NK603 x MON 810 maize

YieldGard® Corn Borer with Roundup Ready®

Corn Borer Protection and
Glyphosate Tolerance

Key Facts

Monsanto Europe-Africa
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Maize, a key crop

After wheat and rice, maize is the third most frequently cultivated crop worldwide. Following European discovery of the Americas where this crop is indigenous, maize was rapidly adopted in Europe, Africa and Asia. Today, it is one of the few intensively cultivated crops in European agriculture. Significant areas of production include the Danube basin from southwest Germany to the Black Sea and southern France through to the Po Valley of northern Italy.

As in other world areas, maize use in Europe is dominated by the demand for animal feed. Maize is also processed into valuable industrial and food products such as ethyl alcohol, maize meal, starch and sweeteners.

In 2005, the area of maize harvested in the European Union (EU) was equivalent to approximately 8.9 million ha, with a maize production of around 63 million tons (FAOSTAT database, http://faostat.fao.org/site/340/DesktopDefault.aspx?PageID=340). The principal use of maize is as livestock feed. Currently, the EU imports around 2.6 million tons of maize gluten for animal feed (http://www.fefac.org/file.pdf?fileID=5071). The EU is not a major exporter of maize.

What is NK603 x MON 810?

NK603 × MON 810 is a traditionally bred maize, produced by the crossing of two genetically modified (GM) maize lines: NK603 (Roundup Ready®[1] corn 2) and MON 810 (YieldGard®[2] Corn Borer) (Figure 1)

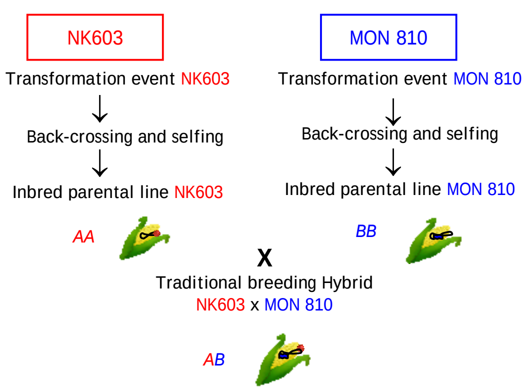

Figure 1: Production of NK603 x MON 810 by traditional breeding of two genetically modified parental lines NK603 and MON 810.

NK603 x MON 810 combines the traits of agronomic interest from the two parental lines, *i.e.* protection against corn borer and tolerance to the broad-spectrum herbicide glyphosate.

Insect protection

MON 810 is genetically modified to confer protection against certain lepidopteran pests, including the European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn stalk borer (*Sesamia spp.*). The larvae of these species damage maize by feeding on the ears and producing feeding tunnels in the stalks. This interferes with the flow of nutrients, enhances infection by stalk diseases, and causes stalk breakage and ear drop prior to harvest, reducing corn yield (Munkvold and Hellmich, 2000; http://www.pgeconomics.co.uk/European_Corn_Borer.htm).

MON 810 carries a gene from the common soil bacteria *Bacillus thuringiensis* coding for the Cry1Ab protein which is selective for the lepidopterans *Ostrinia nubilalis* and *Sesamia spp.* of the Pyralidae and Noctuidae families, respectively (Gonzalez-Nuñez et al., 2000). Susceptible insects contain receptors in their midgut that bind to the specific Cry protein. This leads to the creation of pores which interfere with ion transport systems across the midgut wall, causing lysis of the midgut epithelium and, depending on the dose, subsequent paralysis of the gut or death of the insect (Nester el al., 2002 and Ostlie, 2001). No receptors for these proteins have been identified on intestinal cells of mammals to date.

Herbicide tolerance

NK603 plants are genetically modified to express tolerance to Roundup®[3] herbicide, containing glyphosate, allowing the use of this herbicide for weed control in the crop not just in pre-emergence, but also throughout the growing season.

Glyphosate is a broad-spectrum herbicide that acts via inhibition of the protein "5-enolpyruvyl-shikimate-3-phosphate synthase" (EPSPS) in the green parts of plants. This protein, found naturally in all plants, fungi and bacteria is important in the production of essential aromatic amino acids. Inhibition of EPSPS by glyphosate blocks the production of these amino acids, interfering with growth and leading ultimately to plant death (Alibhai and Stallings, 2001). NK603 plants produce glyphosate-tolerant EPSPS, derived from a common soil bacterium (CP4 EPSPS). This ensures the continued function of the aromatic amino acid pathway, even in the presence of the herbicide (Heck et al., 2005).

[1] Roundup Ready® is a registered trademark of Monsanto Technology LLC
[2] YieldGard® is a registered trademark of Monsanto Technology LLC

[3] Roundup ® is a registered trademark of Monsanto Technology LLC

Worldwide plantings of NK603 x MON 810

Genetically modified crops protected against insect pests and/or tolerant to a specific herbicide were first commercialized in the US in 1997. In 2006, over 100 million hectares of GM crops were grown worldwide, from which 13 million hectares were maize expressing combined traits (http://www.isaaa.org/resources/publications/briefs/35/executivesummary/default.html).
NK603 x MON 810 has been planted commercially for the first time in 2002 in the US.

