Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota

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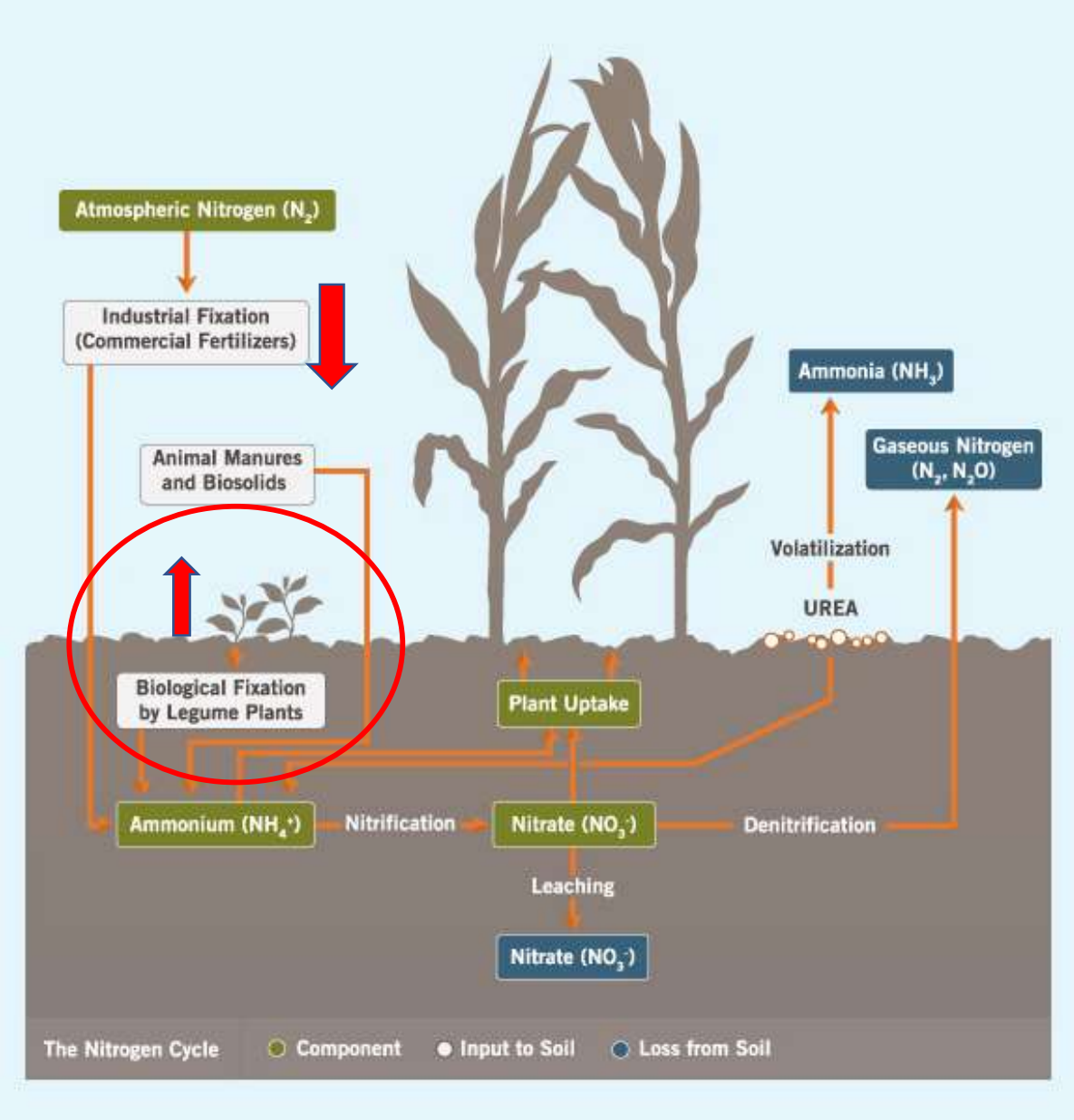
Nitrogen

