GENETIC ANALYSES OF ROOT SYSTEM DEVELOPMENT IN THE TOMATO CROP MODEL

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UNDERSTANDING ROOT ARCHITECTURE

- Increase crop yields
- Increase plant quality
- Reduce irrigation and fertilization needs
- Aid in industry and research
  - Micro propagation
  - Transformation

TOMATO AS A CROP MODEL

- May have less redundancy than Arabidopsis in some genes
- New genomic resources and tools available
- Results applicable to other commercially important crops (e.g. pepper, eggplant, potato, and coffee)

ROOT ARCHITECTURE MUTANTS

- Four tomato mutants exhibiting altered root architecture-
  - *ara 1*
  - *ara 2*
  - *ara 3*
  - *diageotropica (dgt)*

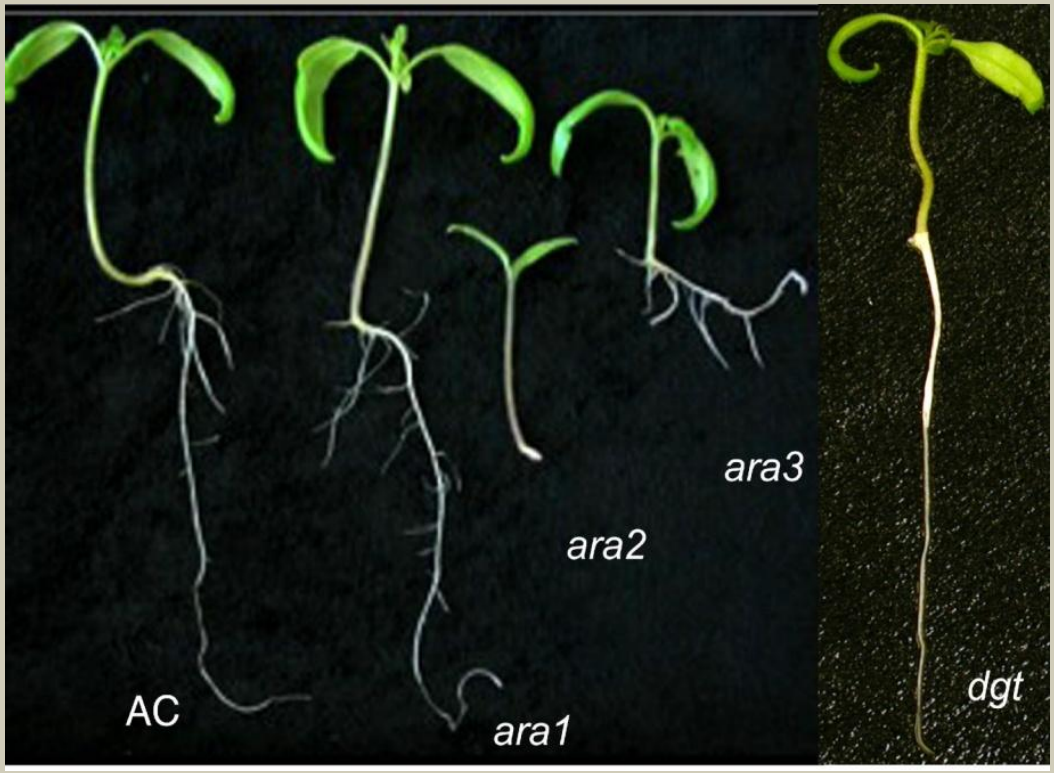
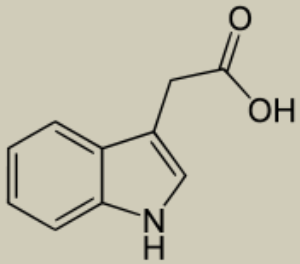


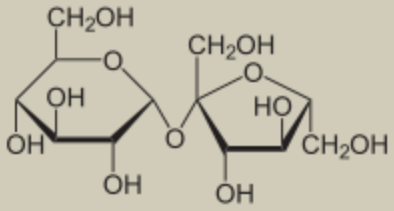
Figure 1- Tomato mutants seedlings grown on agar media

ROOT GROWTH EFFECTORS

- Auxin
  - Vital plant hormone
  - Involved in many plant processes
- Available Nutrition
  - Photosynthesis produces sugars
  - Sugar must be transported to roots



Auxin



Sucrose

HYPOTHESES

- Alterations in auxin response
- Lack sufficient nutrient supply from shoot
- Dgt mutation needs further characterization in cell type-specific effects – need to know precise expression pattern of the DGT protein

EXPERIMENTS

- Test auxin response in adventitious root formation
- Assess auxin response at tissue level
- Analyze starch accumulation and export from leaves
- Analyze nutritional responses
- Identify cell-type specific expression of *dgt* gene

ARA 1

JUVENILE *ARA 1*

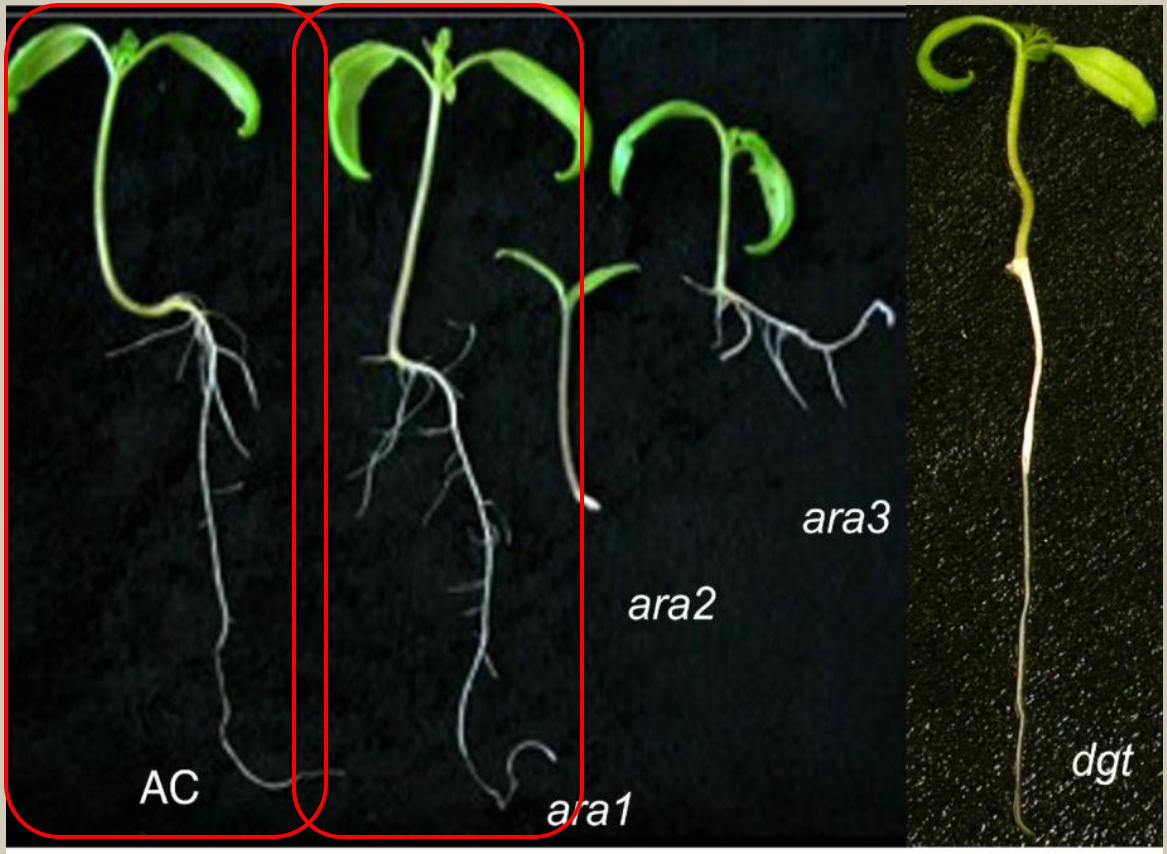


Figure 2- Juvenile WT compared to juvenile *ara 1* grown on agar media for 13 days

