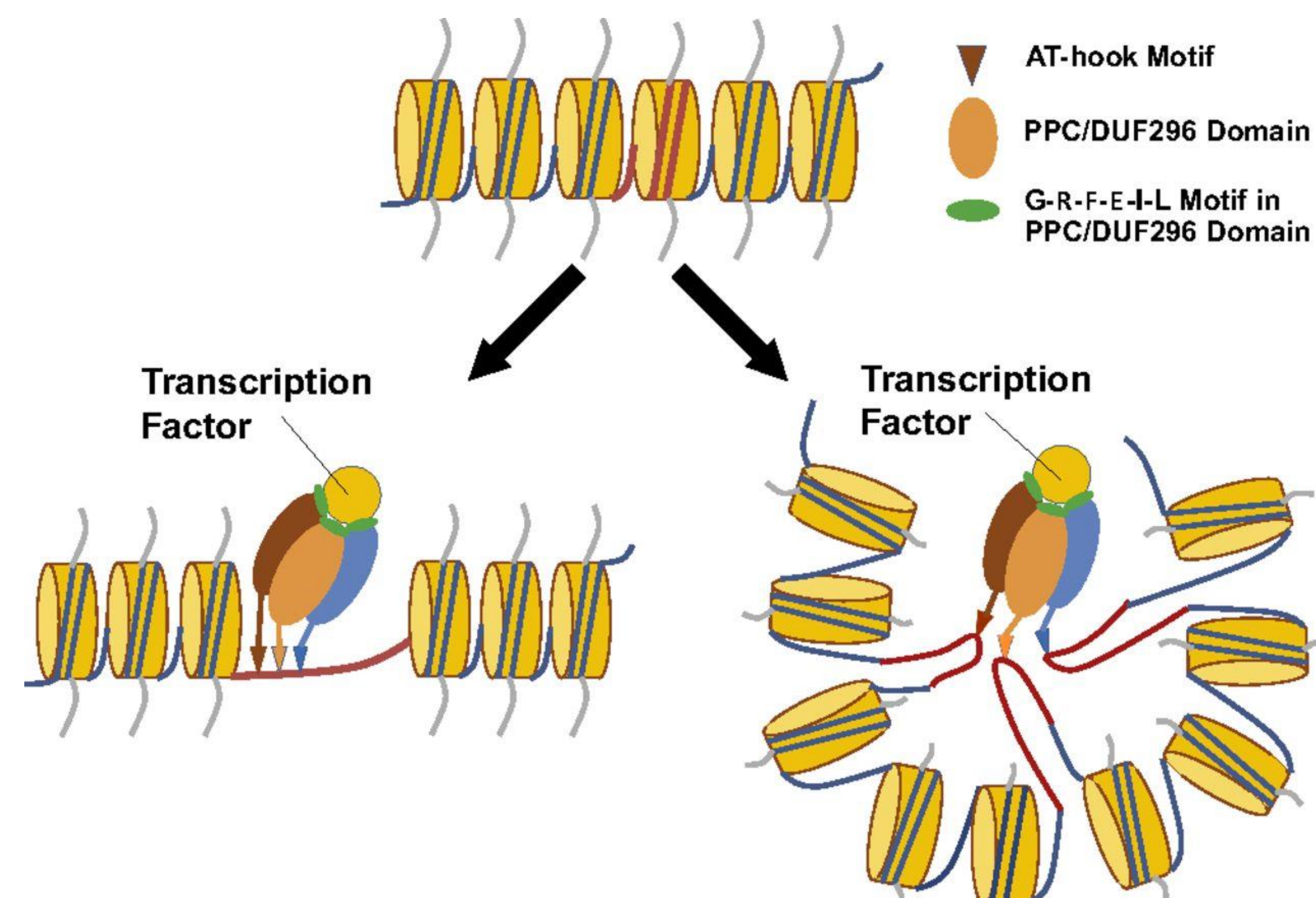
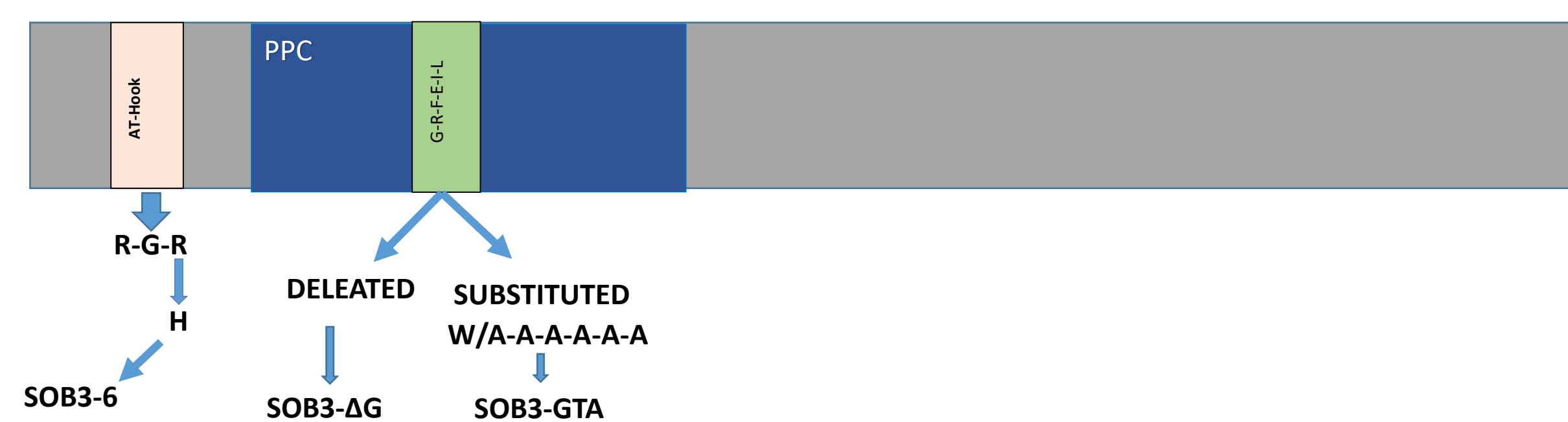