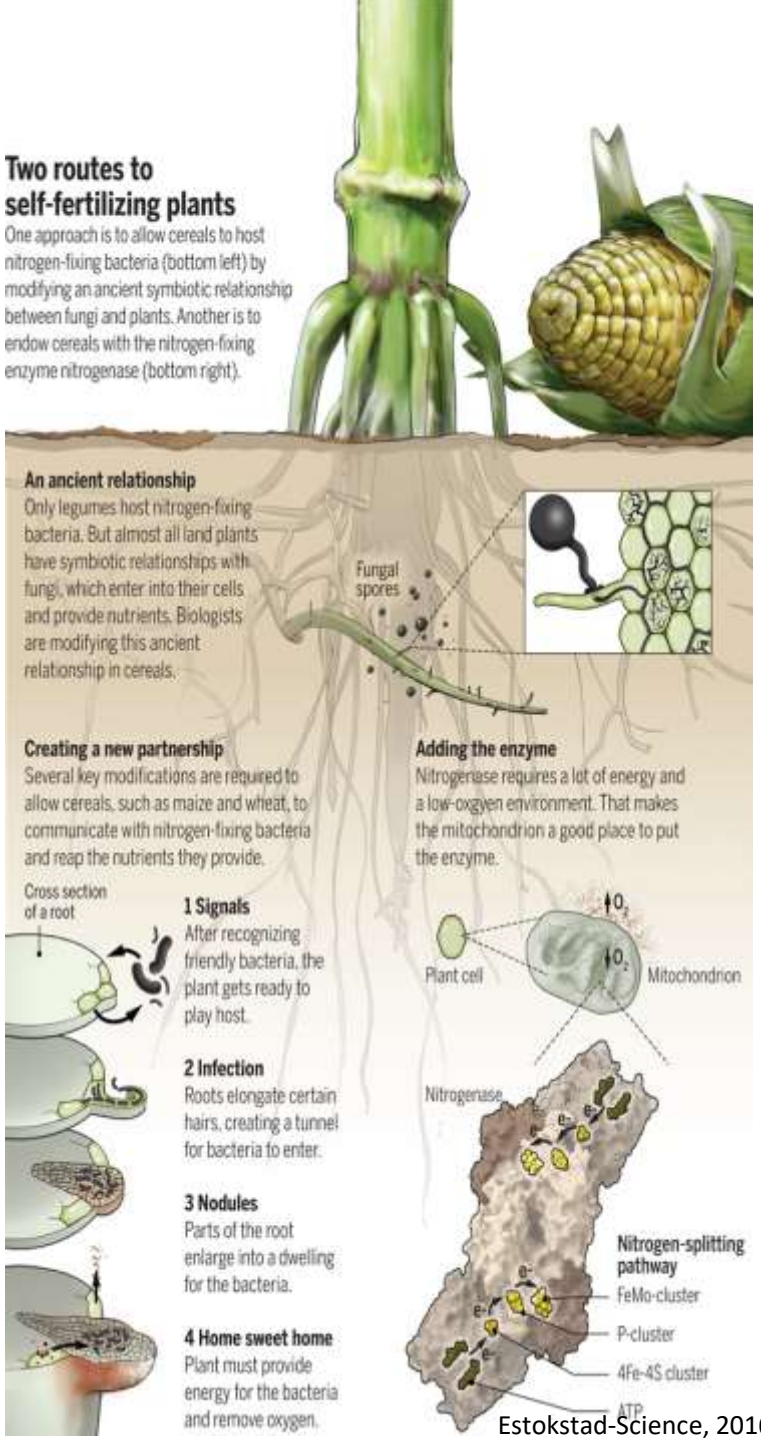
Nitrogen is the major limiting nutrient for crop growth.

Crops needs high inputs of nitrogen fertilizers.

Nitrogen fertilization significantly impacts the environment.

Biological **N**itrogen **F**ixation is safe for the environment.





Estokstad-Science, 2016

PLANT SCIENCE

Future Prospects for Cereals That Fix Nitrogen

Perrin H. Beatty and Allen G. Good

22 JULY 2011 VOL 333 SCIENCE www.sciencemag.org

Nitrogen-fixing bacteria associated with cereal crops

Developing root nodules in cereals

Transfer nitrogen fixation machinery to plant cells

Plant and Soil **186:** 29–38, 1996.

Diazotrophic endophytes: progress and prospects for nitrogen fixation in monocots

Eric W. Triplett
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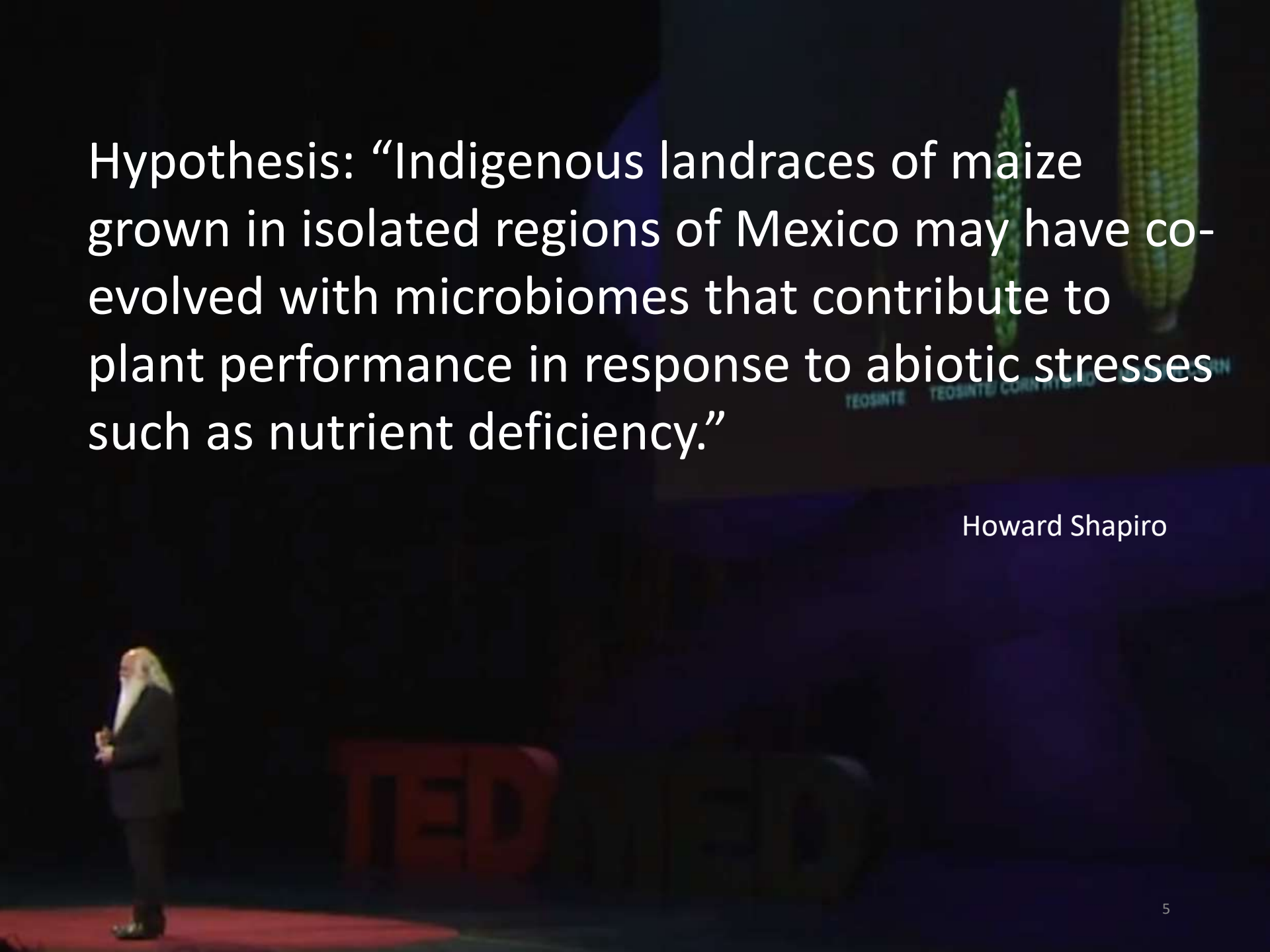
"The development of nitrogen fixation in maize can be considered the "holy grail" of nitrogen fixation research. As nitrogen fertilization is one of the highest costs of corn production, the development of a symbiosis between diazotrophic bacteria and corn would be of enormous economic value."

