When does size matter?

How nitrate supply, storage, sink size and plant stature influence yield and grain size in *Sorghum bicolor*

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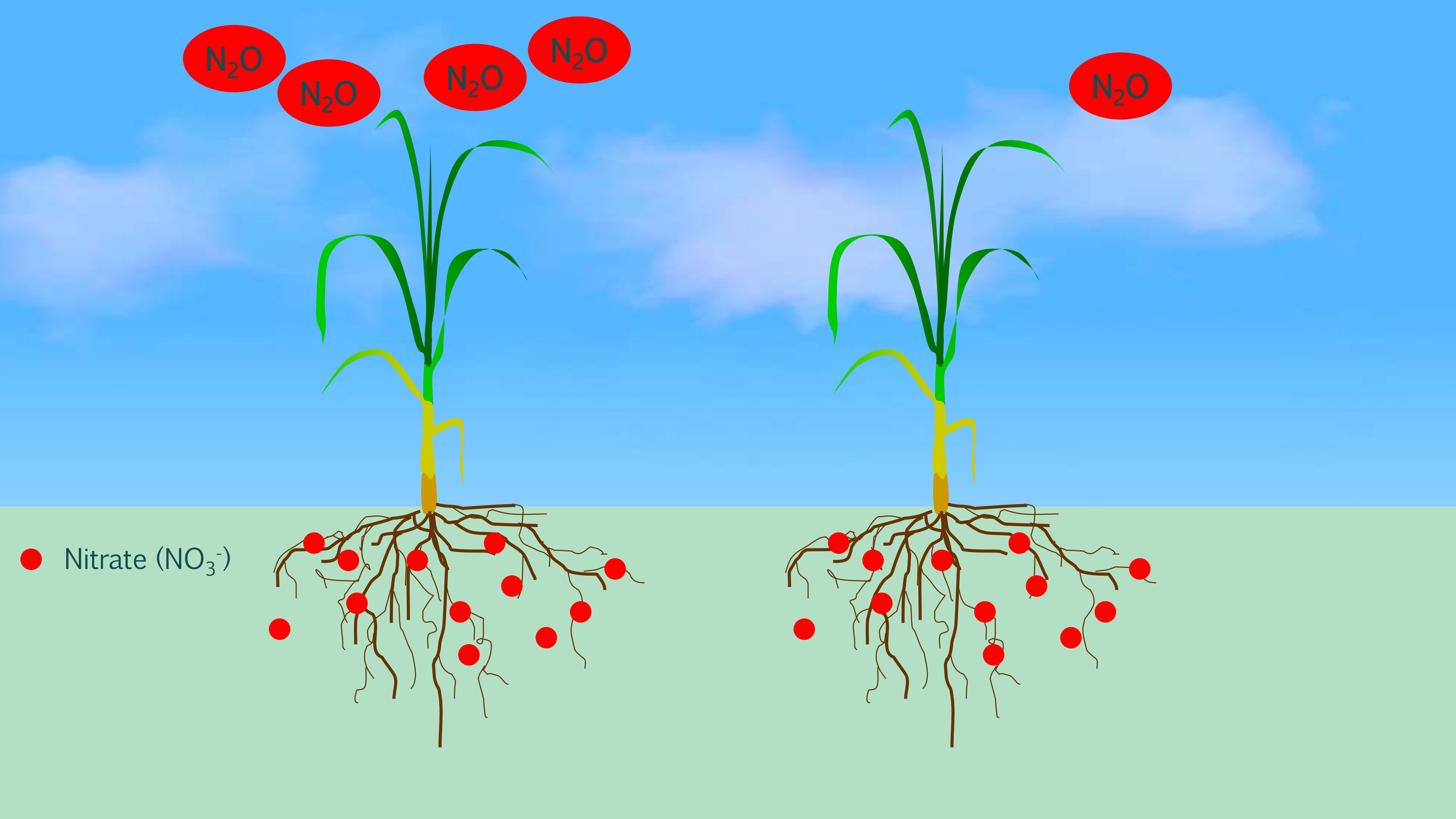


N_2O
N_2O
N_2O
N_2O
N_2O
Nitrate (NO_3^-)

Nitrate accumulation promotes increased yield

"…increased productivity in maize genotypes was due to their ability to accumulate nitrate in their leaves during vegetative growth and to efficiently remobilize this stored nitrogen during grain filling."