## Introduction

The AT-Hook Containing Nuclear Localized (AHL) gene family proteins contains two conserved domains:- 1) one or two AT-Hook motifs and 2) one PPC (Plant Prokaryotic Conserved) domain also called Domain of unknown function, DUF296. The AT-hook domain binds AT-rich DNA and the PPC domain is required for protein- protein interactions, including AHLs with other AHLs and transcription factors (Figure 1).



**Figure 1:** Model of the 3-dimensional manner in which AHLs interact with one another, DNA, and transcription factors (Zhao *et al.*, 2013).



**Figure 2:** This figure illustrates long-hypocotyl-causing mutations located in AHL genes.

- Previous studies have shown that the AHL genes modulate several aspects of *plant growth and development* such as *hypocotyl elongation, flower and fruit development* (Street *et al.*, 2008).

- A conserved palindromic core sequence, R-G-R, in the AT-Hook motif is required to bind AT-rich DNA. Mutations in this region AHL-DNA interaction. Similarly alteration on a conserved region containing G-R-F-E-I-L in the PPC Domain abolishes interaction with AHLs and transcription factors ( Zhao *et al.*, 2013).

-The *Arabidopsis thaliana* genome encodes 29 AHL genes. In this study we examined *AHL29/SOB3* and *AHL27/ESC*.

-SOB3-6, a missense point mutation in the R-G-R region of the AT-Hook, abolishes DNA binding and affects seedling hypocotyl growth (Figure 2).

-Mutations at the conserved region of PPC domain:- SOB3-ΔG (deletion of GRFEIL amino acids) and SOB3-GTA (substitution of alanines for GRFEIL amino acids) also affect seedlings hypocotyl growth (Figure 2).

-ESC-11, a SOB3-6 like mutation in *AHL27/ESCAROLLA (ESC)* also confers long hypocotyl in seedlings because a significant amount of redundancy in *AHL* gene family.