"It may be necessary to isolate maize diazotrophic endophytes from many locations including the areas of maize origin in Mexico."

Diazotrophs – bacteria that fix atmospheric nitrogen.

Hypothesis: “Indigenous landraces of maize grown in isolated regions of Mexico may have co-evolved with microbiomes that contribute to plant performance in response to abiotic stresses such as nutrient deficiency.”

Howard Shapiro



Sierra Mixe, Oaxaca, Mexico

Mountainous region
4,500 – 6,000 feet (~1,500 – 2,000 meters) above sea level
Maize grown on mountain slopes

Sierra Mixe region has a very … very humid climate

Humidity of > 80%
Rains almost everyday
3,180 mm precipitation
Temperature 77 to 88 °F (~25 to 30 °C)

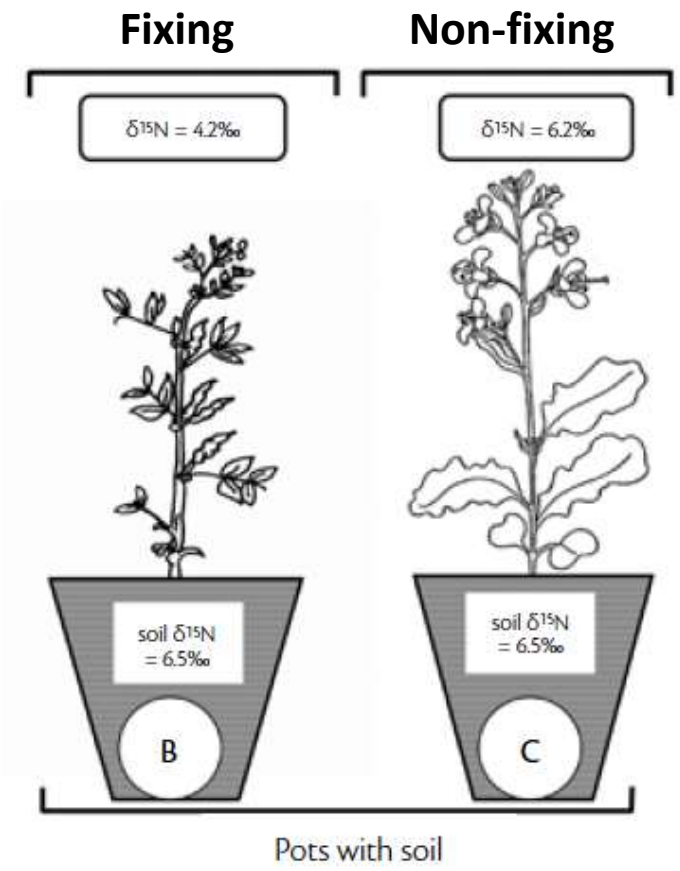
Sierra Mixe maize features

Giant maize:
15-18 feet (~5-6 meters) tall

Long growing season:
8-9 month to maturity
(April - November)

No nitrogenous fertilizer

Determining atmospheric N_2 fixation contribution to plant growth (^{15}N natural abundance)