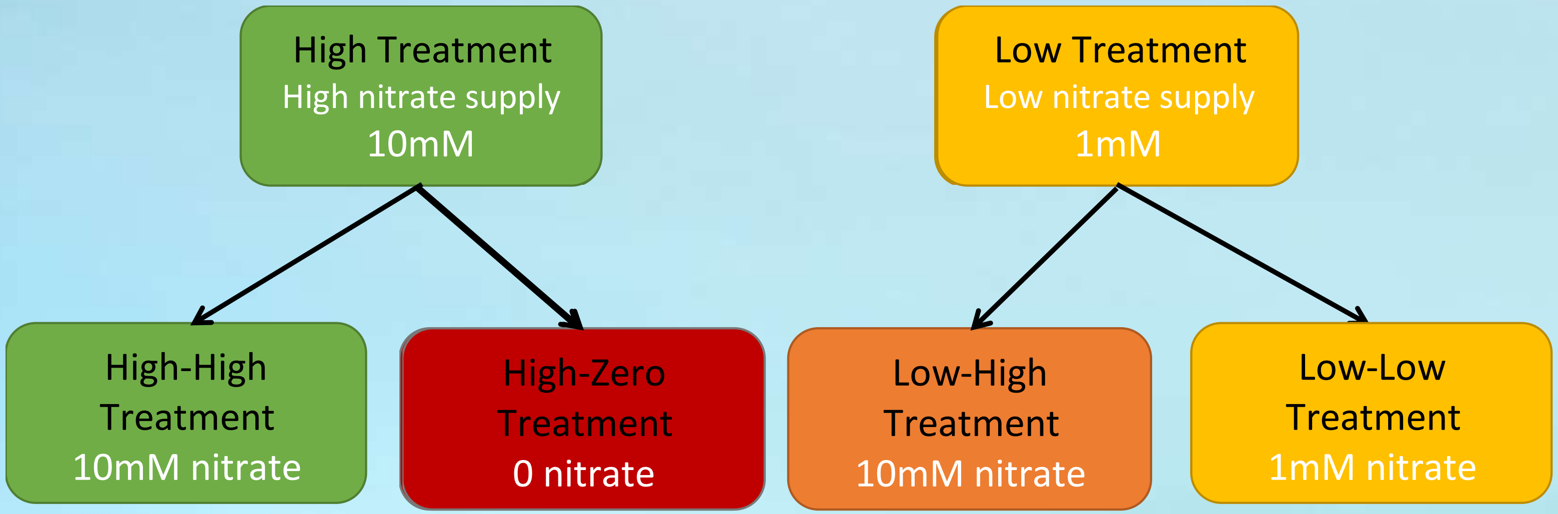
Hirel et al. 2001, *Plant Physiology*

Controlled Glasshouse Experiments

- R931945-2-2 elite Australian breeding line
- Potassium nitrate – sole nitrogen source
- High = 10mM KNO_3^-
- Low = 1mM KNO_3^-
- Inert substrate
- Complete nutrient supply
- Well-watered

