

# Patent disclosure to USDA & Cornell

High N and P Cob Patent Disclosure to USDA and Cornell

Initial concept: March 16, 2019

Submitted: August 27, 2019

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This patent addresses the processes to produce a high nitrogen grain inflorescence that could be used to increase on-farm efficiency of fertilizer use.

## Background:

Grain crops (e.g. maize, rice, wheat, sorghum) are only about 50% efficient in taking up nitrogen fertilizer, and these losses are extremely costly to the farmer and to the environment where they result in water pollution and release of nitrous oxide to the atmosphere (a potent greenhouse gas). Nitrogen fertilizer is used by the plants to synthesize amino acids and protein, which are then used to build biomass and the photosynthetic machinery needed to photosynthesize. At the end of the growing season, a substantial portion of this nitrogen is remobilized to the grain, which is then removed from the field at harvest. A substantial portion also remains in the field within the unharvested biomass. The nitrogen in this unharvested biomass breaks down over the winter and is released into the atmosphere as a potent greenhouse gas, nitrous oxide.

In contrast to these annual crops, perennial crops remobilize most nitrogen back to their roots or bark at the end of the growing season. This results in very high nitrogen use efficiency for perennial species. The fundamental problem is that plant vasculature (specifically the phloem), while bidirectional, generally flows in one direction at a particular time of the year. The ideal approach for remobilizing nutrients in a grain crop would be to move sugars to the harvestable grain, and amino acids to the roots. As annuals are short season, they are presented with a limited time to remobilize nutrients. This leads to grain fill and senescence occurring simultaneously. During this the phloem is transporting both the products of senescence, nitrogen (in the form of amino acids and small proteins) and other nutrients, and the sugars from photosynthesis to the strongest sink in the plant, reproductive structures. Perennial species are able to avoid this problem by temporally separating reproduction and senescence, allowing the products of senescence to be transported to a different sink in the plant than the reproductive structures.

Sink strength in plants is something that can be modified with selective pressures, so while in annuals it might be technically possible to select for remobilization of nitrogen to the roots and sucrose to the kernels it is likely that selecting on these attributes would lead to inefficiency of nutrient partitioning in the plant. This is, while nitrogen would be moved to the roots, because the phloem's largest component is sugars, the movement of nitrogen in the phloem to roots would move sugars with it, decreasing grain yields.

This patent develops a process, where nutrient remobilization across the plant is maintained, but the last steps of nutrient deposition is divided into two products. Sugars are predominantly deposited in the seed (endosperm), while amino acids are stored in the cob (panicle).

Utility of process:

This process would create dual products out of grain crops - 1) a seed (kernel) that is high in starch and/or oil, 2) a high nitrogen cob that could be used for fertilization, feed supplement, or industrial protein.

There are a number of advantages - grain crops such as maize, rice, and sorghum are substantially used for their starch production not their protein - e.g. ethanol, syrup, and feed. While the protein bi-products have lesser value - dried distiller's grains, or require substantial effort to recycle the nitrogen - example collecting from animals, transport, and application of manure. Finally, for feed, separating the two streams allows them to be mixed in the correct proportions for each use.

A high nitrogen cob could be harvested and collected in a separate stream. Cobs could be applied to the field immediately during harvest or the following year as fertilizer. The cob would provide a slow release fertilizer, determined by the rate of nitrification of the nitrogen compounds in the cob, which could act as either a supplement or replacement to the application of synthetic fertilizers. As a supplement of synthetic nitrogen the high nitrogen cobs could work synergistically as a slow release fertilizer may provide needed nitrogen through the season.

This is different from most synthetic nitrogen in the form of ammonia and nitrate. These compounds are readily taken up by plants, but they rapidly move through soil or react with the atmosphere making them quickly unavailable for use.

We claim:

1. A method for creating a crop with a high nitrogen cob product, the method comprising:
  - a. Starting with a diverse population of individuals and applying phenotypic selection for both high nitrogen remobilization to the cob and kernels AND a high differential of nitrogen in the cob relative kernel. Repeating for multiple cycles.
  - b. Selection index includes the terms characterizing: combined nitrogen in cob and kernels, difference (ratio, etc.) in nitrogen between kernel and cob, and remaining nitrogen in plant.
2. Process 1 using mass selection, recurrent selection, pedigree selection, genomic selection, or selection based on progeny testing.
3. Selection could be applied at the level of the kernel and adjacent cob, the plant level, plot, or replicated plots.
4. A method for creating a crop with high nitrogen cob product by disrupting the function of nitrogen uptake and storage by the kernel. Knockout by chemical, transgenic, or CRISPR/Cas9 of genes in the kernel - e.g. storage proteins (Zeins), the transcription factors regulating the storage proteins, and/or the amino acid transporters.
5. A method for creating a crop with high nitrogen cob product by creating expressed storage proteins at high levels in the cob either by modifying their promoters or up regulating an appropriate transcription factor in the cob.
6. A method for creating a crop with high nitrogen cob product by introducing and expressing the bark storage proteins from trees.
7. Application of seed treatment (coatings) to improve germination and deal with low kernel nitrogen.

8. Hybrid system where maternal parents have normal distribution of nitrogen, but the seed of the hybrid nitrogen distribution described in claim 1.

9. Selection for timed release of the nitrogen under agronomic conditions, which could be accomplished by increasing lignin and other compounds in the cell walls of the cob.

10. Combining 1, 2, 3, 4, 5, 6, 7, 8, and/or 9.