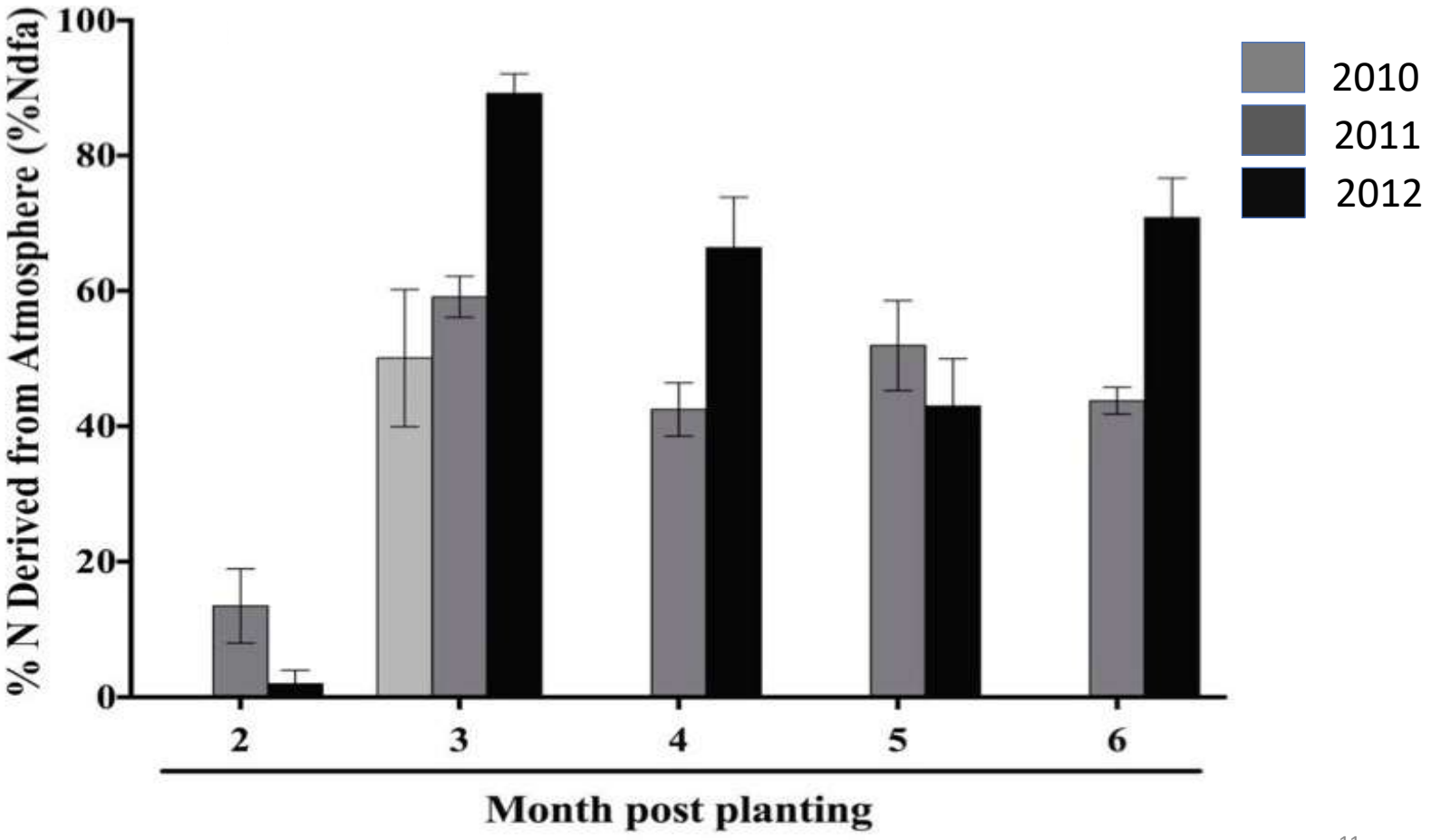


^{15}N atm = 0.336 ppt

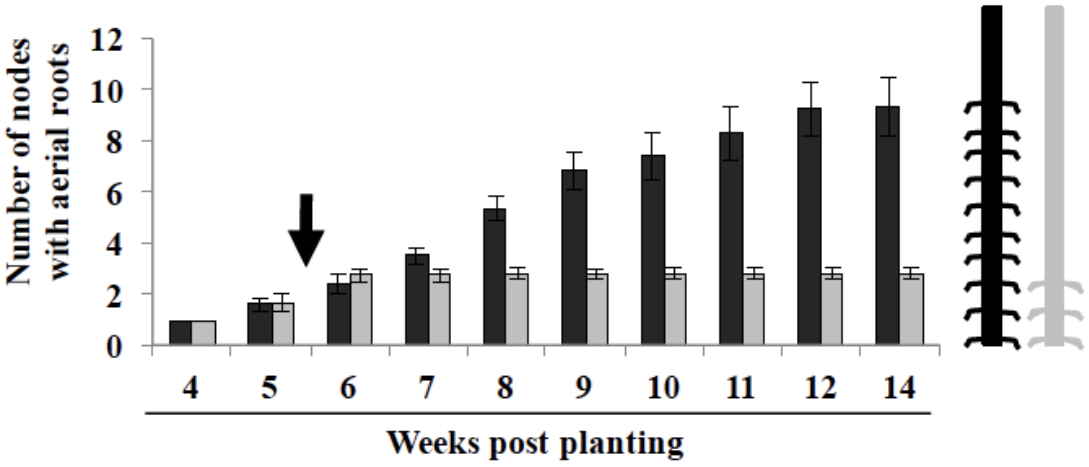
^{15}N soil = 6.5 ppt

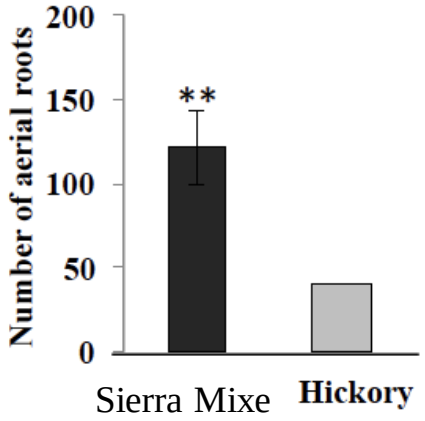
Reference plant provides measure of soil-available N