MATURE *ARA 1*

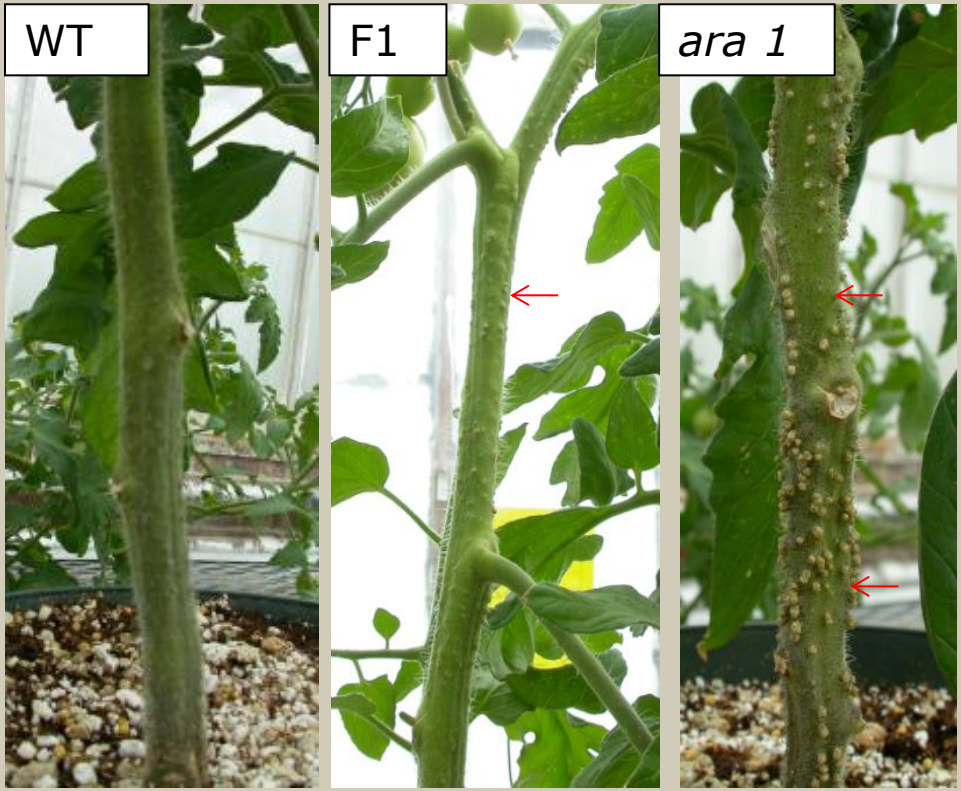


Figure 3- Comparison of adventitious root formation on the stem of mature AC (left), F1 heterozygote (center), and *ara 1* (right).

HORMONAL RESPONSES IN *ARA 1*

- Seedlings grown on agar media
- Stems cut in 4 pieces and placed on media containing different concentrations of auxin
- Cut stems left on media for two weeks
- Adventitious roots counted

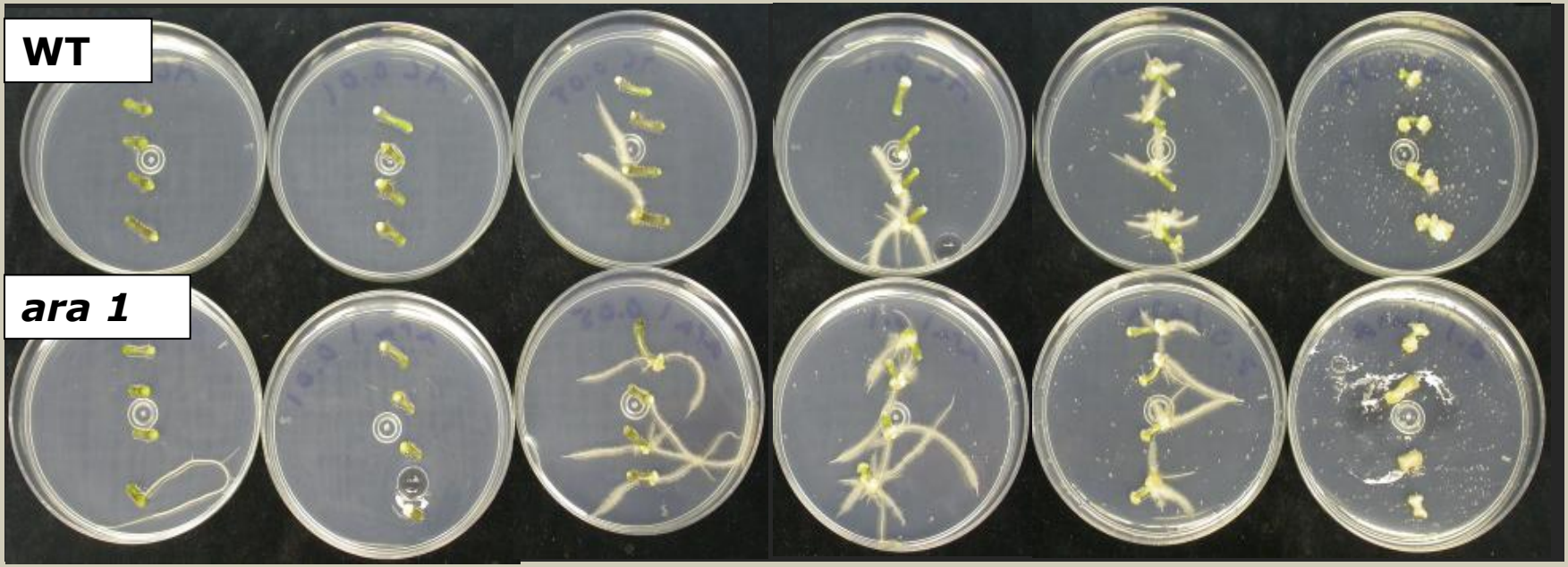


Figure 4- Adventitious root formation of *ara 1* and WT on stems after 2 weeks exposure to auxin

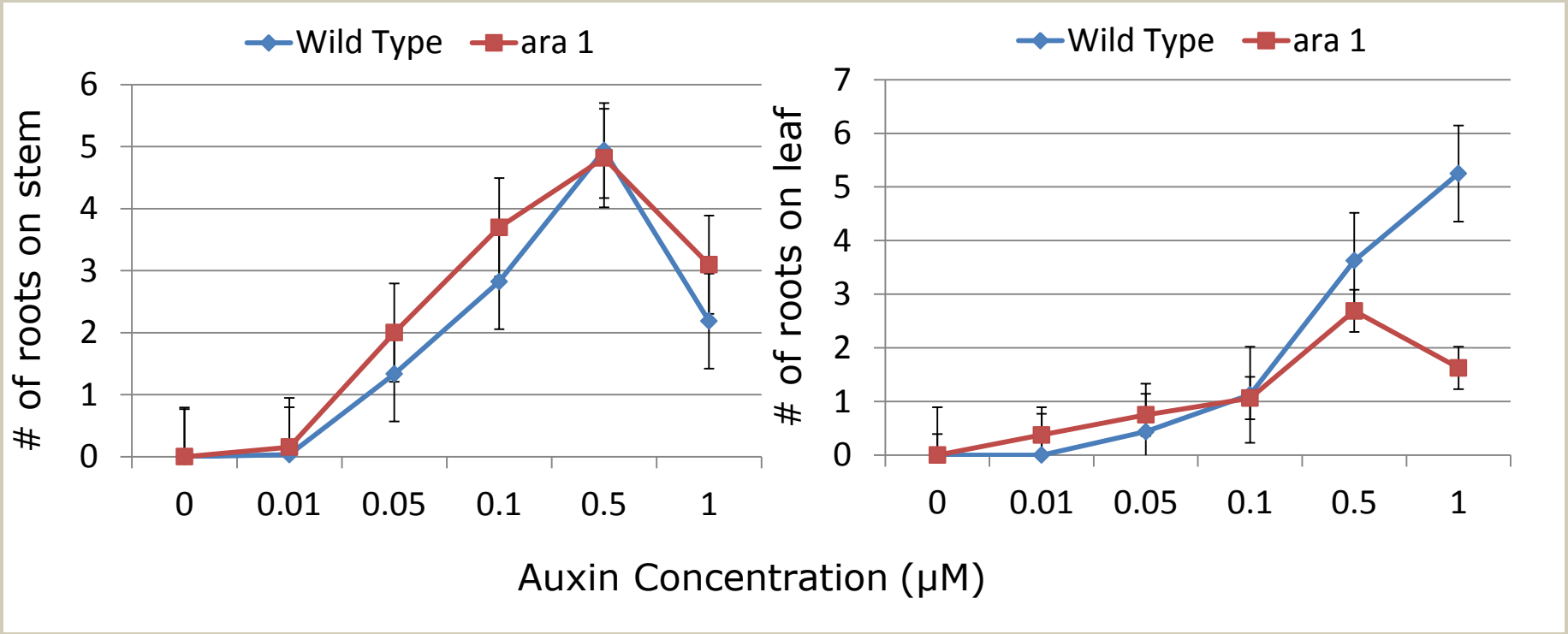


Figure 5- Nearly normal response in adventitious root formation on the leaves and stems of *ara 1* after 2 weeks exposure to auxin

