**RISK ASSESSMENT REPORT OF THE ENVIRONMENTAL SAFETY TECHNICAL TEAM FOR GENETICALLY MODIFIED MAIZE EVENT GA21, TOLERANCE TO THE HERBICIDE GLYPHOSATE**

1. **Introduction**

Since 1996, genetically modified plants with tolerance to the certain herbicides have played an important role in weed management as part of food crop production system. Herbicide-tolerant plants allow more efficience weed management, increase production and reduce soil erosion, so it provides direct benefit for farmers, helps in environmental conservation. In general, farmers have realized and experienced the benefits of using herbicide-tolerant plants so that this technology has become one of the the fastest adopted technology in the history of agriculture (Green, 2012). The adoption of this technology contributes to the sustainability of high agricultural food production that crucial to cope increasing food demand.

Maize plant derived from transformation event GA21 (GA21 maize) contains transgene a double mutated 5-enol pyruvylshikimate-3-phosphate synthase (mepsps), which encodes the mEPSPS enzyme. EPSPS is an enzyme produced by *Zea mays* and other plants, and play role in the the synthesis of aromatic amino acids. Application of glyphosate will inhibit the work of this enzyme that lead to incapability of plant to produce aromatic amino acids and die. Conversely, mEPSPS produced by GA21 maize has a lower affinity for glyphosate than EPSPS, so glyphosate application will not inhibit the aromatic amino acid production and the GA21 maize survive.

The tolerance of GA21 maize to glyphosate and their impact on environmental safety was assessed /evaluated at the Convined Field Trial (CFT) in 2012-2013 in Lampung, Bogor, Yogyakarta and Malang. In conducting the trial, PT Syngenta Seed Indonesia was in collaboration with Universitas Lampung, Institut Pertanian Bogor, Universitas Gadjah Mada dan Universitas Muhammadiyah Malang.

Referring to Government Regulation Number 21 of 2005 concerning Biological Safety of Genetically Engineered Products, and Regulation of the Minister of Environment of the Republic of Indonesia Number 25 of 2012 concerning Guidelines for Preparation of Environmental Risk Analysis Documents of Genetically Engineered Products, the Biosafety Technical Team (BTT) for Environmental Safety has conducted an environmental safety assessment of GA21 maize based on information on environmental safety assessment, environment risks communication, and management and monitoring plans of GA21 maize after commercialization. The results of the assessment are described in the chapters below.

1. **Environmental Safety Assessment**

II.1. Information of Genetically Modified product

GA21 maize express a double mutated/modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS). EPSPS protein is a key enzyme in the shikimic acid pathway involved in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan) and naturally found to be expressed in the all plants, fungi, and bacteria cell, but not expressed in the animal cell. The mEPSPS protein differs from the EPSPS protein in two amino acid residues (Lebrun *et al*., 1996). The mEPSPS and EPSPS protein are functionally comparable and able to function as key enzymes in the shikimic acid pathway but have a different affinity for glyphosate herbicides. The mEPSPS protein has a much low affinity for glyphosate herbicides compared to EPSPS so that it remains functional when exposed to glyphosate herbicides. Therefore, plants that express mEPSPS protein do not lose the function of the EPSPS enzyme when exposed to glyphosate herbicides and are still able to synthesize aromatic amino acids needed by plants. Finally, the plants do not experience damage when exposed to glyphosate herbicides. Conversely, plants that only express EPSPS protein will lose the function of the EPSPS enzyme when exposed to glyphosate herbicides which eventually the plants will suffer damage or even death.

GA21 maize confirmed to have similar character as its conventional maize in terms of physical properties and nutritional composition. GA21 maize has been reviewed and approved for cultivation or use as food and feed processing in 23 countries. Several ASEAN countries have also planted GA21 maize, including Philippines and Vietnam. In all these countries, GA21 maize grown as a main crop or as refuge.

DNA analysis with Southern hybridization technique confirmed the presence of *mepsps* gene inserts in several generations tested, all of which originated from gene inserts on GA21 maize. DNA samples were isolated from leaf tissue of 10 plants per generation for each of GA21 maize (i.e. BC1F1, BC2F1, and BC3F1), to be used for southern analysis. Each DNA samples from these three generations were cut with *Hind*III restriction enzymes and hybridized with OTP-*mepsps*-NOS probes. Hibridization result shows that the insertion in the maize genome originating from the event GA21is proven to be stable at least for three generations (from the BC1F1, BC2F1, and BC3F1 generations).

Phenotypic data obtained from three successive generations of GA21 maize (BC1F1, BC2F1, and BC3F1) and grown in greenhouses, were used to study the segregation model between herbicide-tolerant and sensitive phenotypes and to determine whether the phenotypic ratios observed are match with the phenotypic ratio from the expected segregation model. It was analyzed using the Chi Square distribution (χ2) with the Yates correction factor (Strickberger, 1976).