Evaluation of atmospheric N_2 fixation using ^{15}N natural abundance

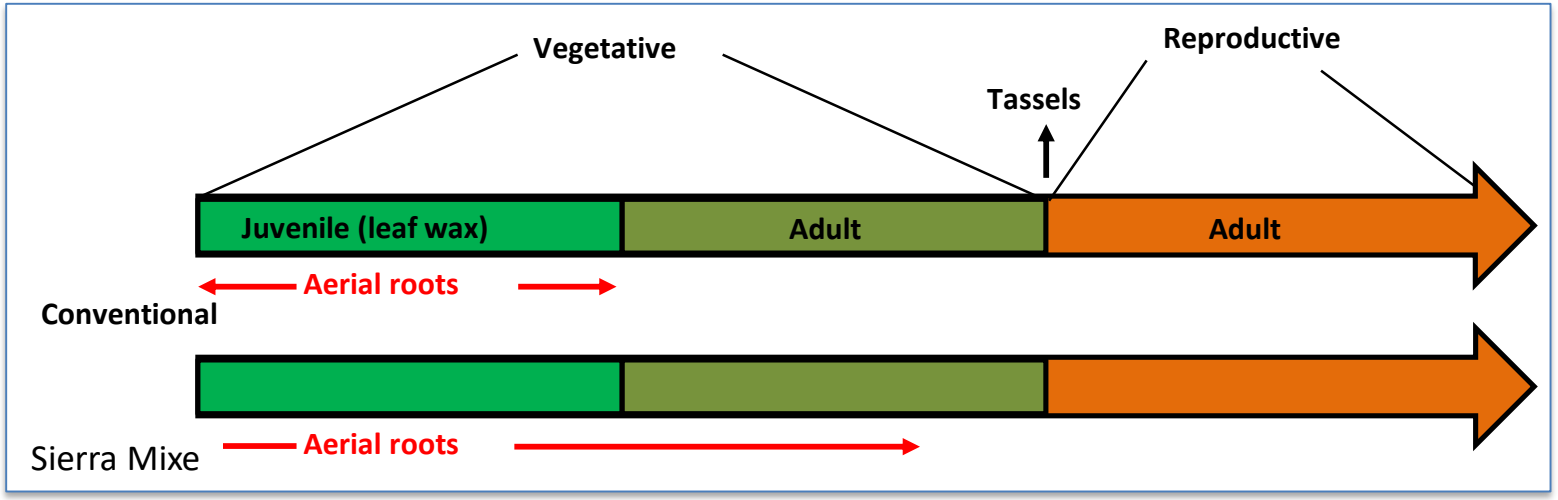


Sierra Mixe maize produces lots of aerial roots

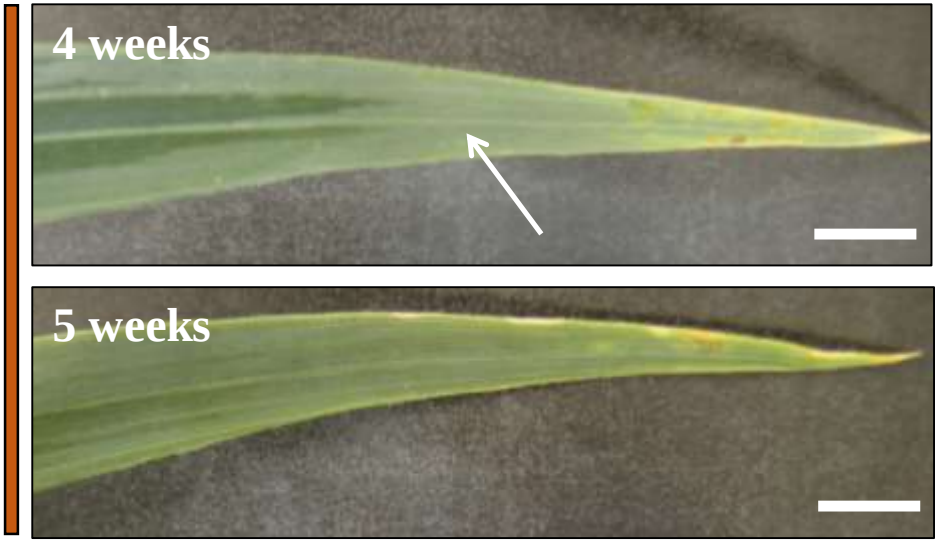




Sierra Mixe maize produces aerial roots during the adult vegetative stage



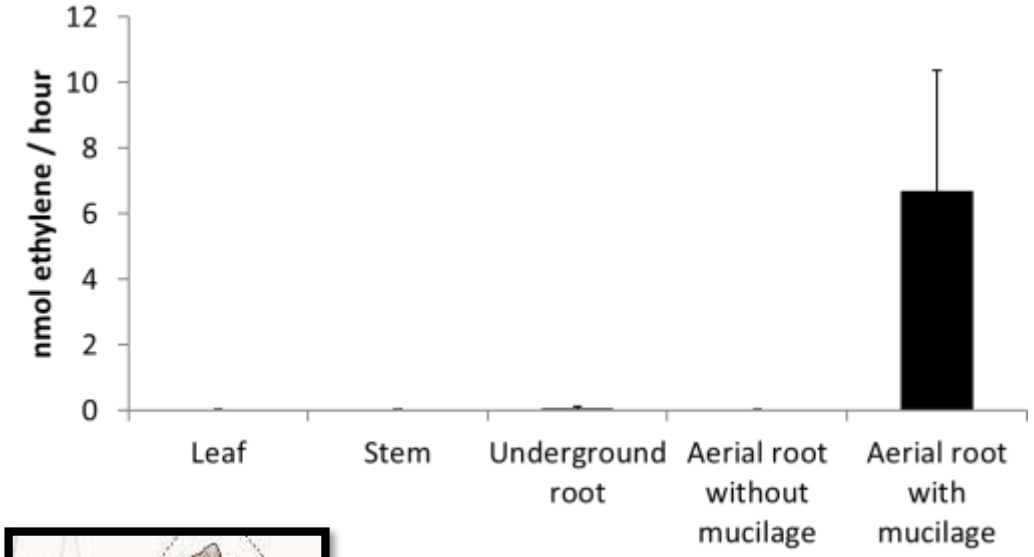
Sierra Mixe maize



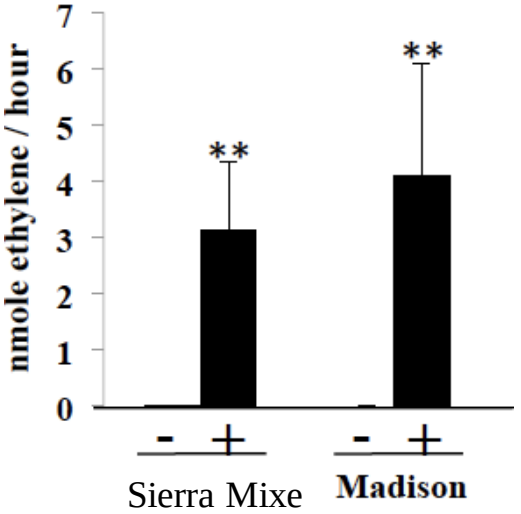
Aerial roots of Sierra Juarez maize produce abundant mucilage after rain