NUTRITIONAL RESPONSES

- Will removing sucrose from media differentially affect *ara 1?*
- Plants grown on optimally nutritional media and media lacking sucrose
- Roots measured at 7 days old

Wild Type

ara 1

No Sucrose

1% Sucrose

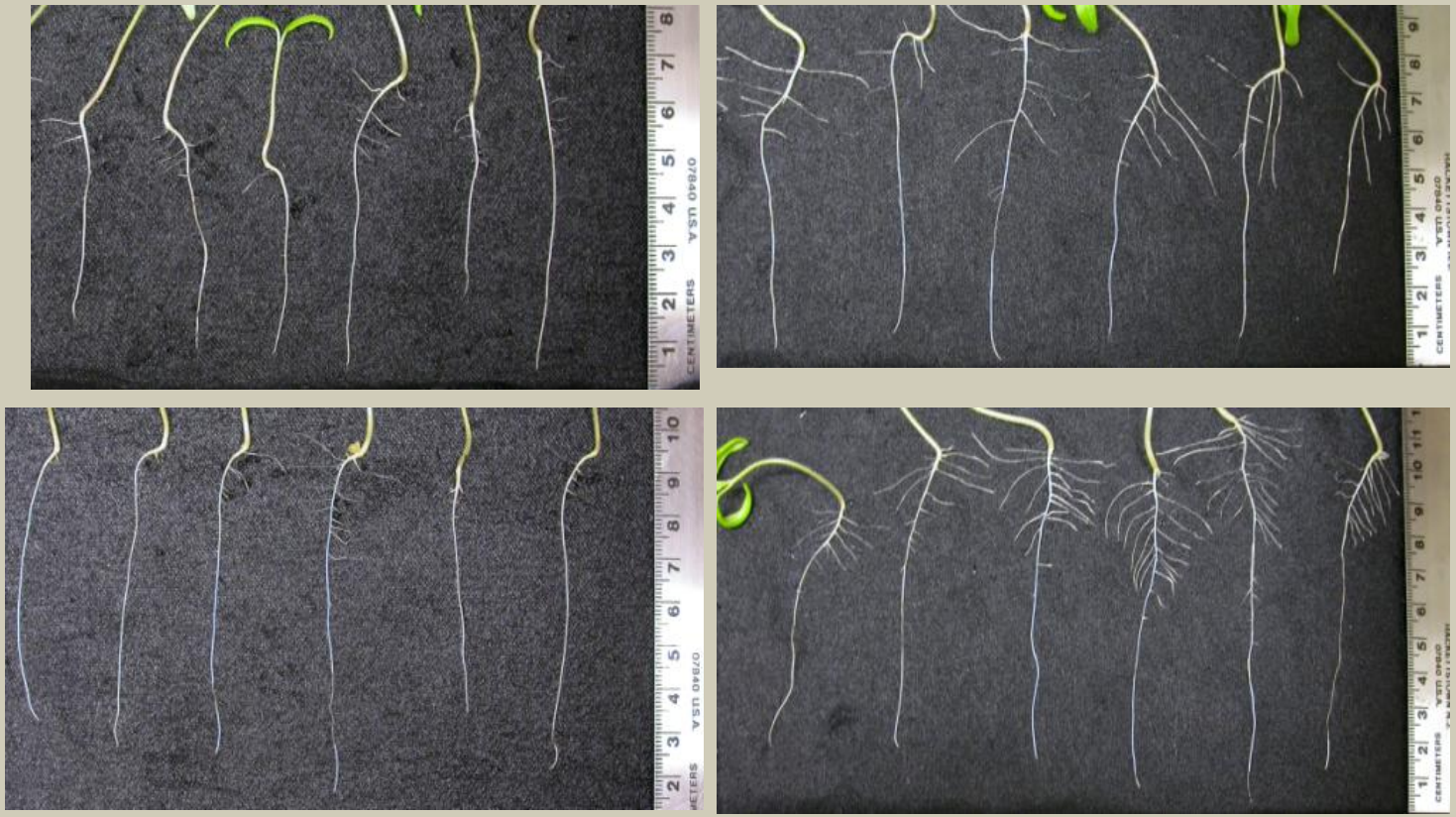
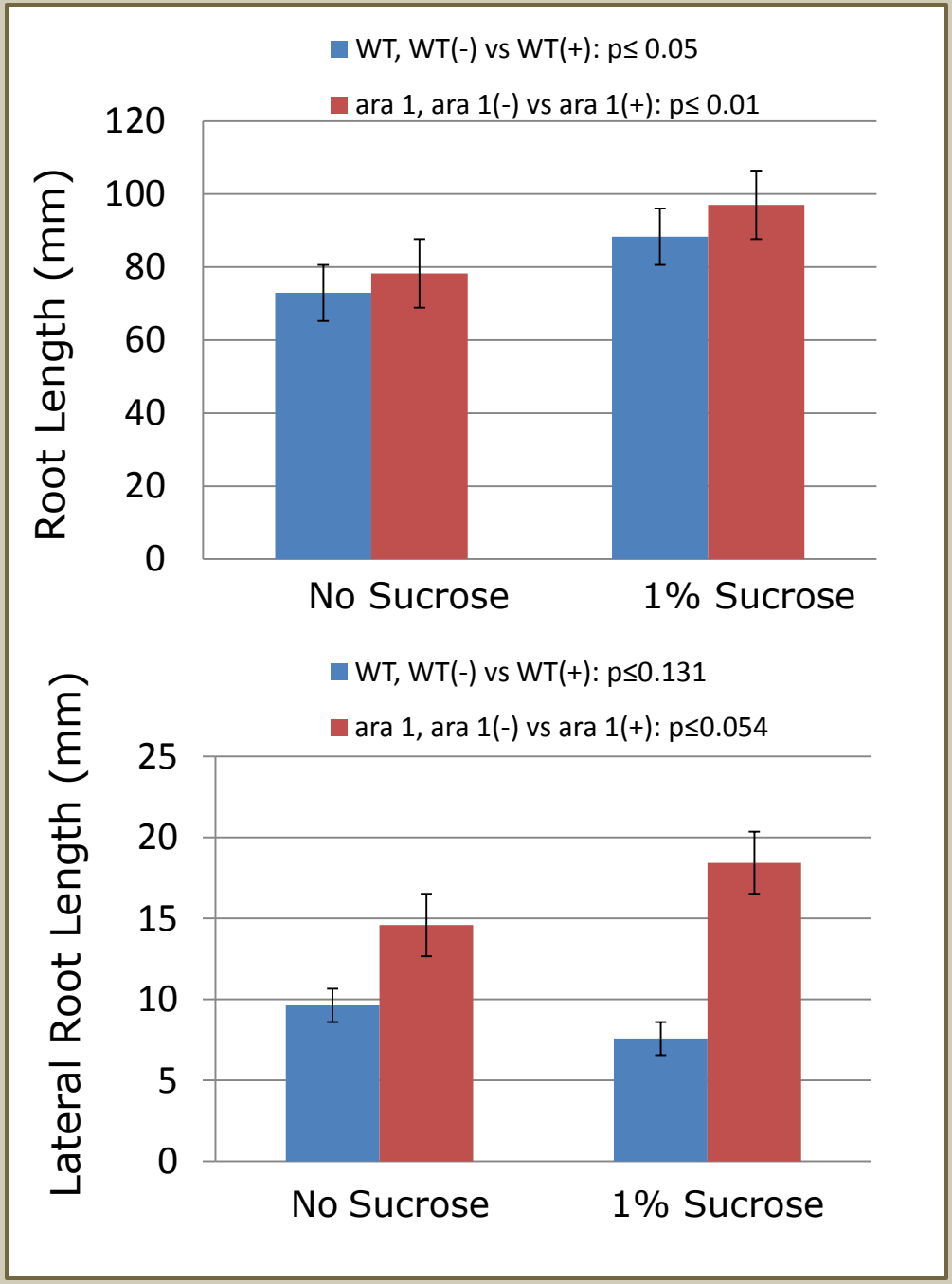