## Acknowledgement

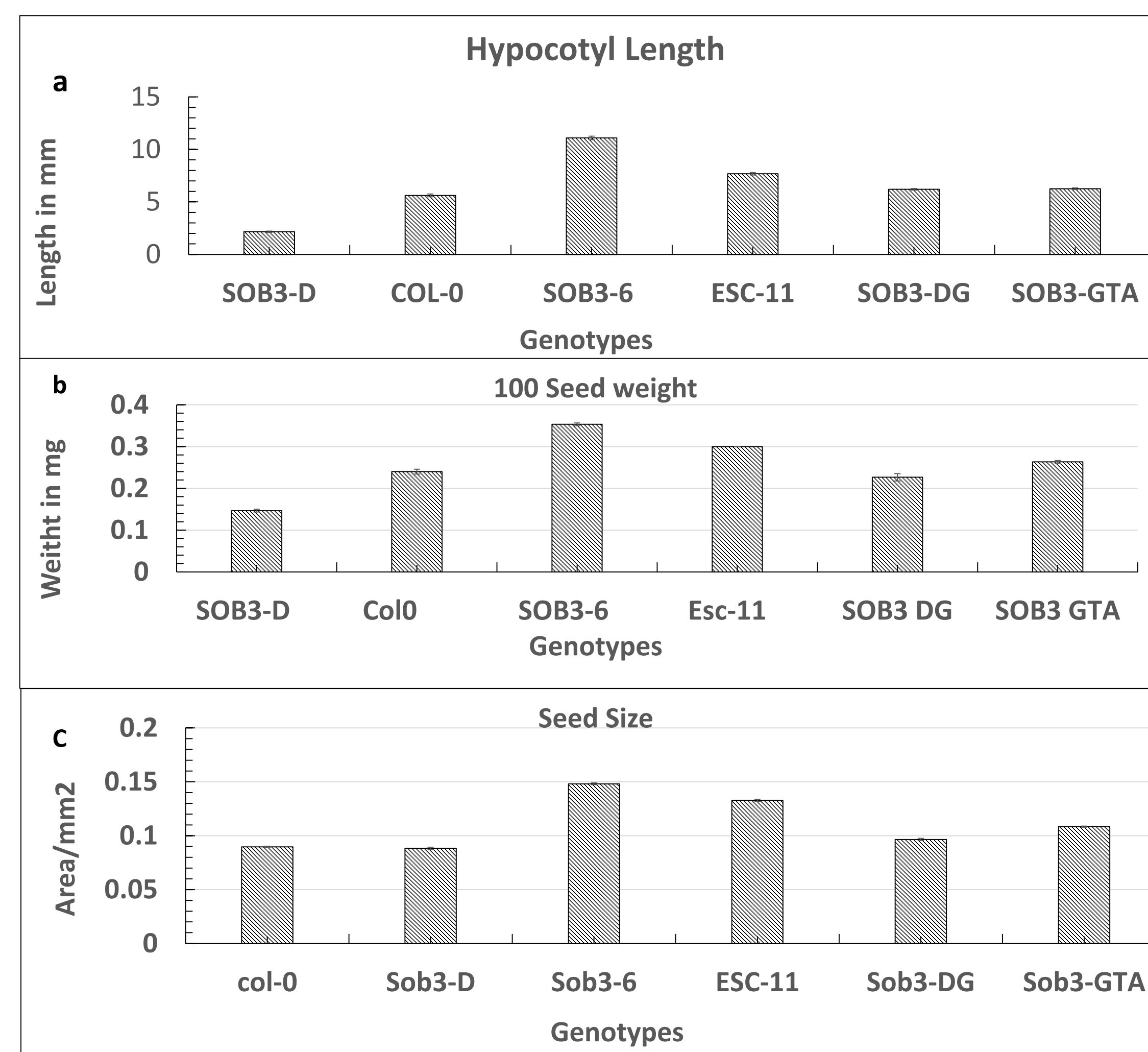
Thank you to my adviser/ mentor, Michael Neff. Thank you to Anna Hulbert, Neff Lab technician, for her help as well as all Neff Lab members for supporting me. This research was supported by funding from Washington State Dept. of Agriculture Biofuel Cropping Systems Project and USDA-NIFA grant #2013-67013-21666 (to M.M.N.).

