**C₄ Photosynthesis**

- Reduction in Photorespiration
  - Exclude O₂ from Rubisco
- Saturates Rubisco with CO₂, so it can achieve maximal catalytic efficiency
- C₄ plants use water and nitrogen more efficiently
- Increases the Radiation Use Efficiency
- More productive per unit area!

**Transferring C₄ into C₃ crops**

- Modeling predicts that it could eventually boost yields by 50% (Sage and Zhu (2011) JXB)
- Engineering specific architecture and cell-cell interactions is considered the major challenge
- Despite 30+ years of research - No known genes that function in Kranz anatomy have been described

**Basics of Kranz anatomy**

- Single cell layer that surrounds the vascular core
- BS cells preferentially accumulate starch
- Increased vein density (only 2 M cells between veins)
  V-BS-M-M-BS-V
- Reduced M size
- BS cells are suberized

**Basics of Kranz anatomy**

- Dimorphic Chloroplasts
- Low PS II in BS chloroplasts

**Formation of minor veins – PIN-YFP expression**

- BS cells form clonal patches along the length of the vein
- BS cells form before the internal Vascular core differentiates

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Slewinski et al (2012) PCP*
Kranz-type C₄ is derived from a conserved tissue found in angiosperms

- **Hypothesis** – Kranz anatomy and C₄ photosynthesis are derived from the projection of endodermal identity onto the bundle sheath within the photosynthetic leaf tissue. This results in a synergistic interaction between photosynthetic cells and the endodermis.

- Would explain how C₄ always arises “fully formed”

- Starch preferentially accumulates in the BS – because it is adapted from the “Starch Sheath” in the stem and petiole

- Suberin synthesis is also characteristic of the endodermis

- In roots, stems, and petioles the endodermis is an Auxin conducting tissue (PIN expression)
  - May explain why there is a shift in vein density?

---

**Evolution of C₄ in plants**

- Kranz-type C₄ evolved at least 70 times independently in both dicots and monocots

- One of the most remarkable examples of convergent evolution

- Evolves “Fully Formed” – little evidence for slow evolutionary progress towards Kranz-type C₄

---

**Kranz-type C₄ is a synergistic interaction between photosynthetic cells and the endodermis**

- Leaf identity projects the photosynthetic program into the endodermis

- Photosynthetic cell (mesophyll) → Endoderm (bundle sheath) → Vascular core

- The endodermal cells project a cortex-like identity onto the mesophyll/photosynthetic cells (CO₂ metabolic shuffling)

- Projecting the endodermal program into C₃ leaves may be one way to engineer C₄ leaves

---

**The endodermis is primed with C₄ metabolism**

- Characteristics of C₄ photosynthesis in stems and petioles of C₄ flowering plants

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**Hypothesis for the evolution of Kranz-type C₄**

Endodermal program projects into the leaf blade - giving rise to Kranz anatomy and preconditioning the metabolism for C₄

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Formation of minor veins – PIN-YFP expression

Development of the minor vein mirrors root formation

Helariutta et al. (2000)

Gene candidates based on endodermis formation in the roots

SCARECROW (SCR) is a primary regulator of endodermis development and identity

Helariutta et al. (2000)

ZmScarecrow Expression

Li et al. (2010) Nature Genetics

mutant alleles

ZmScarecrow GRMZM2G131516

Slewinski et al. (2012) Plant Cell Phys

zmscr mutants produce extra BS cell files in leaves

IKI stained

zmscr

Slewinski et al. (2012) Plant Cell Phys

zmscr mutants have defective minor veins

Wild type IKI stained zmscr

Slewinski et al. (2012) Plant Cell Phys

Veins in the scr mutant leaves frequently collide

IKI stained

BS cells merge into a continuous structure that displaces mesophyll cells

Slewinski et al. (2011) Plant Cell Phys
**ZmSCR regulates BS formation and identity**

Wild type | IKI stained | ZmSCR
---|---|---

**Disruption of ZmSCR results in undifferentiated BS cells**

ZmSCR

**Gene candidates based on endodermis formation in the roots**

Stele | Endodermis | Cortex
---|---|---

--- What role does SHORTROOT (SHR) play in Kranz anatomy and C4 physiology?

**zmshortroot1 mutant**

Mutant allele of ZmSHR1

ZmSHR1 expression

WT | IKI stained | zmshr1
---|---|---

**Incomplete Kranz anatomy in the zmshr1 mutant**

WT | zmshr1
---|---

**Altered M cells in the zmshr1 mutant**

WT | zmshr1
---|---
BS without veins in the zmshr1 mutant

Arundinella hirta
*distinctive cells*

Model of minor vein formation in developing WT leaves based on PIN-YFP expression

The Phyllode Theory and grass leaf development

The Phyllode Theory and grass leaf development

Morphological shift that led to the grasses also preconditioned Kranz-type C₄
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