Figure 6- Root growth on media containing standard sucrose and no sucrose

Figure 7- Root growth (top) and lateral root growth (bottom) on media containing standard sucrose and no sucrose



STARCH ACCUMULATION AND EXPORT FROM LEAVES

- Main product of photosynthesis in leaves is sucrose
  - Sucrose is converted to starch
  - Starch is converted back to sucrose at night and transported to other organs

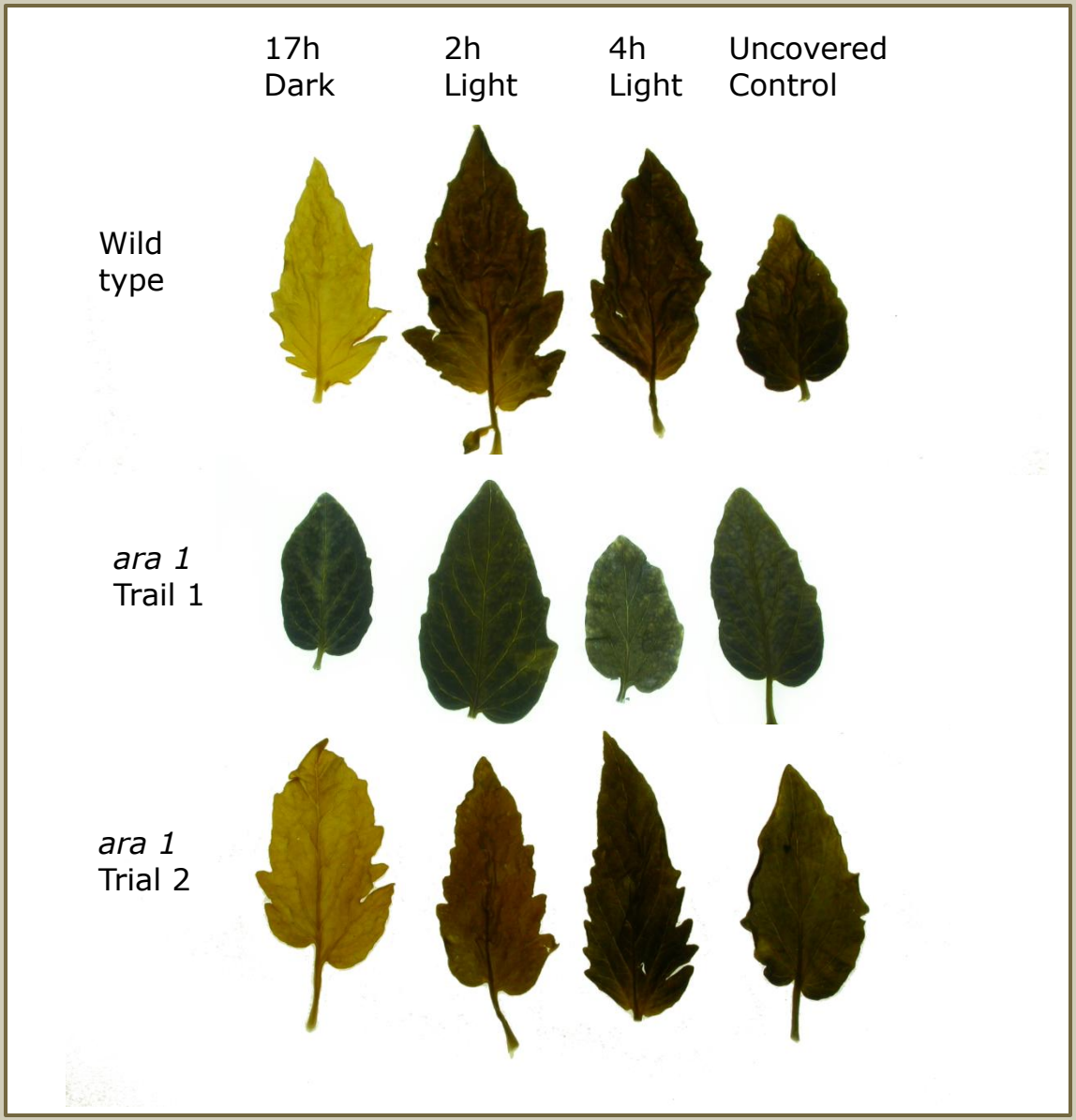


Figure 8- **Starch accumulation and export throughout the day in *ara 1***

ARA 1 DISCUSSION

- Small to no difference in auxin response, not a probable explanation for the mutation
- Root growth insensitive to nutritional depletion
- Leaves may not be transporting sucrose
- Possible mutation in sucrose biosynthesis

ARA 2

JUVENILE *ARA 2*

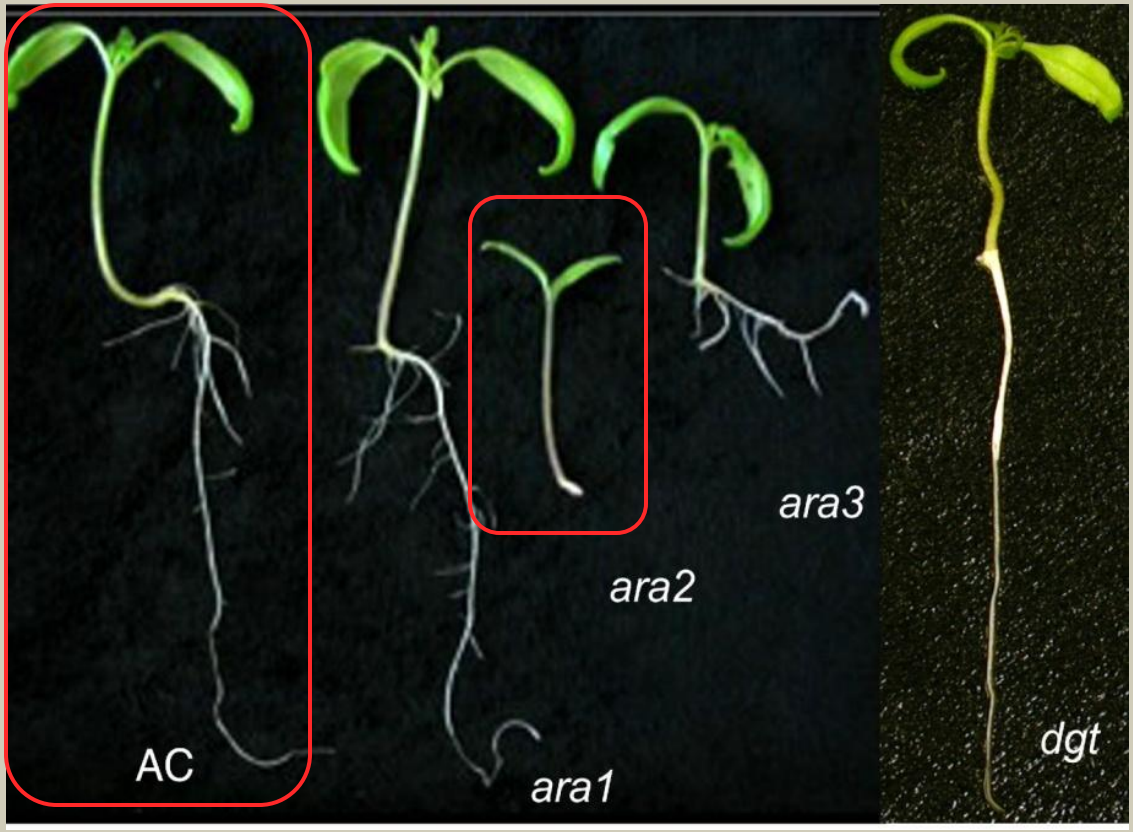


Figure 9- Juvenile WT compared to juvenile *ara 2* grown on agar media for 13 days