## Objectives

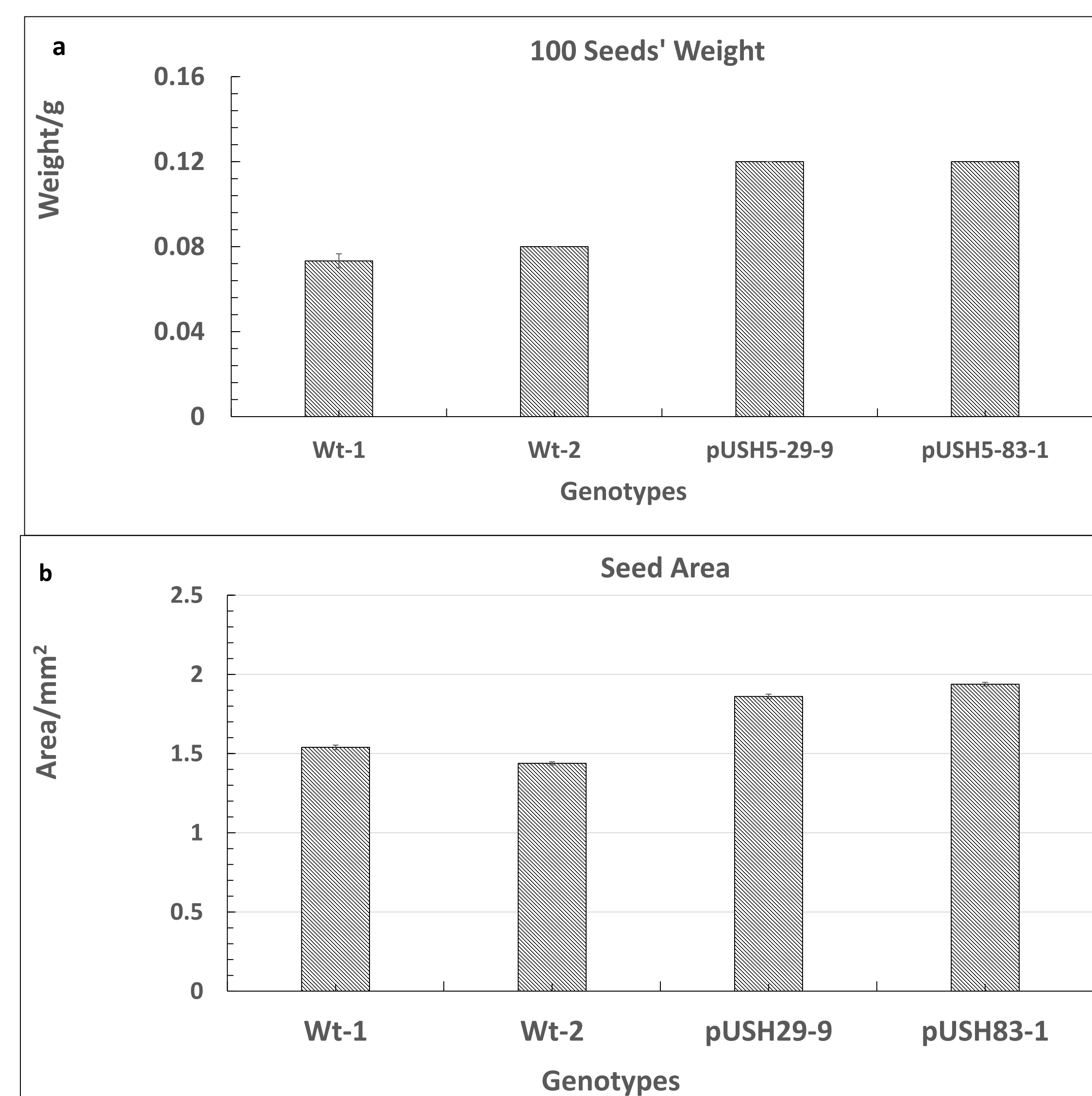
SOB3-6, SOB3-6 like (ESC-11) and other mutations in SOB3 confer long hypocotyls in seedlings (Figure 3) and our preliminary result have shown that the SOB3-6 mutation also confers big-seeds. The purpose of this project is to test:

- 1) Whether SOB3-6 and other mutations of AtAHL29/SOB3 confer big-seeds and tall seedlings
- 2) Whether SOB3-6 like mutation in other AHLs also confer similar phenotypes as SOB3-6.
- 3) Whether this knowledge would be transferred to other brassica oil seed crops .