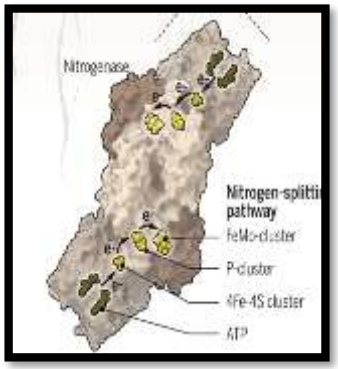
Aerial roots with mucilage exhibits significant nitrogenase activity.

Nitrogenase activity on different plant parts



Nitrogenase activity on mucilage collected in Sierra Mixe and in Madison, WI

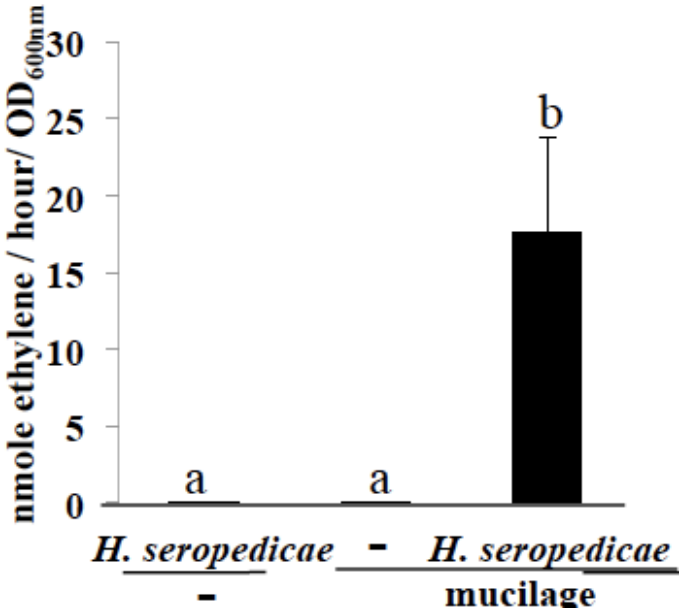


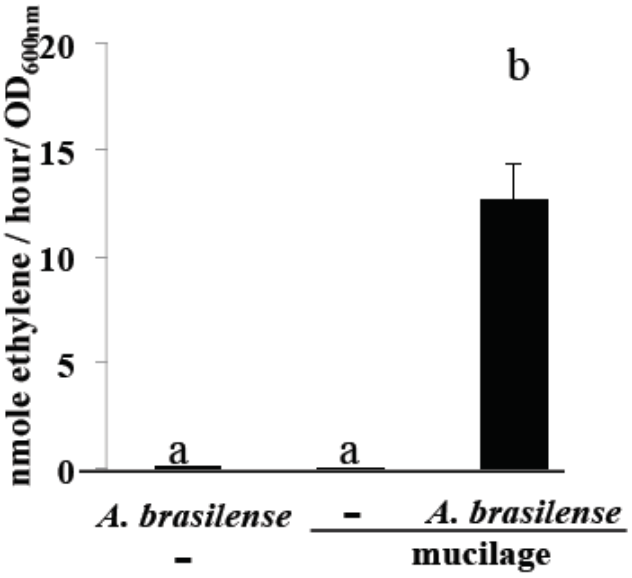


NITROGENASE – enzyme that converts N_2 into ammonium.

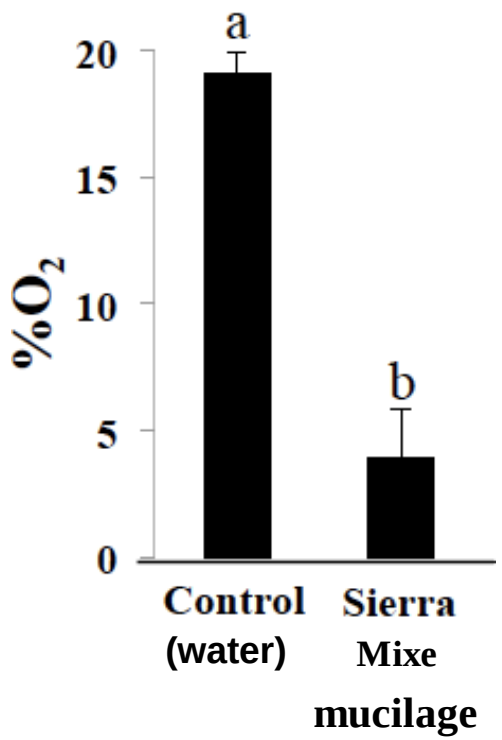
Sierra Mixe maize mucilage provides a proper environment for nitrogen fixation