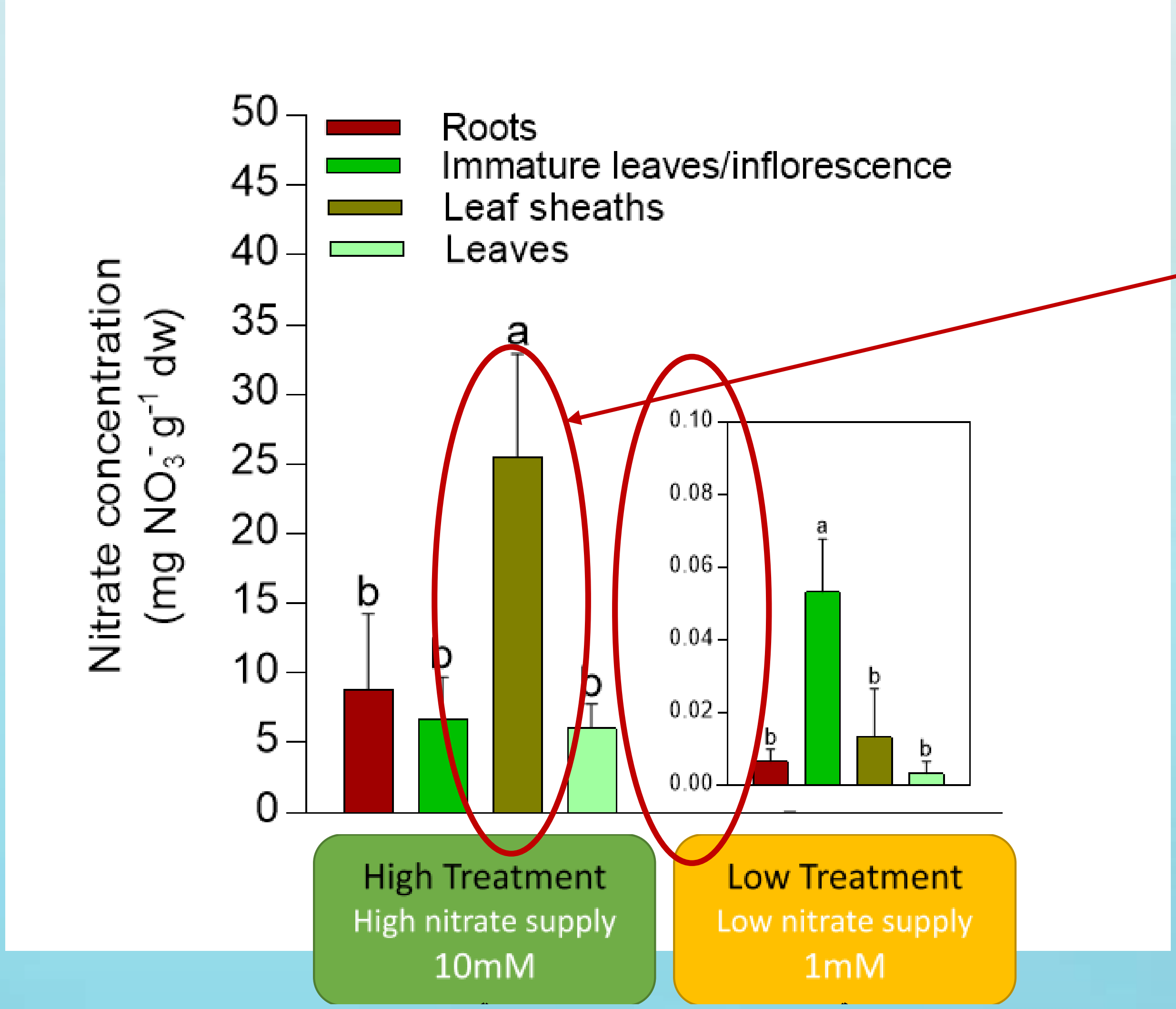
55 DAS pre-panicle exsertion

Nitrate supply changed



55 DAS – high or low nitrate supply

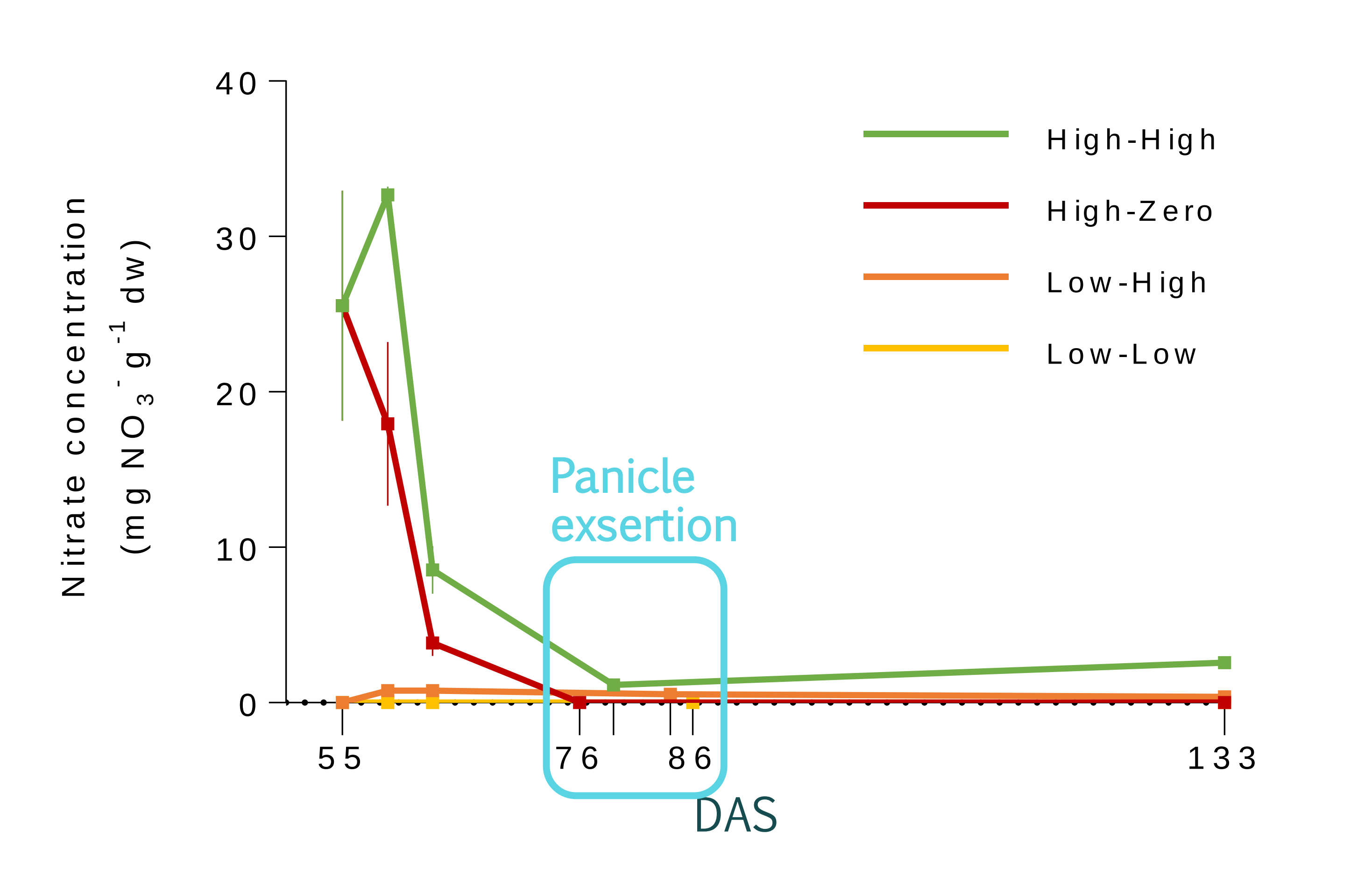
Nitrate concentration highest in LEAF SHEATHS at 55 DAS