## Results



**Figure 3:-** (a)-Hypocotyl length of 6 days old Arabidopsis seedlings grown under 20um white light, (b)-weight of 100 Arabidopsis seeds, (c)-Arabidopsis seed area



**Figure 4:-** (a)- Weight of 100 seeds of *Camelina sativa*, (b) size of *Camelina sativa* seeds

## Methods

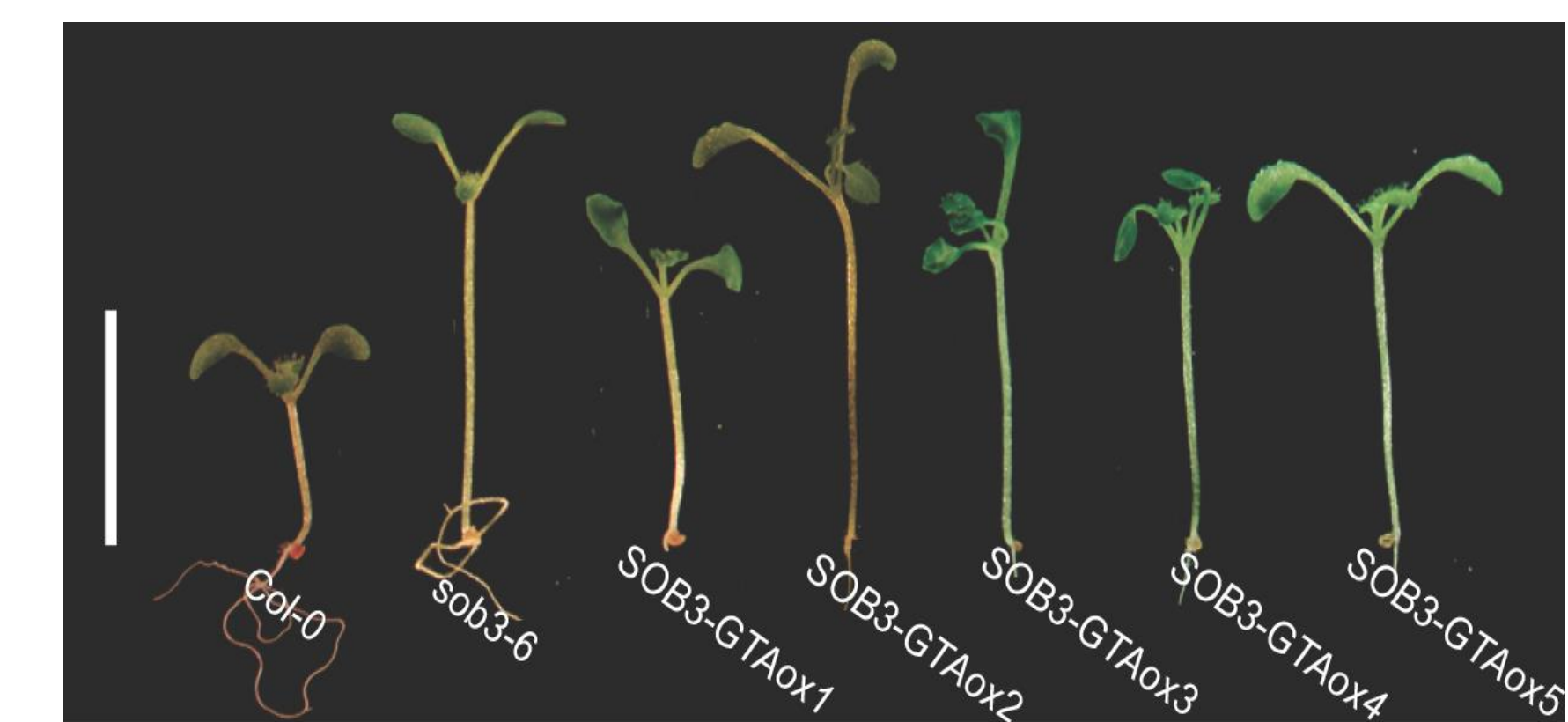
- Transgenic *Arabidopsis thaliana* expressing different AHL mutant lines under the CaMV 35S promoter were generated by *Agrobacterium*-mediated flora dip transformation (Clough *et al.*1998). Transgenic *Camelina sativa* expressing SOB3-6 were also generated using flora dip transformation(Lu *et al.*).
- Surface sterilized Arabidopsis seeds were sown on media containing 0.06% (wt/vol) Gellan Gum (Phytotechnology lab), 1.5% (wt/vol) sucrose, and 0.5x Linsmaier and Skoog-modified basal medium, incubated at 4 °C in the dark for 3 d, and grown under 20um white light at 25 °C.
- Six-day-old seedlings were then scanned into a TIFF format at 1,200 dpi using a flatbed scanner. Hypocotyl lengths were measured using National Institutes of Health (NIH) ImageJ and analyzed using Excel software.
- 100 seeds of Arabidopsis and camelina were counted and weighted using a Mettler Toledo analytical balance. Arabidopsis and Camelina seeds were also scanned into a TIFF format at 4,800 dpi using a flatbed scanner. Seed size was then measured using SmartGrain7 software National Institute of Agrobiological Sciences, Japan (NIAS).