Nitrogenase activity on **sterile** mucilage inoculated with *Herbaspirillum seropedicae or Azospirillum brasilense*





Sierra Mixe maize mucilage provides a low oxygen, high sugar and low nitrogen environment

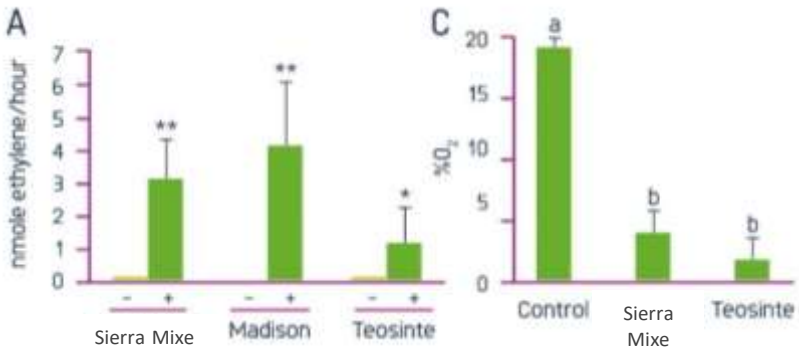


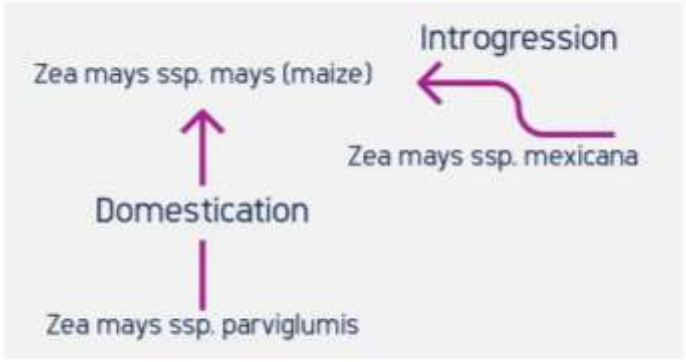
Residue	Weight (ug)[1]	mole%
Arabinose(Ara)	113.7	13.8
Ribose(Rib)	0.0	0.0
Rhamnose (Rha)	0.0	0.0
Fucose (Fuc)	377.0	41.8
Xylose (Xyl)	27.1	3.3
Glucuronic Acid(GlcUA)	28.6	2.7
Galacturonic acid (GalUA)	0.0	0.0
Mannose (Man)	27.1	2.7
Galactose (Gal)	354.3	35.8
Glucose (Glc)	0.0	0.0
N Acetyl Galactosamine (GalNAc)	0.0	0.0
N Acetyl Glucosamine (GlcNAc)	0.0	0.0
Heptose(Hep)	0.0	0.0
3 Deoxy-2-manno-2 Octulsonic acid (KDO)	0.0	0.0
Sum	927.8	100

[1]Values are expressed as mole percent of total carbohydrate.

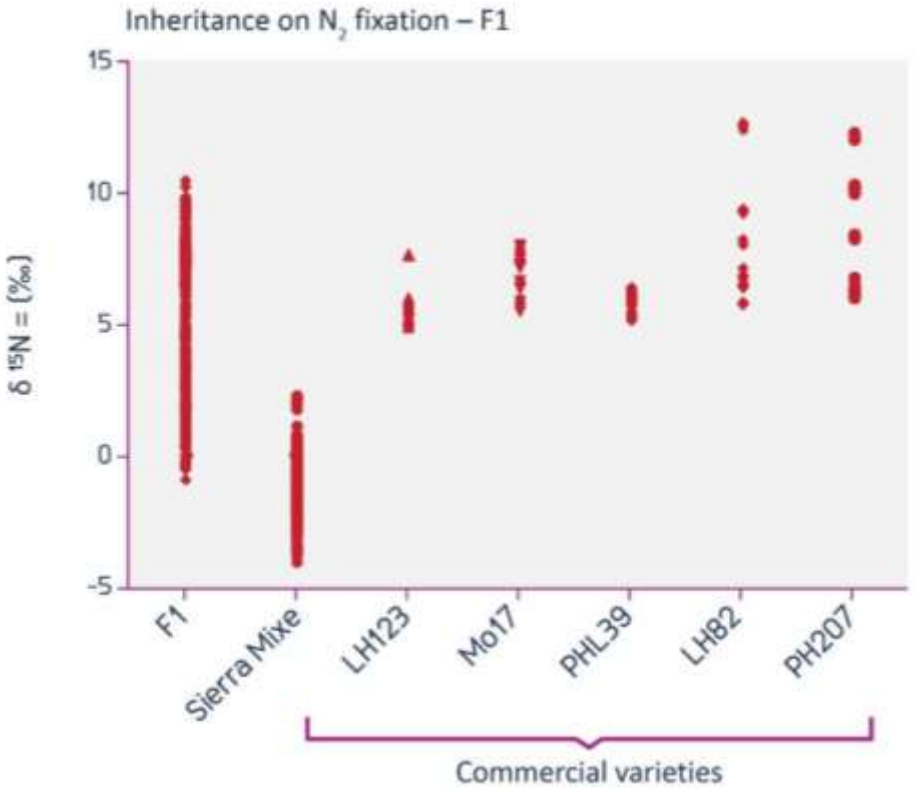
Where is this trait coming from?