~4x higher nitrate concentration

Worland et al., *Journal of Plant Physiology*, 2017

Nitrate was assimilated in all plant organs prior to panicle exsertion



Worland et al., *Journal of Plant Physiology*, 2017

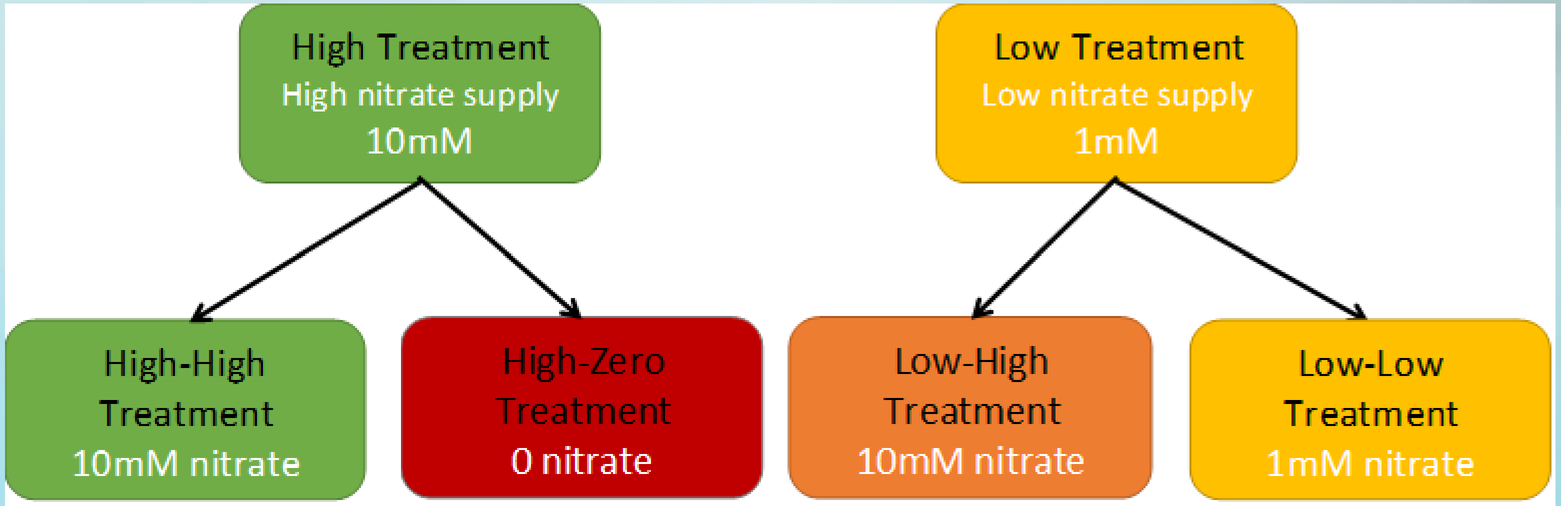
Nitrate supply post-anthesis critical to yield and grain protein

High-High Treatment 10mM nitrate	versus	⇧ Low-High Treatment 10mM nitrate

- Nitrate supply increased pre-panicle exsertion
- Panicle 61% smaller than High-High
- 24% less nitrate applied than High-High
- 29% larger grains than High-High
- Similar yield and protein content