In that analysis, the hypothesis that the herbicide tolerant trait was segregated matching with the Mendelian inheritance rules was tested. The critical value χ2 for rejecting hypotheses at the 5% level is 3.84 (Strickberger, 1976). If the value of χ2 is less than 3.84 for all generations tested, then the hypothesis that the herbicides tolerant trait follows the Mendelian inheritance rules and segregates at a ratio of 1:1 is accepted.

The yield potential and agronomic traits of GA21 maize were tested at 4 locations in Indonesia that representing different agroecological zones. The characteristics chosen for comparison of agronomic traits are those commonly observed by professional corn breeders and agronomists and represent characteristics during maize growth. The results of the study show that there is no statistically significant difference in yield or agronomic traits between the GA21 maize hybrid and the hybrid counterpart. These results are in line with previous studies conducted in the United States in 2005.

GA21 maize was introduced firstly to the market in 1998 and has been approved to support maize cultivation activities in nine countries. Syngenta has combined event GA21 with other event and currently there are ten new event combinations that have been reviewed and approved globally for cultivation and/or food/feed processing purposes.

The only new protein produced in GA21 maize is the mEPSPS protein. The mEPSPS protein is a variant of EPSPS which naturally exists in maize cell/tissue. In maize, EPSPS protein involved in the biosynthesis of aromatic amino acids and does not toxic to other organisms.

Analysis of proximate (including flour, TDF, ADF and NDF), mineral content, amino acids, fatty acids, vitamins, secondary metabolites and anti-nutrients have been carried out on the grain of GA21 maize hybrids and its counterpart. No statistically significant differences were observed between GA21 maize hybrids and its counterpart, except for the levels of β-carotene, folic acid and linoleic acid 18:2 which were statistically significant different. However, the average values ​​of all variables observed in the analysis of GA21 maize hybrids and its counterpart were all within the range of natural variation as reported in the literature. Forages from GA21 maize hybrids and its counterpart have been analyzed for proximate (including ADF and NDF), calcium and phosphorus content. In general, no statistically significant differences were observed from the analysis result, except for carbohydrate content. However, the average values ​​for all variables observed in the forage, including carbohydrates, were all within the range of natural variation as reported in the literature. From data in 2004 and 2005, it could be concluded that nutritient composition of GA21 maize hybrids are substantially equivalent with its counterpart (conventional maize hybrids).

II.2. Information on Genetic Properties

II.2.1. Transformation Method

The GA21 maize is created using transformation techniques by microprojectile bombardment in suspension cell culture of maize. Details of the transformation activities are described in US Patent 6,040,497 (Spencer *et al*., 1997). The explants that used in the transformation were immature embryos from elite AT strains derived from B73 strains (Spencer *et al*., 2000). The DNA components that inserted into the explant were consists of rice actin promoter (including the first intron and exon), optimized Chloroplast Transit Peptide (CTP) which is optimized based on CTP sequences of sunflower (*Helianthus annus*) and corn (*Z. mays*), coding region of modified 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps*) derived from corn (*Z. mays*), and the Nopaline Synthase (NOS) terminator from *Agrobacterium tumefaciens*. The DNA fragments were separated from the pDPG434 plasmid backbone using the *Not*I restriction enzyme and only DNA fragments containing the *mepsps* gene were used for transformation so that in the transformation and integration process there is no plasmid backbone sequence.

II.2.2. Inserted gene

The gene inserted in GA21 maize chromosome is the *mepsps* gene (modified 5-enolpyruvylshikimate-3-phosphate synthase gene) encoding mEPSPS which is not sensitive to glyphosate. The mEPSPS protein produced was fused with the Optimized Transit Peptide (OTP) encoding sequence which will target the mEPSPS protein to the chloroplast organelle. The promoter used to regulate the expression of *mepsps* gene was Act + intron promoter from rice (Rice Actin promoter and intron) while the terminator was the NOS-3 terminator which is the terminator of the nopaline synthase gene from *A. tumefaciens*.

II.3. Environmental Safety Information

II.3.1. Potential Impacts on Non-Target Organisms and Biodiversity

Genetic materials of GA21 maize are not derived from pathogenic organisms. The donor organisms of functional elements that used in GA21 maize transformation are *Oryza sativa*, *H. annus*, *Z. mays* and *A. tumefaciens*. *O. sativa*, *H. annus* and *Z. mays* have been known to be safe for human consumption, whereas *A. tumefaciens*, although it is classified as a plant pathogen, the NOS terminator sequence does not have virulent properties. This sequence is commonly used in the production of genetically modified plants and has a safe history in its use. Acute oral toxicity studies in mice show that mEPSPS is not toxic at exposure levels up to 2000 mg / kg body weight, the highest level examined in this test (Barnes, 2005).

