Historical Perspective on Cereal Transformation:

Plant transformation was first achieved in the early 1980s as a major scientific breakthrough that has changed world agriculture and plant biology fundamentally. Initial plant transformation relied on *Agrobacterium*-mediated gene transfer and was restricted to dicot plants while gene transfer to cereals remained elusive for nearly a decade despite extensive efforts. The first reports of fertile transgenic cereal plants were from the DeKalb maize project that involved Dr Albert Kausch. Kausch played a seminal role in the development of the first stably transformed and fertile maize transformation through his work on the critical recipient cell systems. This work clearly established the practical possibility of cereal transformation and identified the critical roles of not only DNA delivery, but other multifactorial aspects to a successful transformation protocol, including genotype dependency, somatic embryogenic callus, time in culture, appropriate selection criteria and media addenda. *Agrobacterium*-mediated transformation of cereals, such as maize, rice, wheat, barley, sorghum, millet, oats, Triticale, and rye, is the current method of choice for cereal transformation, recently superseding direct DNA transformation methods for generating transgenics.

It is often assumed that significant improvements to monocot transformation were the result of developments of enhanced *Agrobacterium* strains for DNA delivery. What is not as well appreciated, however, is that a number of synergistic factors were essential for reliable and efficient cereal transformation including: i) advances in monocot tissue culture of "early'" embryogenic cells, such as as transformation competent recipients; ii) identification of suitable explant sources that produce these types of cell cultures (e.g., immature embryos and embryogenic callus); iii) careful selection of genotypes amenable to production of such embryogenic cultures; iv) use of appropriate selectable makers for monocots; v) complex media alterations; and vi) a number of species-specific refinements including the identification of genotype dependent cell culture responses.

In most other cereal crops (i.e., maize, wheat, barley and sorghum), however, *Agrobacterium* transformation remains restricted primarily to genotypes selected for their ability to produce somatic embryogenic callus such as the maize inbred line A188 , and A188 × B72 Hi II the Bobwhite line in wheat, and Schooner in barley . Some success has been achieved with extension of transformation capabilities to elite cereal germplasm through media modifications to enhance embryogenic culture production, but *genotype-independent cereal transformation* has been an elusive target.

"Transformability" is now understood as a complex of interdependent and inclusive systems characteristics. The systems comprise a number of biological processes such as: i) accessibility to DNA introduction; ii) and stable chromosomal integration of the transgene; iii) genotype-specific cell culturability, which may translate into developmentally specific gene expression variation; iv) selectability of totipotent transfected cells; and, v) regeneration of fertile plant from stably transformed cells. As a result successful monocot transformation methods are often, *complex, multi-step protocols,* whereby small improvements are accrued over time, minute method compliance is mission-critical, and hands-on experience matters significantly.

Current Limitations of Cereal Transformation: For many cereals, reproducible and reliable transformation efficiency is still to be quite low. For most minor species and highly recalcitrant maize inbred lines including B73, Mo17, and W22 that are critically important for genomics studies, neither regeneration nor transformation systems have been available in public transformation laboratories. The limitation of genotype dependence and/or low transformation efficiencies in some species is due to several mutually inclusive criteria such as the capability to produce 'early' embryogenic cultures from a

single transformed cell, the proliferation amidst the senescing cells of the untransformed culture during selection, and retaining the potential for subsequent whole plant regeneration to fertile plants (totipotency). So while transformation may be achieved at reasonably high frequencies in many cell types, the ability to transform and recover totipotent (i.e. developmentally competent) target cells may be a significant limiting factor. Furthermore, *Agrobacterium*-mediated transformation involves only recipient target cells at the surface of tissues. For many monocot systems, transformation procedures rely on scutellar epidermal cells of immature embryos. The problem of low transformation efficiencies are compounded by the fact that some events can contain multicopy, rearranged or poorly expressed insertions due to chromosome position effects. Hence, the relative low transformation efficiency may be the major obstacle in order to recover the number of independent transgenics necessary to biologically replicate and address a given experimental questions. For translational research, the required number of independent transformation events may reach large numbers (over 20) to recover simple insertions with stable transgene expression.

Challenges to improved cereal transformation include an increased and integrated understanding of the culture systems and the genetic basis of transformation parameters. Factors which influence transformation efficiencies, such as temperature, recipient cell desiccation, centrifugation, necrosis inhibition and other media addenda, need to be tested in multifactorial designs and extended to other species and cultivars. Another limitation for cereal transformation is the relatively few number of effective selectable markers available for cereal species. The ability to test multiple constructs and gene stacking capabilities rely on multiple selection strategies. Finally, more efficient methods are needed to enhance the ability to rapidly and efficiently identify desirable transformation events. New genome editing approaches such as ZNF, TALENs and CRISPR technologies have recently been demonstrated in crop plants and provide site specific stable mutagenesis facilitated by nonhomologous end joining (NHEJ) repair of induced double stranded breaks. These technologies may also provide reliable insertion of transgenes at targeted sites. The leading challenges and opportunities for cereal genome research is the need to extend and facilitate the implementation of new transformation biology tools important to genomic analyses.

TO UNDERSTAND THE BIOLOGY OF TRANSFORMATION WE NEED TO UNDERSTAND THE BIOLOGY OF SOMATIC EMBRYOGENESIS IN MONOCOTS.