The regulatory process for GM crops that combine traits varies across countries. The US, Canada and Australia, for instance, do not require new authorisations on combined traits developed by conventional breeding if the single trait products have completed the regulatory process and if the traits are unrelated (e.g. NK603 x MON 810 since the two proteins expressed have different mode of action). In the case of NK603 x MON 810, the inserted genes and the gene products (proteins) have a history of safe use and have been thoroughly reviewed and approved by regulatory agencies worldwide. In contrast, the EU and Argentina require new authorisations for all combined products. Further information on regulatory status of biotech crops, can be found on the agbios website

(http://www.agbios.com/main.php).

A strict regulatory system for genetically modified crops in the EU

In the EU, the regulatory system for genetically modified (GM) crops comprises several regulations and directives, including Directive 2001/18/EC for deliberate release of GMOs in the environment (repealing Directive 90/220/EEC) and Regulation (EC) N° 1829/2003 on GM Food and Feed (replacing Regulation (EC) N° 258/97 on novel foods and novel food ingredients for GM products).

Regulation (EC) N° 1829/2003 includes procedures for the authorisation of deliberate release (cultivation and/or import, and processing), in addition to food and feed use, according to the "one door, one key" principle.

A regulation on traceability and labeling of GMOs and products produced from GMOs (Regulation (EC) N° 1830/2003) entered into force on 18 April 2004.

Regulatory status of NK603 x MON 810 in the EU

In June 2004, Monsanto submitted an application for food and feed use of NK603 × MON 810 maize as any other maize (excluding cultivation) under Regulation (EC) N° 1829/2003 to the European Food Safety Authority (EFSA), via the UK Competent Authority.

EFSA evaluated this applications and adopted favourable scientific opinions on 13 October 2005, concluding that NK603 x MON 810 maize "*is unlikely to have adverse effects on human and animal health or the environment in the context of its proposed uses*"

(http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770082.htm).

The Community Reference Laboratory (CRL) considers that the detection methods validated on the parental lines, NK603 and MON 810, show a comparable performance when applied to NK603 x MON 810. The validation report was published on 16 March 2006

(http://gmo-crl.jrc.it/statusofdoss.htm).

The EFSA overall opinion, which fulfils the requirements of Articles 6 and 18 for the placing on the market of NK603 x MON 810, was published on 31 March 2006

(http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620784153.htm).

After consideration by the Standing Committee on the Food Chain and Animal Health (SCFCAH) on 8 June 2007, and the Council of Agriculture Ministers on 26 September 2007, NK603 × MON 810 was approved by the European Commission on 24 October 2007 (Commission Decision 2007/701/EC), which adopted the proposal for 10 years authorisation. This authorisation also concludes the notification and renewal procedures for "existing products" (Articles 8 and 20 of Regulation (EC) No 1829/2003), including feed materials, feed additives and food additives

(http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm).

Regulatory status of the parental single-trait lines

NK603 was authorized for import, processing and feed use under Directive 2001/18/EC by Commission Decision 2004/643/EC on 19 July 2004, published by the Lead Member State Spain. The use for food and food ingredients under Regulation (EC) N° 258/97 was authorized by the Commission on 3 March 2005 (Commission Decision 2005/448/EC).

MON 810 was authorized for placing on the market under Directive 90/220/EEC by Commission Decision 98/294/EC of 22 April 1998 and notified for food use under Regulation (EC) N° 258/97 on 6 February 1998 (98/ C 200/08).

Traceability, labelling, unique identifier

Operators importing, handling or using NK603 × MON 810 grain and derived foods and feeds in the EU should be informed of the legal obligations regarding traceability and labelling, laid down in Regulation (EC) N° 1830/2003 and in the conditions of placing on the market of the consent. The unique identifier of YieldGard® with Roundup Ready® is
MON-ØØ6Ø3-6 X MON-ØØ81Ø-6

Food, feed and environmental safety of NK603 x MON 810

NK603 x MON 810 is a traditionally bred maize, produced by the crossing of two GM maize lines: NK603 and MON 810. The safety assessment was essentially carried out in two steps:

- Demonstration that the characteristics of the parental single-trait lines are maintained in NK603 x MON 810.
- Assessment based on that of the parental single-trait lines.

Molecular analysis of the DNA inserts present in the NK603 x MON 810 confirmed that the insert structures of the parental single-traits were retained. Also, CP4 EPSPS and Cry1Ab protein levels in forage and kernels of NK603 x MON 810 were comparable to the levels in the corresponding single-trait maize (NK603 and MON 810).

The conclusions of safety for the Cry1Ab and CP4 EPSPS proteins, as already demonstrated in the context of the previously approved NK603 and MON 810 maize lines, remain applicable when these proteins are produced in combination in NK603 x MON 810.

