Executive Summary

Syngenta Crop Protection LLC, on behalf of Syngenta AG and its affiliates, has developed MZIR098 corn (maize; *Zea mays* L.), a new cultivar that has been genetically modified to provide dual modes of action for corn rootworm control in a single event. In addition, MZIR098 also offers commercial levels of tolerance to glufosinate-ammonium herbicide products.

MZIR098 corn plants contain the transgenes *ecry3.1Ab* and *mcry3A,* which encode the insecticidal proteins eCry3.1Ab and mCry3A, and the transgene *pat-08,* which encodes the enzyme phosphinothricin acetyltransferase (PAT). The native Cry3A from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* is active against certain coleopteran pests. The modified protein mCry3A produced by MZIR098 corn has enhanced activity against western corn rootworm *(Diabrotica virgifera virgifera)* and other related coleopteran pests of corn. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab that is also active against *D. virgifera virgifera* and other related pests of corn. The native Cry1Ab from *B. thuringiensis* subsp. *kurstaki* is active against certain lepidopteran pests; however, the portion of Cry1Ab included in eCry3.1Ab has not preserved the activity of Cry1Ab against lepidopterans. The transgene *pat-08* was derived from the soil bacterium *Streptomyces viridochromogenes*. PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. PAT was used as a selectable marker in the development of MZIR098 corn.

MZIR098 corn was produced by transformation of immature embryos of proprietary variety NP2222 via *Agrobacterium tumefaciens* mediated transformation. The region of the plasmid vector, pSYN17629, intended for insertion into the corn genome included gene-expression cassettes for *ecry3.1Ab, mcry3A,* and *pat-08*. The *ecry3.1Ab* expression cassette consisted of the *ecry3.1Ab* coding region regulated by a CMP promoter from cestrum yellow leaf curling virus (CMP-04) and the nopaline synthase (NOS) terminator sequence from *A. tumefaciens* (NOS-05-01), as well as the NOS enhancer sequence (NOS-02). The *mcry3A* expression cassette consisted of the *mcry3A* coding region regulated by a corn ubiquitin promoter (Ubi1-18) and NOS terminator (NOS-20). The *pat-08* expression cassette consisted of the *pat-08* coding region regulated by the 35S promoter from cauliflower mosaic virus (35S-04) and the NOS terminator (NOS-05-01).

Genetic characterization studies demonstrated that MZIR098 corn contains, at a single locus within the corn genome, a single copy of each of the following functional elements: *ecry3.1Ab*, *mcry3A*, *pat-08*, NOS-02 enhancer, CMP-04 promoter, Ubi1-18 promoter, NOS-20 terminator, 35S-04 promoter and two copies of the NOS-05-01 terminator as expected. No extraneous DNA fragments of these functional elements occur elsewhere in the MZIR098 corn genome. Similarly, plasmid backbone sequence from transformation plasmid pSYN17629 is not present in the MZIR098 corn genome

Southern blot analyses demonstrated that the MZIR098 T-DNA insert is stably inherited from one generation to the next and that the MZIR098 corn genome contains a single T-DNA insert. Further, the observed segregation ratios for *ecry3.1Ab*, *mcry3A*, and *pat-08* in three generations of MZIR098 corn plants, using real-time PCR analysis indicated that these genes are inherited in a predictable manner, according to Mendelian principles. Analyses of grain and forage demonstrate that MZIR098 corn is nutritionally and compositionally similar to, and as safe and nutritious as, conventional corn.

Well-characterized modes of action, physicochemical properties, and a history of safe use demonstrate that the eCry3.1Ab, mCry3A, and PAT proteins present in MZIR098 corn present no risk of harm to humans or livestock that consume corn products or to wildlife potentially exposed to MZIR098 corn.