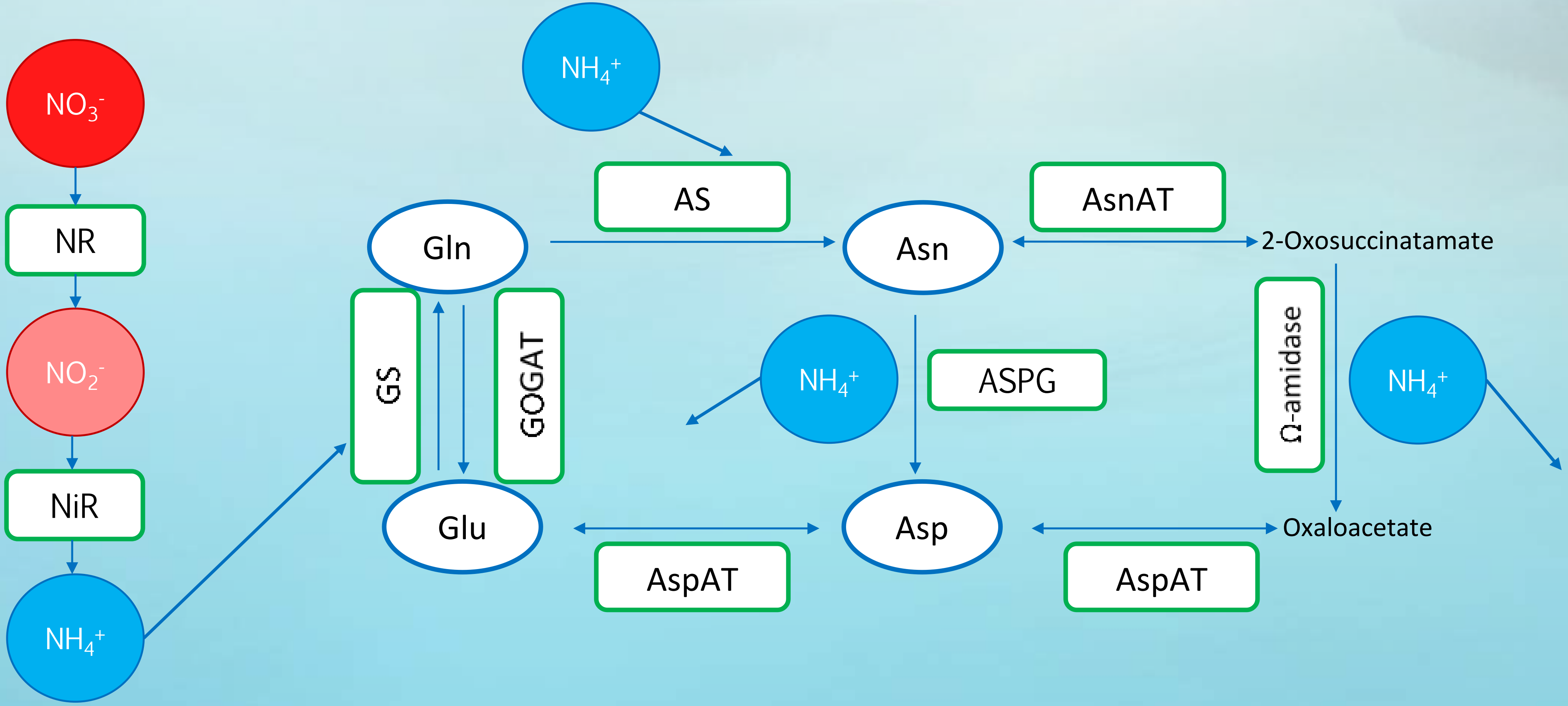
Worland et al., *Journal of Plant Physiology*, 2017

Transcriptomes of leaf sheath



- Harvested 4 times, every 2 days after change in nitrate supply
- Transcriptome produced at 4 days after change in nitrate supply

Nitrate & Ammonium assimilation pathway



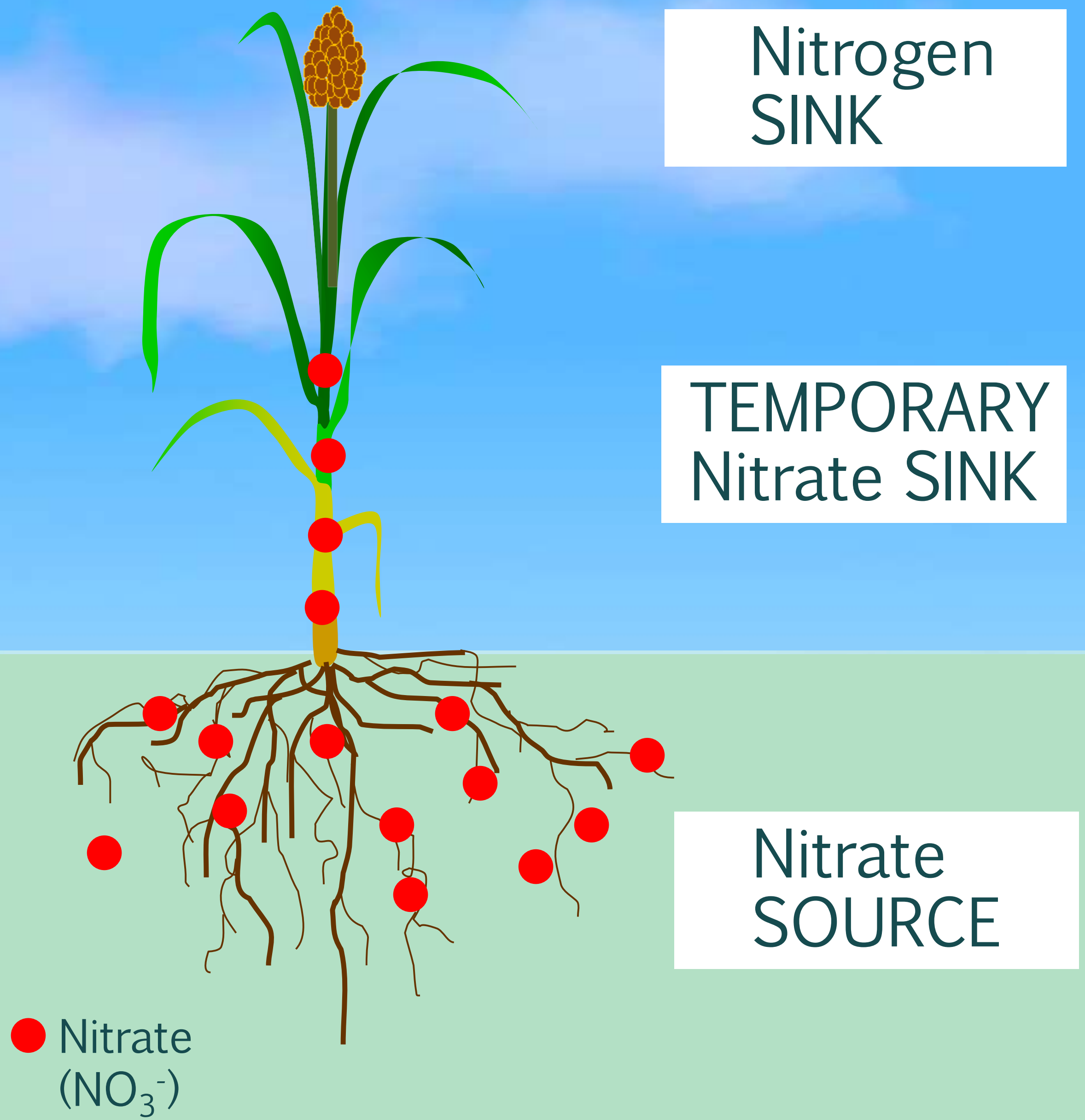
Nitrogen assimilation and mobilization in leaf sheaths