Furthermore, detailed analysis has shown that, apart from the deliberately introduced traits (herbicide tolerance and insect protection), NK603 x MON 810 is agronomically, phenotypically, compositionally, and nutritionally equivalent to conventional maize

http://www.efsa.eu.int/science/gmo/gmo_opinions/1284/gmo_op_ej309_maizenk603xmon810_en1.pdf

Food and feed safety

The food and feed safety of NK603 x MON 810 was established through:

- The long history of safe use of *Bt* Cry (including members of the Cry1 class) and CP4 EPSPS proteins;
- The evaluation of CP4 EPSPS activity and its homology to EPSPS proteins present in a diversity of plants, including those used for foods;
- The rapid digestibility of the introduced proteins in *in vitro* digestive models;
- The lack of toxicity or allergenicity of the introduced proteins, as demonstrated with bioinformatics as well as *in vitro* and *in vivo* safety studies of the protein;
- A large margin of safety resulting from the low dietary exposure to the introduced proteins.

NK603 x MON 810 was found to be as safe and nutritious as the parental lines and conventional maize by analysis of key nutrients, including protein, fat, carbohydrates, amino acids, fatty acids and minerals, as well as by a feed performance study using grain fed to broiler chickens.

Environmental safety

The environmental safety of NK603 × MON 810 was established through extensive laboratory and field testing of plant tissue or purified CP4 EPSPS and Cry1Ab proteins, and with a wide range of non-target species. No adverse effects have been observed in non-target species exposed to CP4 EPSPS and Cry1Ab proteins. In addition, these proteins are expected to degrade rapidly in the environment. Furthermore, agronomic, morphological and pest susceptibility data have been recorded in multiple field trials conducted in major corn growing regions of the US. Results from these trials confirm that NK603 x MON 810 is phenotypically equivalent to conventional corn except for its protection against corn borer and its tolerance to Roundup® herbicide.

NK603 × MON 810, the benefits

NK603 × MON 810 will benefit both farmers and the environment.

Its potential benefits combine the benefits provided by both parental lines: NK603, which confers tolerance to glyphosate, and MON 810, which confers protection against certain lepidopteran insect pests. These benefits have been proven by several years of commercial plantings with NK603[4], MON 810[5] and the traditionally bred hybrid combining both traits, NK603 × MON 810[6].

The benefit expected for NK603 × MON 810 will provide:

- A method to control corn borers, compatible with integrated pest management (IPM) approaches, that offers improved pest control and higher yields, while at the same time being safe for humans and the environment. This is combined with a successful broad-spectrum weed control option in maize that provides flexibility to treat weeds on an "as needed basis" (Heimlich et al., 2000; Johnson et al., 2000; Marra et al., 2002; Pilcher et al., 2002);
- A significant reduction in herbicide use (70% herbicide use reduction per hectare estimated for glyphosate resistant corn in the US) (Gianessi, 2005);
- Increased benefits for farmers linked to the reduced exposure to insecticides, ease of use and handling, time and labor savings, as well as better pest control (Marra et al., 2002; Alston et al., 2002);
- Negligible to no risks for adverse effects on beneficial, non-target organisms when

4 In 2006, NK603 was planted on 5 million hectares in four countries (US, Canada, South Africa, Argentina).
5 In 2006, MON 810 was planted on 6 million hectares in ten countries (US, Canada, South Africa, Argentina, Uruguay, Spain, France, Germany, Czech Republic and Philippines).
6 In 2006, NK603 X MON 810 was grown on 5 million hectares in the US and Canada.

compared to fields treated with conventional pesticides or with untreated controls, attributed to the reduction in insecticide use, low toxicity of glyphosate and compatibility with conservation tillage practices (Ammann, 2003; Eckert, 2006; Fawcett and Towery, 2000; Giesy et al., 2000; Lozzia, 1999; Orr and Landis, 1997; Pilcher et al., 1997; Reyes, 2005);

- Decreased occurrence of fungal mycotoxins associated with adverse health effects, as a result of lower damage to maize plants by lepidopteran pests (Munkvold and Desjardins, 1997; Thiel et al., 1992; Bakan et al., 2002; Rossi et al., 2005; de la Campa et al, 2005);
- The opportunity to replace several selective herbicides by a single broad-spectrum herbicide with a favorable human health and environmental profile. The active ingredient glyphosate is non-persistent and has limited mobility as it binds tightly to soil. The compound presents very low toxicity to humans. Furthermore, it does not bioaccumulate and presents minimal risk to terrestrial and aquatic species including fish, birds, mammals and invertebrates (Giesy et al., 2000; Williams et al, 2000);
- Resource conservation linked to reduced insecticide and herbicide use, *e.g.* less diesel fuel consumed in the manufacture and delivery of insecticides, less water used for insecticide application, conservation of aviation fuel and reduced use of insecticide containers (Carpenter et al., 2002; Phipps and Park, 2002; Heck, 2005; NCGA & USGC, 2005);
- An excellent fit with reduced tillage systems, which are linked to many environmental advantages including improved soil and water quality, reduced soil erosion and runoff, improved wildlife habitat and reduced fuel use and CO_2 emissions (Fawcett and Towery, 2002);

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