ARA 2 PHENOTYPE

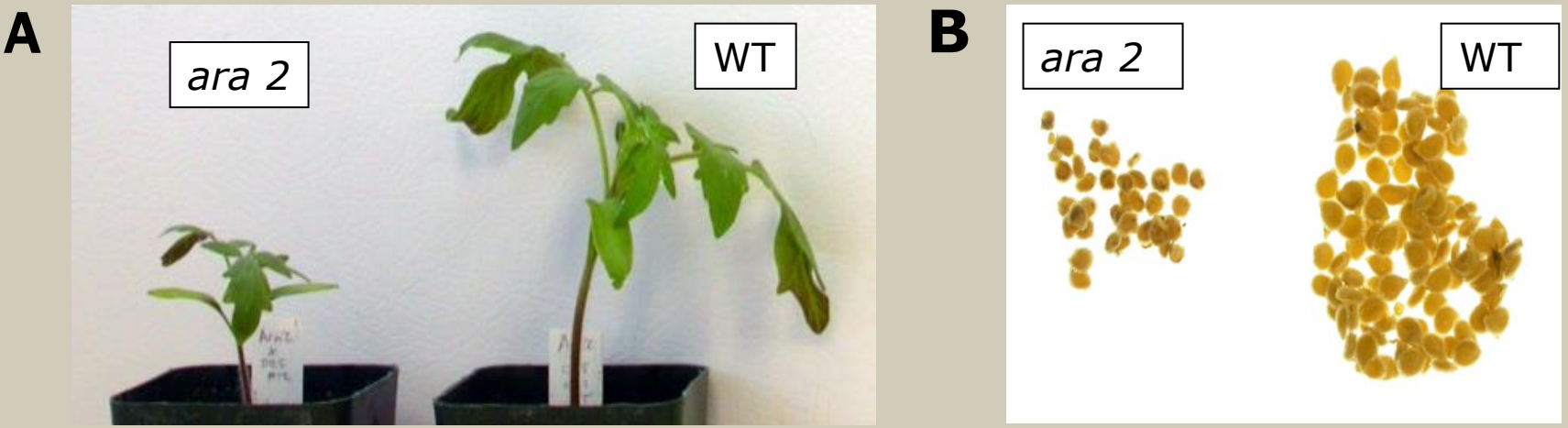


Figure 10- Mature *ara* 2 grown in soil (A) and comparison of wild type and *ara 2* seed (B)

HORMONAL RESPONSES IN *ARA 2*

- Seedlings grown on agar media
- Stems cut in 4 pieces and placed on media containing different concentrations of auxin
- Cut stems left on media for two weeks
- Adventitious roots counted

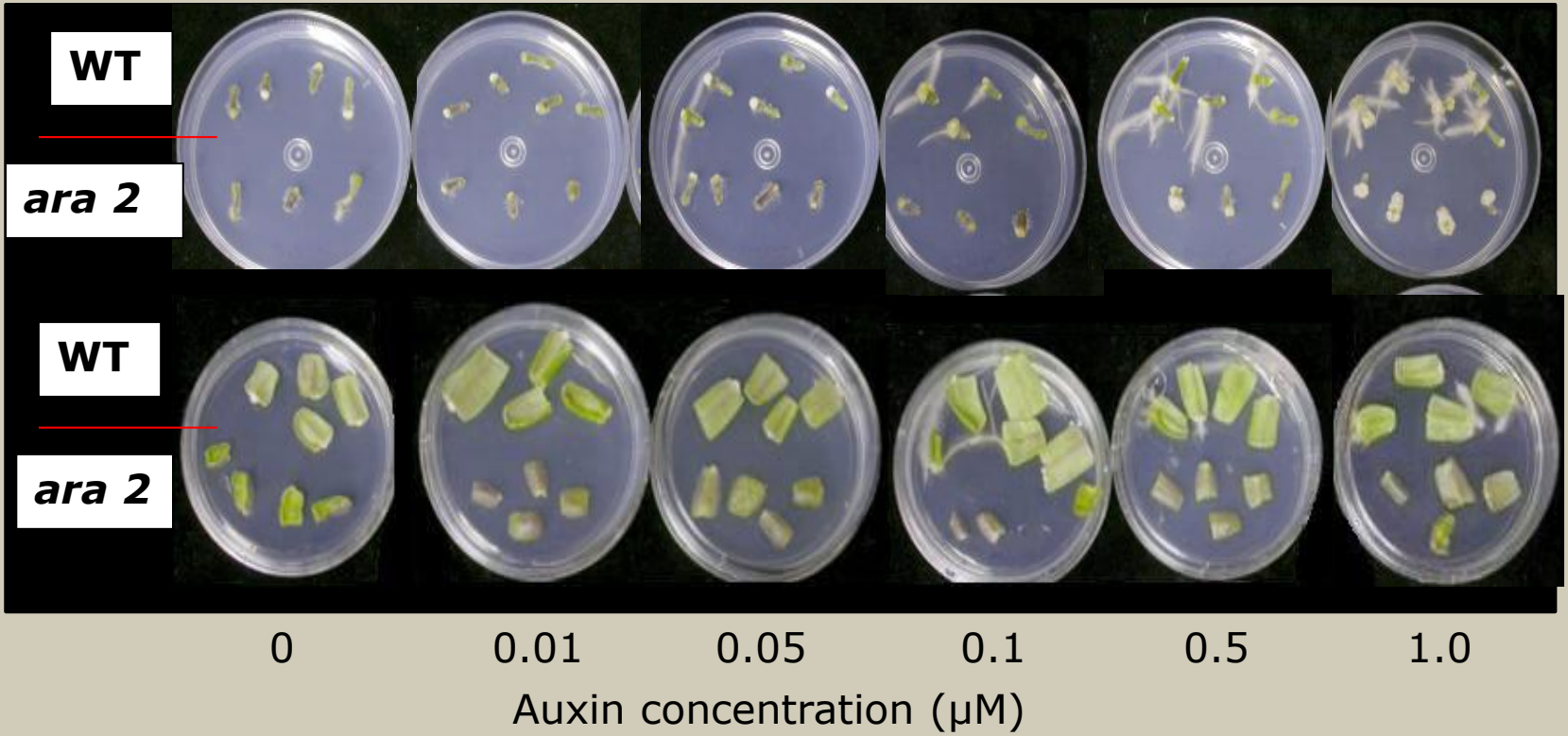


Figure 11- Adventitious root formation of *ara 2* and WT on leaves and stems after 2 weeks exposure to auxin