- Differential gene expression of N assimilation enzymes being validated (qPCR)
- Quantification of free amino acids & ammonium (UPLC)
- Differential gene expression of
  - 1 ammonium transporter
  - 6 low and 1 high-affinity nitrate transporters
  - 5 peptide transporters being validated (qPCR)
- Creation of hypothetical nitrogen assimilation and mobilization pathways under variable conditions of nitrate supply

Nitrogen sink size
- yield vs grain size
- protein content

Nitrate storage & assimilation
- maximise nitrate storage by increasing leaf sheath size

Nitrate supply & timing
- critical POST-anthesis



R931945-2-2
Australian elite breeding line

High-High Treatment
10mM nitrate

R931945-2-2
dw3 mutant

Near-isogenic-line (NIL)

Increased temporary nitrate sink

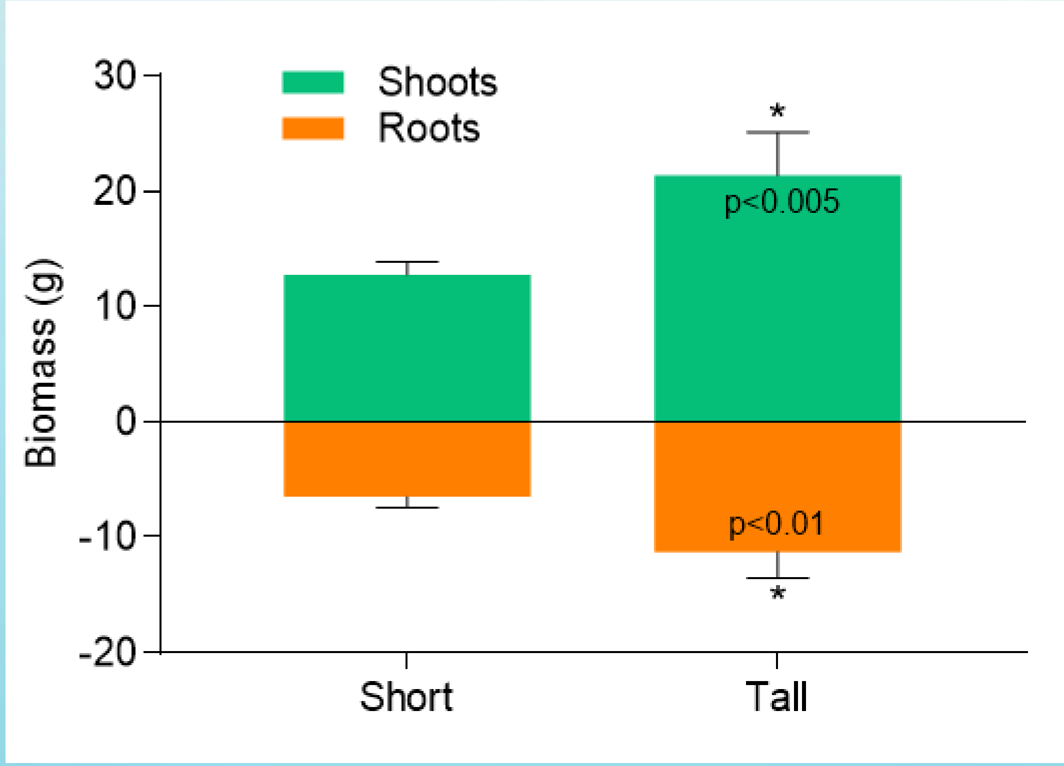
46% shorter $p<0.0005$

Smaller, greener leaves $p<0.005$

Narrower stems $p<0.01$

~6 days slower to flag leaf $p<0.05$

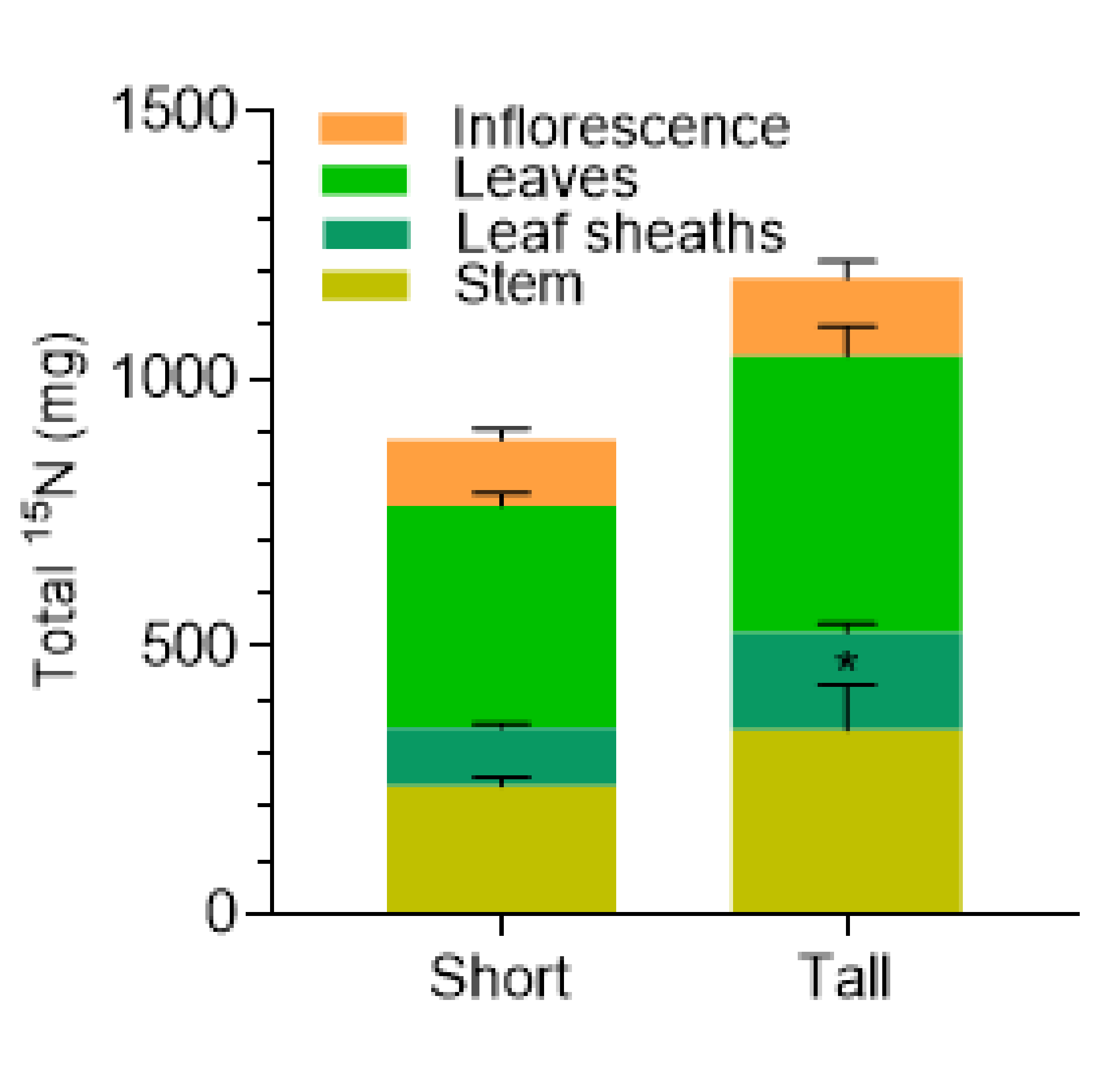
10 days AFTER flag leaf



Tall plants had:-

- 67% larger shoot biomass $p<0.005$
- 25% more total nitrogen $p<0.01$
- 70% longer leaf sheaths $p<0.005$