Teosinte mexicana may be an ancient source of the diazotrophic association in Sierra Mixe maize landrace.

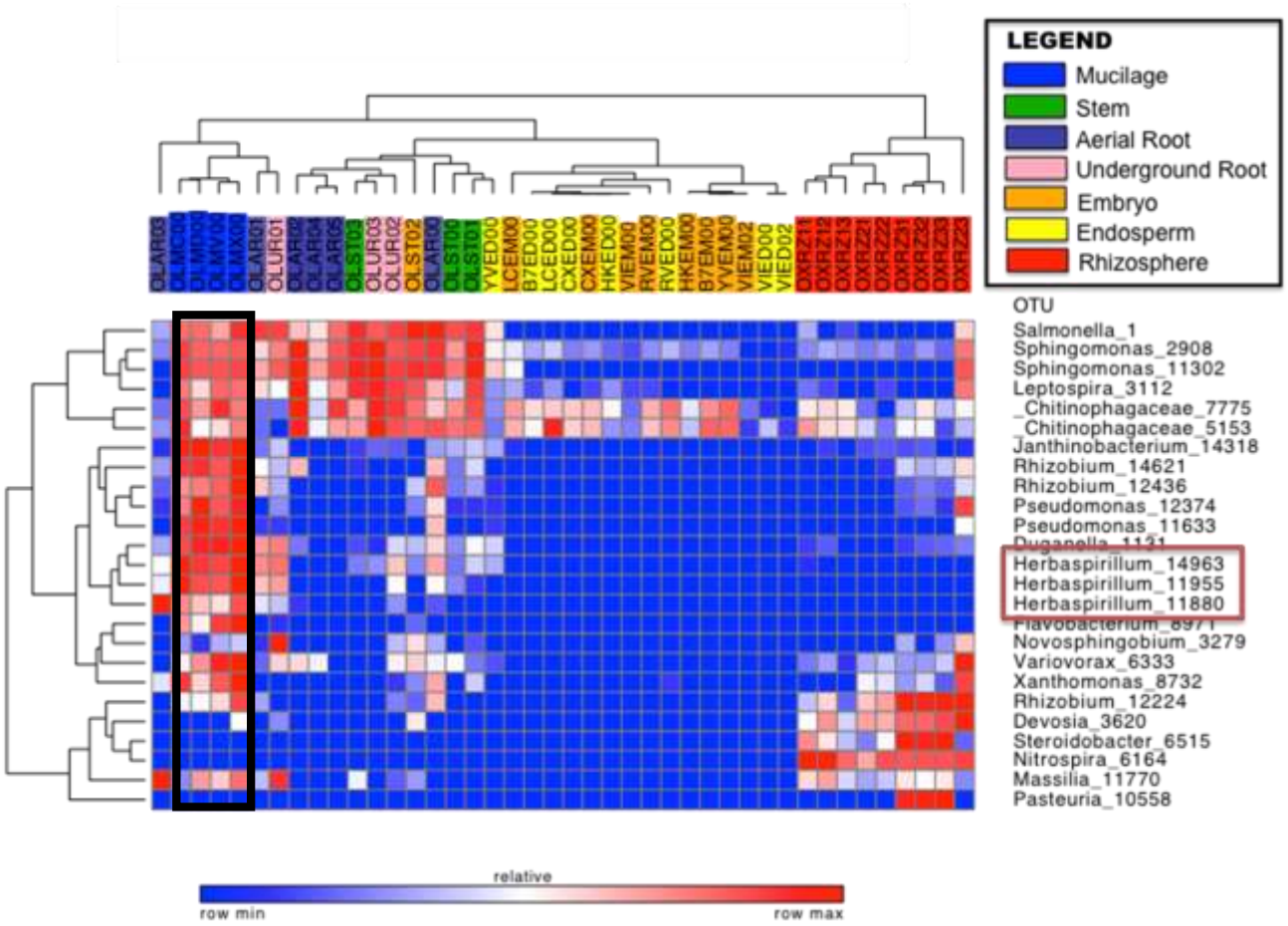




Inheritance of N_2 fixation – inbreed lines



Metagenomics show that corn mucilage has a unique bacterial community, high in N_2-fixers



Conclusions

Researchers are close to obtaining nitrogen-fixing cereal crops through natural diversity

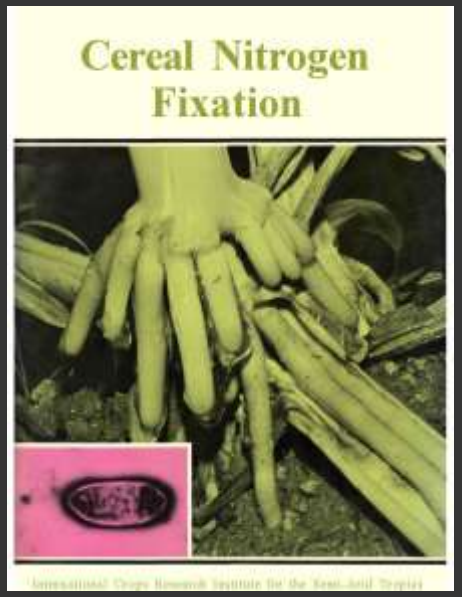
Breed the trait from Sierra Mixe corn into conventional varieties adapted to the Midwest

Need to specific agronomic studies

Identify microbes that maximize the rates of nitrogen fixation

Study mechanisms of aerial root development and mucilage production

Analysis of this trait in other cereal crops



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