Optimized binary vector design and molecular event characterization increase the efficiency of transformation and consistency of gene expression in corn and sorghum.

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The introduction of the binary system in 1983 revolutionized *Agrobacterium*-mediated plant transformation and opened up the field of plant genetic engineering. Prior to the advent of the binary systems, researchers were limited to using a cumbersome and inefficient design to create vectors for plant transformation that involved recombining a modified T-DNA cassette into the T-DNA region of the large Ti-plasmids.T-DNA binary vectors simplified the design and production of transformation vectors considerably. In the last 30 years, the binary system has been enhanced for transformation of monocot plant species, with the development of “Japan Tobacco” (JT) super-binary co-integrate vectors (harboring additional *vir*genes) and ternary vectors (containing an additional helper plasmid with extra copies of the Vir genes). We have developed a highly improved binary vector system (the pVir super-binary) based on reverse engineering of the JT super-binary vectorsand resulting in vectors with improved stability and transformation performance. The pVir binary vectors significantly improved transformation efficiency and increased production of high quality transgenic events in corn and sorghum.

We have also performed detailed DNA sequence-level characterization of transgenicevents produced by *Agrobacterium*-mediated transformation to identify perfect single copy insertions. After careful gene expression analysis of multiple events representing a variety of different transgenes, we have demonstrated that single copy intact (quality) events generated through random *Agrobacterium*-mediated transformation and distributed across the maize genome haveconsistent, uniformtransgene expression with low variation from site to site for the constructs tested.