## Broader Impacts

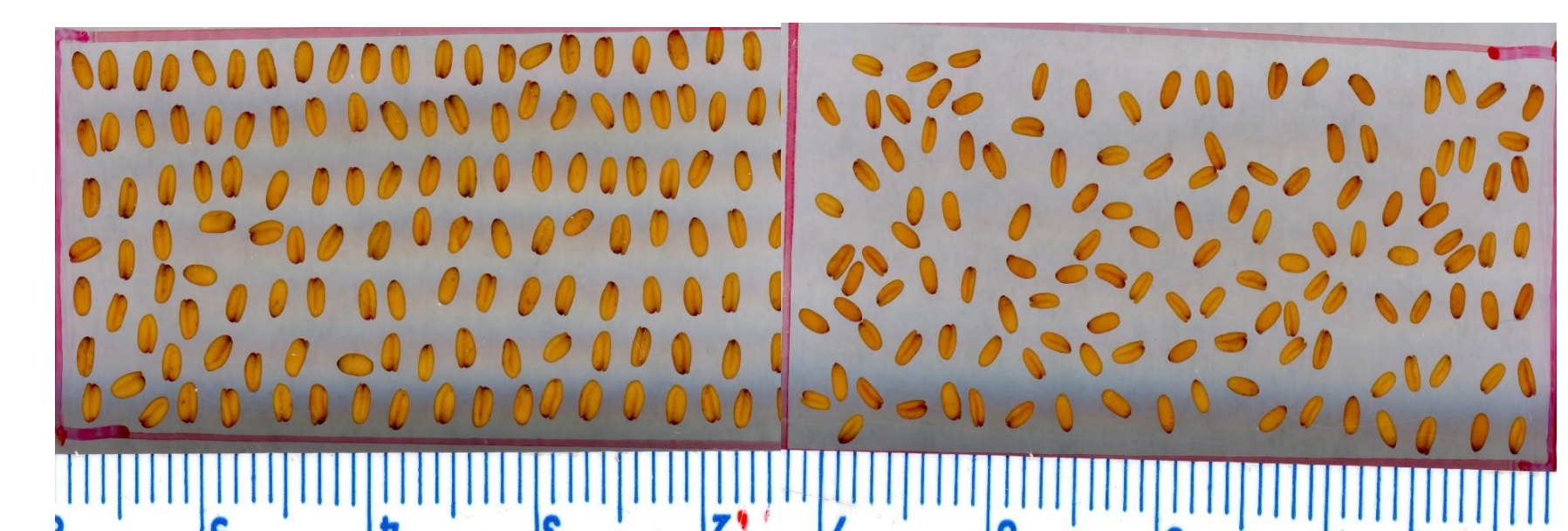
Plants that have AHL mutations that lead to a longer hypocotyl length in seedlings, also have larger seeds.

Crops that have bigger seeds with longer hypocotyls can be planted deeper into soils that are dry, in order to access moisture lower in the soil profile.

Understanding the role of AHL genes in seed size may help accelerate the process of breeding larger-seed varieties in other brassica crops.



**Figure 6:** *Arabidopsis* seedlings comparing the hypocotyl length of wild type to mutants in the AT-hook (*sob3-6*) and in the GRFEIL (*SOB3-GTA*) domains (Neff Lab, unpublished).



**Figure 7:** (Left) Scanned seeds of Camelina transgenic- expressing Arabidopsis Sob3-6 allele,(right) wild type ((Neff Lab, unpublished).

## Future Work

Work has been done to clone CRISPR constructs to create mutations which may confer big-seeds and tall seedlings in Camelina. Some constructs have been transformed and some need to be transformed.

Analysis and characterization of CRISPR lines need to be done in the future.

## References

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