. The protein interactions moderately differ from the *Arabidopsis* counterpart.

GENE EDITING

The BnaREMs under study were targeted for editing. We plan to first get single orthologs mutants, and later generate higher level mutants by crossings or re-transformation. Several gRNAs per gene were designed and tested by a protoplast destructive assay. The most efficent were cloned in PTG constructs that were transformed in *Bna* by *Agrobacterium* trasformation. 8 T_0 plants have been obtained.

CONCLUSIONS

- Double mutants in *AtREM34*, *AtREM35* and *AtREM36* show a yield increase. It is meaningful to get similar mutants in crops such as *Brassica napus*.
- Bioinformatic analysis suggest the homolog cluster in *B. napus* to be constituted by 10 genes. This is coherent with the species allotetraploidy and the genome evolution.
- The *B. napus* genes show functional conservation in interspecifc complementation tests and conserved gene expression when compared to the *Arabidopsis* context. However, some proteins interaction ability, diverge.
- Single homolog mutants are being generated by CRISPR multiplex. Higher level mutants will be developed subsequently.

REFERENCES

[1]Caselli et al, REM34 and REM35 control female and male gametophyte development in *Arabidopsis thaliana,* Frontiers in plant science (2019).
[2]Mantegazza O., et al. Analysis of the *Arabidopsis REM* gene family predicts functions during Flower development. Annals o f Botany, (2014).