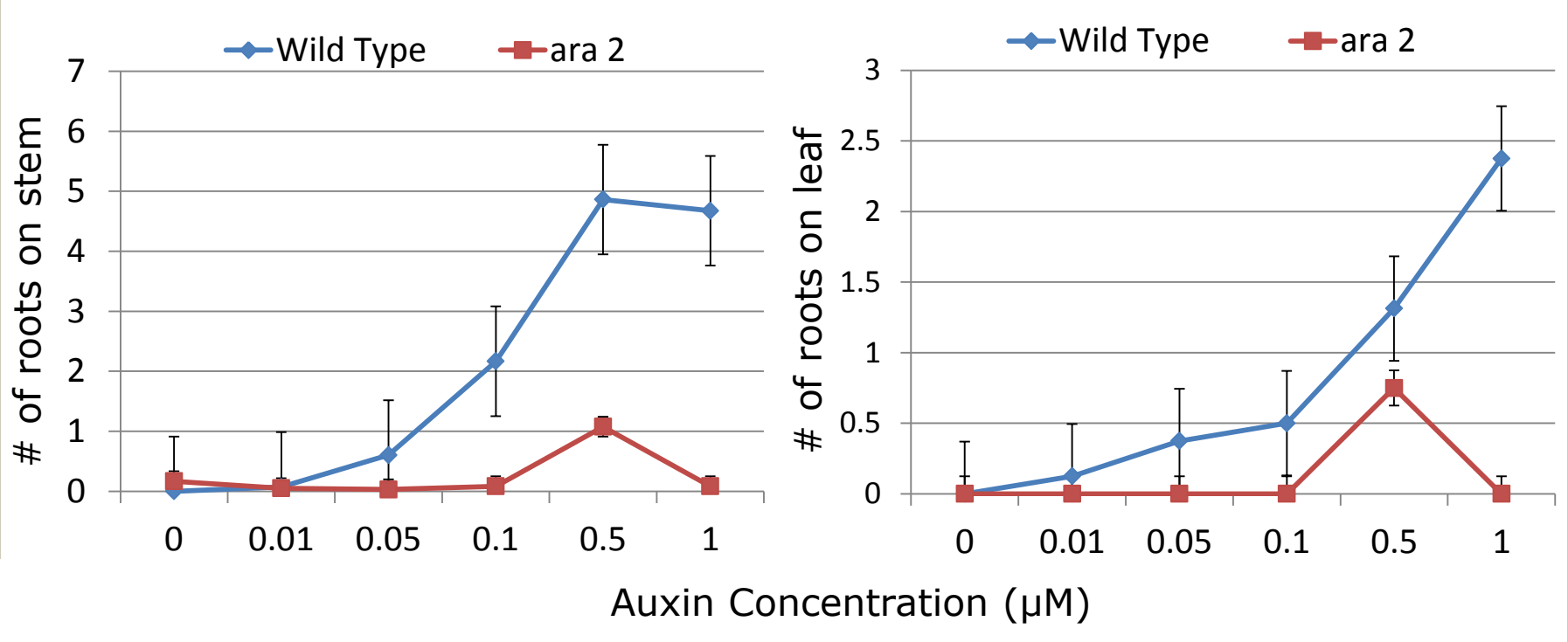


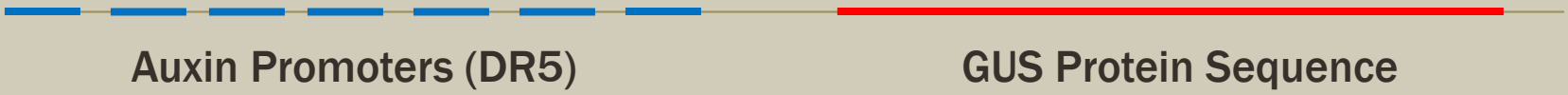
Figure 12- Decreased response in adventitious root formation on leaves and stems of *ara 2* after 2 weeks exposure to auxin

AUXIN RESPONSE AT THE TISSUE LEVEL

- Can *ara 2* respond to auxin?
- Selectively breed *ara 2* to incorporate DR5:GUS into it's genome
- DR5:GUS allows visualization of auxin response at the tissue level

DR5:GUS

- Plants with the DR5:GUS construct produce the GUS protein in cells where auxin-regulated genes are expressed
- When incubated with a substrate, DR5:GUS creates a blue stain that remains within the cell
- Auxin expression can therefore be seen at the tissue level as it will appear blue

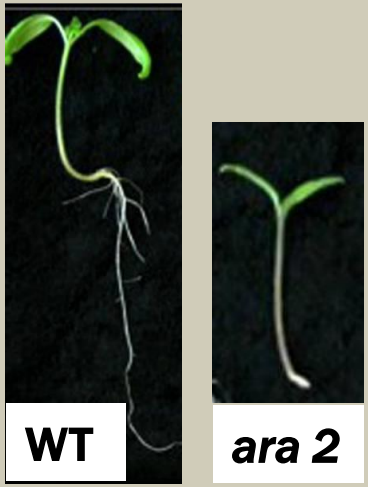


AUXIN RESPONSE AT THE TISSUE LEVEL

- Cross homozygous WT plant with the DR5:GUS construct to *ara 2*
- Selectively breed plants to create homozygous WT and *ara 2* plants that are also homozygous for DR5:GUS
- Compare WT and *ara2* staining intensity and pattern

AUXIN RESPONSE AT THE TISSUE LEVEL

- WT and *ara 2* seedlings selected
- Seedling root tips incubated with substrate
- Plants with strongly stained blue root tips grown to maturity in greenhouse
- Seed from mature plants used to verify homozygosity



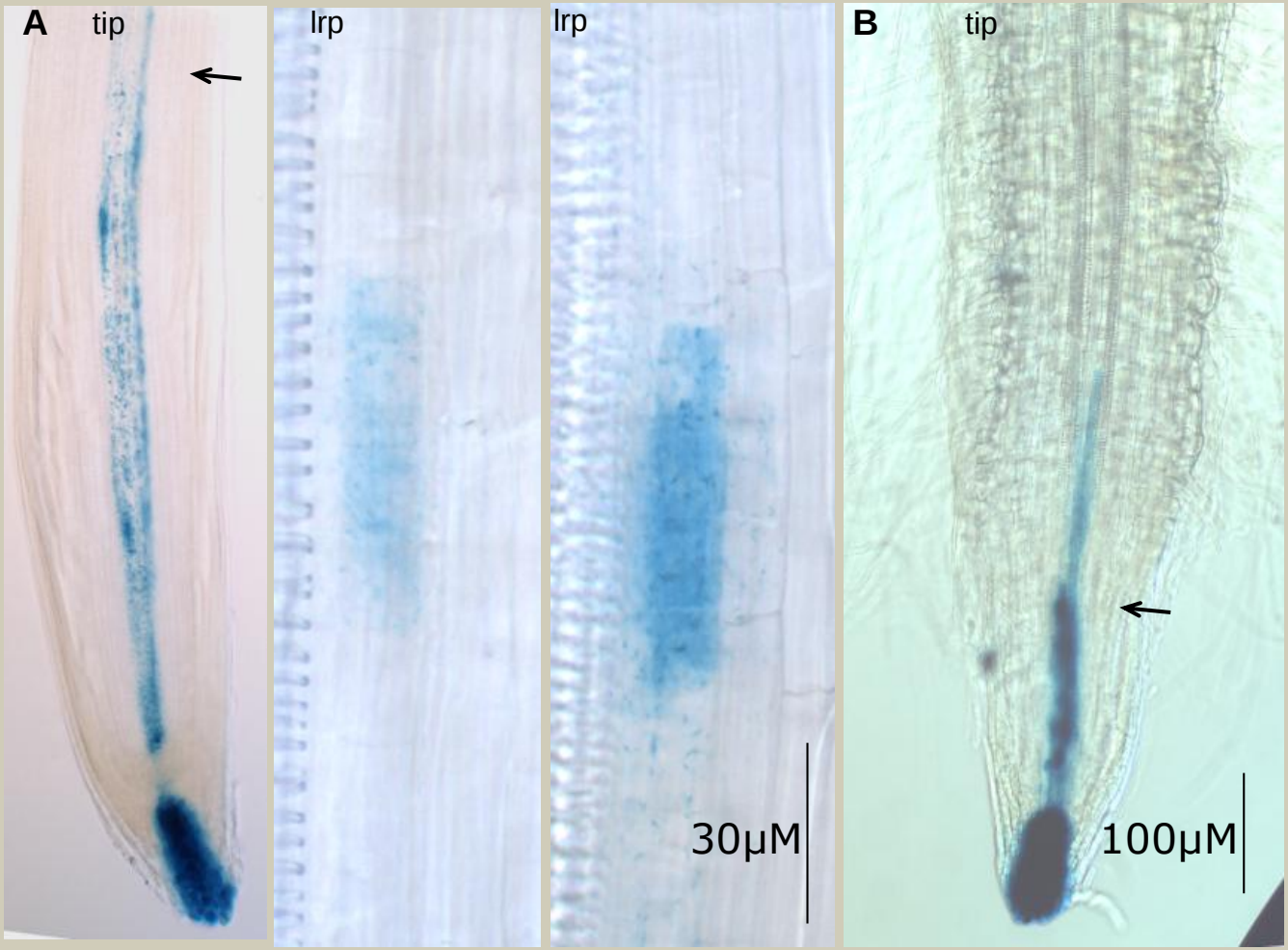


Figure 13- Comparable DR5 response between the WT and *ara 2* in root tips and a lack of DR5 response in lateral root formation in *ara 2*