- 98% more leaf sheath biomass $p<0.01$
- therefore larger potential stores of nitrate

Total ^{15}N-nitrate uptake & mobilization



- ^{15}N labelled KNO_3^- for 3 hours
- 34% more ^{15}N-nitrate in Tall plants
- 67% more ^{15}N-nitrate in Tall leaf sheaths $p<0.01$

Half-head treatment

10 days AFTER flag leaf appeared

24 days after flag leaf

(i.e. 14 days after half-head treatment)

- Larger shoot biomass in Tall genotype $p<0.0005$
- Full-head unripe grain yield larger than Half-head $p<0.05$
- TKW significantly different between treatments $p<0.05$
- Grains are larger in Half-heads by 10% (Tall) & 23% (Short)

50 days after flag leaf – mature grain

- Short genotype has lower yield*
- HI – higher in Short but does not offset effect of shoot biomass*
- Grain number was reduced in Short genotype
- Tall Full-head 30-42% smallest grains $p<0.05$

* Same results in field George-Jaeggli et al., *Field Crops Research* 2011

	Grain number	Yield (g)	TKW	Harvest Index
Short Full-head	323	10.5	32.7	45%
Short Half-head	198	6.3	32.0	29%
Tall Full-head	630	14.6	23.1	33%
Tall Half-head	148	4.4	30.0	14%

