

## Practical applications and implementations:

Research conducted indicates that within maize populations there exists little to no variation for end of season nitrogen content in the maize cob. The variation that was found was driven, mostly, if not entirely by the seed set. Plants that did not set many kernels had higher end of season cob nitrogen content. This is in accord with what is known about maize physiology: At the end of the season maize catalytically dismantles protein complexes in the leaves and stalks into amino acids which are then transported via phloem to the strongest sink in the plant. The most effective way to increase cob nitrogen was to prevent the development of the maize's strongest sink tissue, the developing kernels. This research highlights problems that will be associated with the creation of a high nitrogen cob via phenotypic selection. It is likely that heavy selection on end of season cob nitrogen will also select for low seed set, something that the last 100 years of maize breeding has been working against and a trait that in most breeding populations likely displays a low amount of phenotypic variance. While it is theoretically possible to reach the goal of a high seed set, high yielding, and a high nitrogen cob it is likely that phenotypic selection would take many generations of breeding, something that even with methods such as genomic selection or speed breeding would take many years. The most efficient method for the creation and testing of high cob N maize lines is likely to be via gene editing.

Via genome editing a high end of season cob nitrogen content maize plant can be envisioned being created without affecting the sink strength of the kernels, or incurring a yield penalty. We suggest that the implementation of this would involve a maize cob specific gene promoter on at least two types of genes. Firstly a gene (or set of genes) that increases the number of amino acid transporters to the interior of cells in the cob tissue, and, secondly, the increased expression of storage proteins in the maize cob cell. It is likely that other adjustments and tuning would need to be made to each of these genes and their combinations, but the basic idea of developing a molecular filter for amino acids as they move through the cob would remain the goal of this genome editing. In this way a large portion of the amino acids could be intercepted, decreasing the grain nitrogen content with the cob tissues retaining the entrained nitrogen. Implementing this may cause issues related to maize seedling vigor, however these could be envisioned being solved by modifying existing seed treatment protocols to incorporate needed nutrients. In another method the various genetic components could be compiled in separate inbred cultivars such that the high cob nitrogen trait is expressed only in the hybrid. Since maize is bred, grown, and sold in a hybrid system this easily would fit into the existing market structure without affecting seedling vigor.

A main potential benefit of separating nitrogen and the grain product is the potential of a 'circular economy' of the nutrient inputs to the field. If a maize crop can effectively leave nutrients that it has taken up in forms that are unavailable for leaching or volatilization, but that can be made available to the next season's crop, nutrient recycling can be achieved. The first step at a circular nutrient economy would be to separate the nutrients from the harvested portion of the crop. A high nitrogen cob has the ability to do this. The high nitrogen cob could either be applied directly to the field as a source of fertilizer or taken off the field for further processing. To avoid problems with high NO<sub>x</sub> production storage of the cobs may be considered over winter and then the application of cobs could occur in the summer. There is the potential that the mineralization of the N compounds in the cob could be achieved at predetermined rates based on the properties of the storage proteins chosen, and the matrix of the cob (which could be modified with transgenes again). Another potential would be the use of industrial processing of the cob tissue to make nutrients more or less labile, depending on the situation. Any implementation of a high nitrogen, or high other nutrient cob would require more research.