ARA 2 DISCUSSION

- Exogenous auxin application creates no response
- Auxin response within the tissue appears functional
- Further testing needed to determine whether auxin signaling or transport is disrupted

ARA 3

JUVENILE *ARA 3*

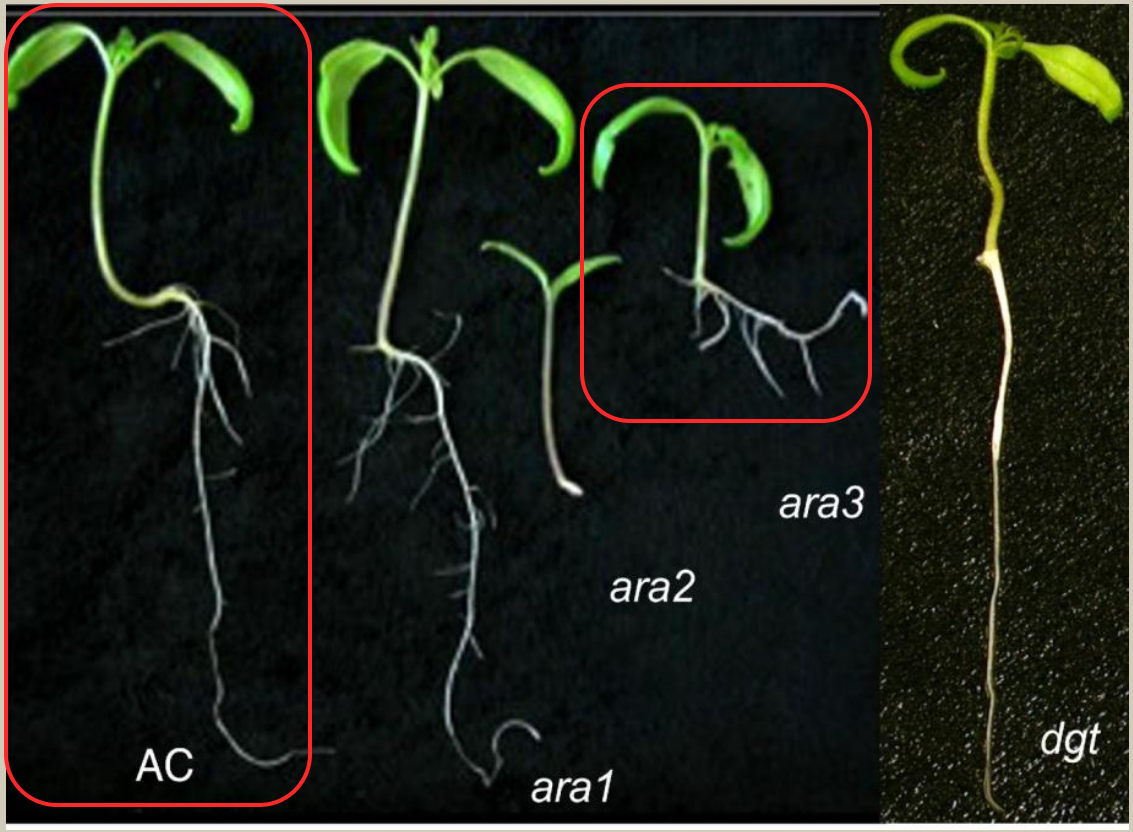


Figure 9- Juvenile WT compared to juvenile *ara 3* grown on agar media for 13 days

ARA 3 PHENOTYPE

Figure 14- Decreased apical dominance (A) and comparison of wild type and *ara 3* fruit (B)

ISOLATION OF ROOT MUTATION IN *ARA 3*

- *Ara 3* phenotypic characteristics segregating in progeny
- *Ara 3* contains more than one mutation

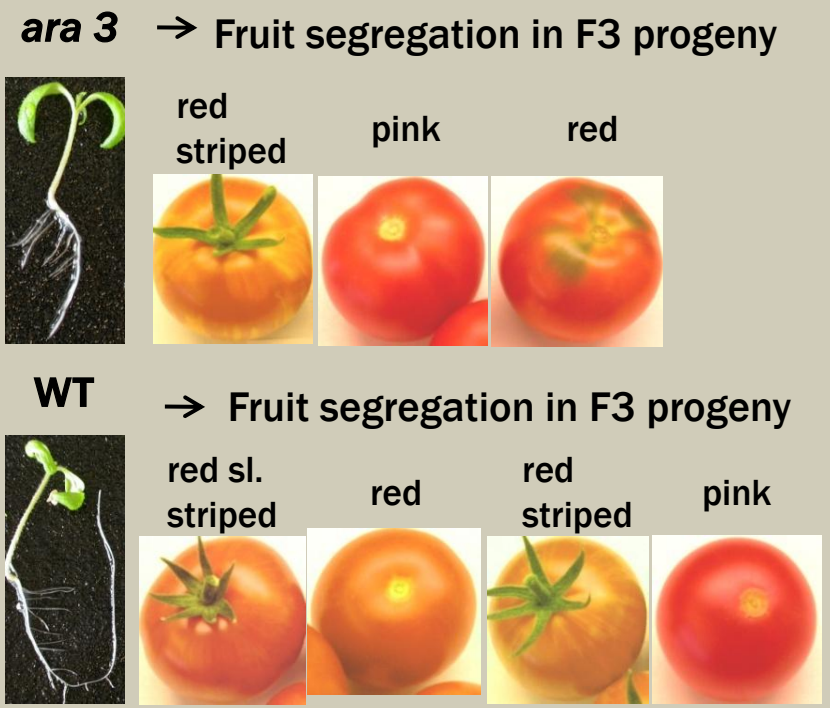


Figure 15- Segregation between WT and *ara 3* of fruit color and striping

ISOLATION OF ROOT MUTATION IN *ARA 3*

- Results with *ara 3* may not be related to the root mutation, experimentation haulted
- Selective breeding of *ara 3* to isolate root mutation new focus
- Once *ara 3* contains only the root mutation, experimentation could continue

ARA 3 DISCUSSION

- Isolation of *ara 3* root mutation not yet successful, further selective breeding needed
- Pink fruit due to *colorless fruit epidermis* (Y) gene on chromosome 1
- Striping due to *green stripe* (GS) gene on chromosome 7
- Apical dominance largely controlled by auxin, multiple genes could be responsible

DGT

DGT PHENOTYPE

- Juvenile *dgt* has no lateral roots
- Auxin resistant
- *Dgt* gene responsible for mutation
- Viewing *dgt* gene expression pattern may help understand it's role in root development

Figure 16- AC (WT) and *dgt* grown on agar media at 13 days old

TOMATO TRANSFORMATION (DGT)

- Transform *dgt* mutant with tagged DGT protein
- DGT protein expression identified as red fluorescence
- Similar to how DR5:GUS works, but no selective breeding

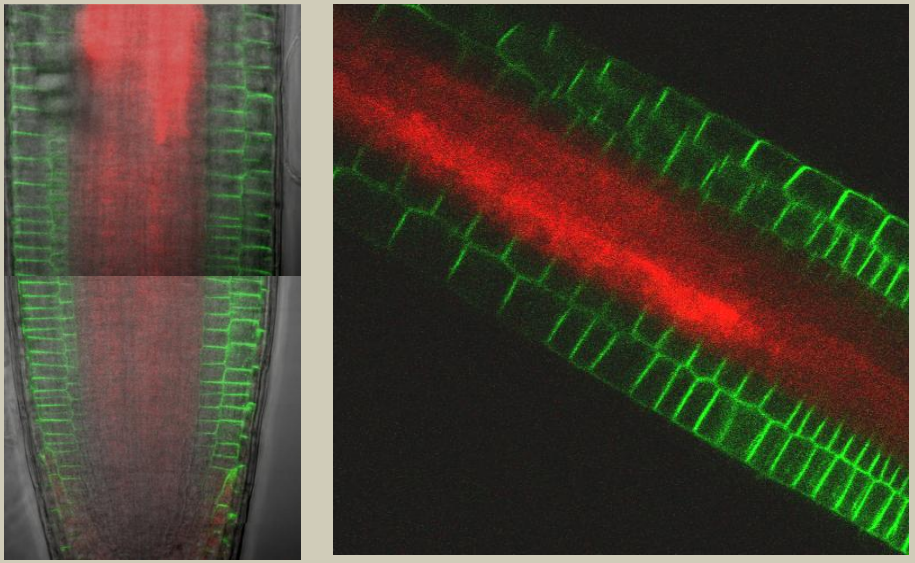


Figure 17- Arabidopsis transformed with *DGT*:mCherry-DGT construct

TOMATO TRANSFORMATION

- Young stems cut and exposed to *Agrobacterium* previously transformed with *DGT*:mCherry-DGT
- Stems placed on containing hormones
- Organogenesis forms new stem that may have the *DGT:*mCherry-DGT construct

