Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota
Nitrogen is the major limiting nutrient for crop growth.

Crops need high inputs of nitrogen fertilizers.

Nitrogen fertilization significantly impacts the environment.

Biological Nitrogen Fixation is safe for the environment.
Nitrogen-fixing bacteria associated with cereal crops

Developing root nodules in cereals

Transfer nitrogen fixation machinery to plant cells
“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
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 $\text{N}_2$ fixation contribution to plant growth ($^{15}\text{N}$ natural abundance)

$^{15}\text{N}$ atm = 0.336 ppt

$^{15}\text{N}$ soil = 6.5 ppt

Reference plant provides measure of soil-available N
Evaluation of atmospheric N\textsubscript{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.
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 activity in Sierra Mixe and Madison:

- Sierra Mixe: 4 nmol ethylene/hour, significant
- Madison: 5 nmol ethylene/hour, significant

NITROGENASE – enzyme that converts N₂ 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 mucilag 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 Octulosonic acid (KDO) | 0.0   | 0.0 |
| Sum                            | 927.8             | 100    |

\(^1\)Values are expressed as mole percent of total carbohydrate.
Teosinte mexicana may be an ancient source of the diazotrophic association in Sierra Mixe maize landrace.
Inheritance of $N_2$ fixation – inbreed lines

![Graph showing inheritance of $N_2$ fixation in F1 generation.](image)
Metagenomics show that corn mucilage has a unique bacterial community, high in $\text{N}_2$-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.
The Team

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