50 days after flag leaf – mature grain

	Grain number	Yield (g)	TKW	Grain Protein Conversion
Short Full-head	323	10.5	32.7	12%
Short Half-head	198	6.3	32.0	13%
Tall Full-head	630	14.6	23.1	10%
Tall Half-head	148	4.4	30.0	13%

- Tall genotype –
  - highest grain number
  - highest yield
  - smallest grains
  - lowest protein

24 days after flag leaf - Gene expression analysis (qPCR)

- Genes associated with grain size and grain number
- Under selection in multiple cereals
  - GW8, PBF1, Bt2, GW5, GIF1
- Under selection at gene level in sorghum
  - DEP2, IPT2, SRS3, SMG1, BRD2
- Also associated with improved NUE in rice
  - GS3, DEP1
- Seed number in rice - TH1
- Kernel N transport in maize – Gln-4

Genes selected from Tao et al., *Frontiers in Plant Science*, 2017

Poster Presentation on Wednesday & Thursday

Basam Tabet – P26 – Breeding

- Transgenic field trials – breaking the nexus between yield and grain size
- *GS3* &*DEP1* orthologues
- RNAi and CRISPR –Cas9 technologies

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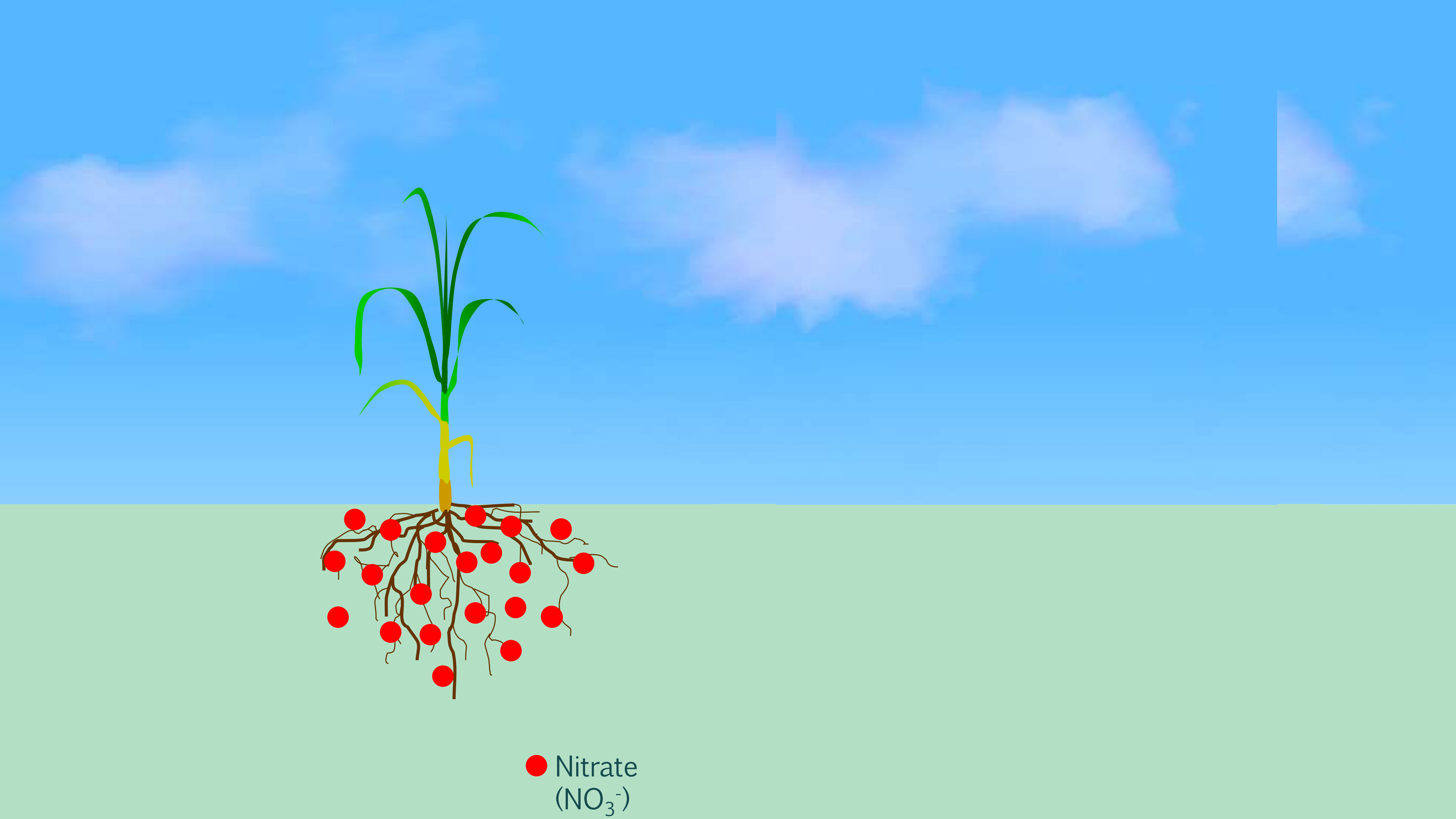
Nitrate
(NO_3^-)