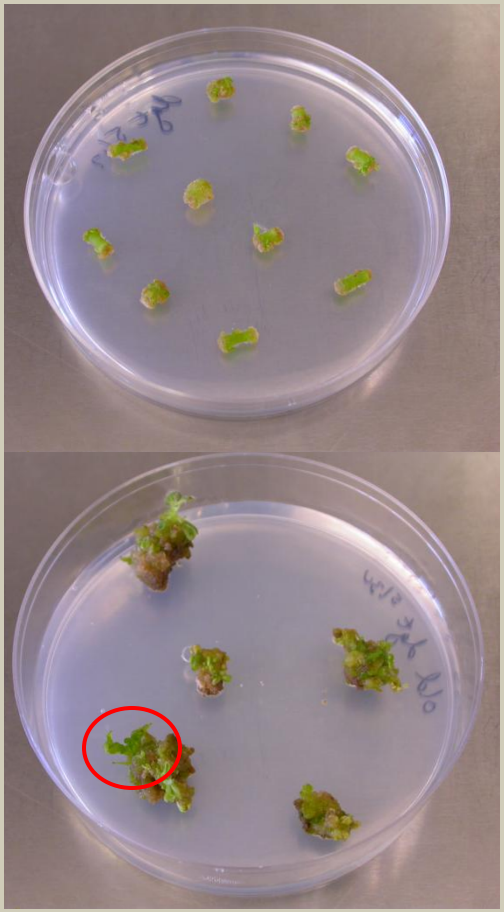


Figure 18- Cut *dgt* stems growing on hormone media

TOMATO TRANSFORMATION

- Newly formed stems cut and placed on different hormone media
- Stem produces roots and becomes self sustaining
- Full plants grown in greenhouse
- Seed from this plant grown and *dgt* expression pattern viewed

Figure 19- Cut *dgt* stems formed by organogenesis growing on different hormone media

TOMATO TRANSFORMATION

- Limited success in transformation; only wild type transformed
- Construct is effective, *dgt* gene is highly expressed within the root tip
- *Dgt* may be difficult to transform; auxin resistant
- Several crosses performed to selectively breed construct into *dgt*

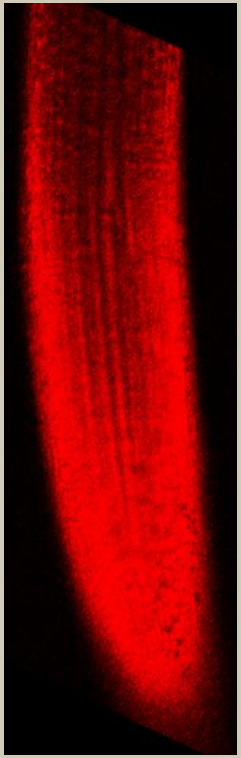


Figure 19- ***DGT***:mCherry-DGT expression in transformed seedling

SUMMARY

- *Ara 1* may have a mutation effecting sucrose biosynthesis and does not appear to have abnormal auxin sensitivity
- *Ara 2* has an altered auxin response, but auxin expression within the root tip is normal
- *Ara 3* contains multiple mutations and further selective breeding is needed to isolate the root mutation
- The *dgt* gene is highly expressed in the root tip and the *DGT*:mCherry-DGT construct is effective

CONCLUSIONS

- *Ara 1* homozygousity needs to be confirmed, starch accumulation needs more repeat experiments.
- If starch accumulation results confirmed, sucrose biosynthesis patway and sucrose effects on root development could be better understood

CONCLUSIONS

- *Ara 2* could elucidate genes involved in auxin signaling or transport, further experimentation in auxin response is needed
- Results could broaden the understanding of how auxin regulates root development

CONCLUSIONS

- *Ara 3* could help illuminate regulation of lateral root initiation
- Once root gene isolation is successful experimentation can continue
- *Dgt* currently being crossed with transformed WT, results could reveal importance of *dgt* gene
- Better understanding the *dgt* gene could also increase understanding of the regulation lateral root initiation

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REFERENCES

- **Ballester, A., & Molthoff, J.** (2010). Biochemical and molecular analysis of pink tomatoes: Deregulated expression of the gene encoding transcription factor slmyb12 leads to pink tomato fruit color. *Plant Phys.* **152**, 71-84.
- **Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G. And Friml, J.** (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591-602.
- **Cline, M.** (1991). Apical dominance. *The Botanical Review*, *57*(4), 318-346. Retrieved from http://mw8xt6bj7r.scholar.serialssolutions.com/?sid=google&auinit=MG&aulast=Cline&atitle=Apicaldominance&id=doi:10.1007/BF02858771&title=Botanicalreview&volume=57&issue=4&date=1991&spage=318&issn=0006-8101
- **Dubrovsky J. G., Napsucialy-Mendivil S., Duclercq J., Cheng Y., Shishkova S., Ivanchenko M. G., Friml J., Murphy A. S., and Benkova, E.** (2011) Auxin minimum defines a
- developmental window for lateral root initiation. *New Phytol.* **191**, 970-983.
- **Dubrovsky, J. G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M. G., Friml, J., Shishkova, S., Celenza, J. and Benková, E.** (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 8790-8794.
- Ermel FF, Vizoso S, Charpentier JP, Jay-Allemand C, Catesson AM, Couée I. (2000) Mechanisms of primordium formation during adventitious root development from walnut cotyledon explants. *Planta* **211**, 563-574.
- **Galtier, N., Foyer, C., Huber, J., Voelker, T., & Huber, S.** (1993). Effects of elevated sucrose-phosphate synthase activity on photosynthesis, assimilate partitioning, and growth in tomato (Lycopersicon esculentum var uc82b). *Plant Phys. 101*, 535-543.
- **Hodge A., Berta G., Doussan C., Merchan F. and Crespi M.** (2009) Plant root growth, architecture and function. *Plant Soil* **321**, 153–187.
- **Ivanchenko, M. G., Coffeen, W. C., Lomax, T. L. and Dubrovsky, J. G.** (2006) Mutations in the Diageotropica (Dgt) gene uncouple patterned cell division during lateral root initiation from proliferative cell division in the pericycle. *Plant J.* **46**, 436-447.
- Kircher S. and Schopfer P. (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **109**, 11217-11221.
- **Malamy J.E. and Benfey P.N.** (1997) Organization and cell differentiation in lateral roots of Arabidopsis thaliana. *Development* **124**, 33-44.
- **Negi S., Sukumar P., Liu X., Cohen J.D. and Muday G. K.** (2010) Genetic dissection of the role of ethylene in regulating auxin-dependent lateral and adventitious root formation in tomato. *Plant J.* **61**, 3-15.
- **Oh K., Hardeman K., Ivancehnko M.G., Ellard-Ivey M., Nebenführ A., White T.J. and Lomax T.L** (2002) Fine mapping in tomato using microsynteny with the *Arabidopsis* genome: The diageotropica locus. *Genome Biol.* **3**, research0049.
- **Oh, K., Ivanchenko, M. G., White, T. J. and Lomax T. L.** (2006) The diageotropica gene of tomato encodes a cyclophilin: a novel player in auxin signaling. *Planta* **224**, 133-144.
- **Park S.H., Morris J.L., Park J.E., Hirschi K.D. and Smith, R.H.** (2003) Efficient and genotype-independent Agrobacterium-mediated tomato transformation. *J. Plant Physiol.* **160**, 1253-1257.