GA21 maize does not have any chronic effect on key species of insects or soil organisms. The new protein produced in GA21 maize is a double-mutated 5-enol pyruvylshikimate-3-phosphate synthase (mEPSPS) protein. mEPSPS protein is a mutant variation from the original EPSPS that found in maize. The original EPSPS on *Z. mays* plays a role in the synthesis of aromatic amino acids. EPSPS enzyme is not associated with toxicity of non-target organisms.

The mEPSPS exposure will occur mainly through consumption of GA21 maize or contact with the soil where GA21 maize is planted. EPSPS enzyme is ubiquitous, can be found in plants and microorganisms (ILSI, 2011) and GA21 maize has been deregulated by the United States Department of Agriculture (USDA) since 1997. Therefore, wild species that have the potential to be exposed to mEPSPS through GA21 maize or soil, most likely have been exposed to the same enzyme or enzymes with the same function. There are no harmful effects caused by exposure to the EPSPS enzyme at concentrations found in nature or from the cultivation of GA21 maize that has been used for more than 15 years in various countries.

The mEPSPS producted in GA21 maize is not expected to change the soil compound content. mEPSPS is a mutant that has two changes in amino acids compared to the original EPSPS protein sequence. The first change at amino acid position 102, converting threonine to isoleucine and another at amino acid position 106, converting proline to serine. Changes in those two amino acids cause the affinity of mEPSPS to glyphosate is lower than EPSPS, although the two enzymes are functionally equivalent (Lebrun, 1996), therefore as with EPSPS exposure, mEPSPS exposure to soil is not expected to change the content of soil compounds.

Study on the impact of GA21 maize on key species has been carried out at four CFT locations with different agroecological zones in Indonesia. Results show that generally there is no difference in the impact of GA21 maize and its counterpart cultivation, in terms of total population and diversity index of insect families, total abundance and diversity index of soil fauna and number of soil microbes (total soil microbes, phosphate solvent microbes, cellulolytic microbes and nitrogen-fixing *Azospirillum*). Data shows that there is no anticipated negative impact from GA21 maize and its counterpart. The insect families found in the GA21 maize plot is comparable to the insect families found in its counterpart (CFT Report, 2016).

II.3.2. Potential to be Weed

In Indonesia, members of the genus of unmodified parental plants are not known as weeds. This is likely due to the spread of recombinant proteins outside of maize cultivation through volunteers and survival GA21 maize populations is very small. Maize (*Zea mays* ssp. *mays*) hybridizes a goups of taxon that are collectively known as Teosinte. Some types of Teosinte are classified as sub-species of *Z. mays*, while others are recognized as separate species from Zea. Teosinte is a native plant that grows in Mexico and Central America and have existed along with cultivated maize for thousands of years. They remain genetically separated from cultivated varieties despite interrogations from time to time (US EPA, 2010; Baltazar *et al*., 2005).

Maize has an ear with a cob enclosed with husks. Consequently, seed dispersal of individual kernels does not occur naturally (Hallauer, 2000; OECD, 2003).

Maize has lost the ability to survive without cultivation efforts (OECD, 2003). Maize cannot survive in habitats outside of agricultural habitats, maize requires cultivated land to germinate and is highly uncompetitive with perennial vegetation (Raybould *et al*., 2012).

II.3.3. Potential for Gene Transfer

Maize is wind-pollinated plants (OECD, 2003). Maize pollen is only viable for several hours (Luna *et al*., 2001; Muui *et al*., 2007). Pleasants *et al*. (2001) found that the deposition of pollen on grass (milkweed) which is 5 m from the edge of the field is approximately one tenth compared to grass that found at the edge of the field. Pollen density is 300, 200, 75 and 25 grains / cm2 at 0, 1, 2 and 4-5 m from the edge of the land, this shows that only a few pollens moved away from the land.

The duration of pollen viability will greatly depend on environmental conditions. Low heat and humidity will cause a short period of viability. Transgene in GA21 maize is not expected to influence other genes that affect pollen viability.

If GA21 maize hybridises wild relatives, the trait of glyphosate tolerance theoretically can be inherited. Nevertheless, the teosinte species (wild relatives of maize) are not known as aggressive weeds both in their natural habitat and in their introduced habitat (US EPA, 2010). Same as conventional maize, GA21 maize does not have any potential to become a weed. Therefore, GA21 maize x wild relatives will not be more invasive as weeds than conventional maize x wild relatives. Considering that wild relatives of maize are not aggressive, the glyphosate herbicide does not appear to be crucial to control wild relatives of maize if glyphosate tolerant trait is inherited. It is not possible for Maize to hybridize its wild relatives naturally because; 1) the biological properties of maize that make it impossible, or 2) wild relatives of maize not found naturally in the agricultural system in Indonesia.

The distribution of pollen from Maize sources varies depending on environmental conditions (for example: wind direction and speed, rain). There is a possibility of pollen to pollinate neighboring corn if environmental conditions are favorable and the flowering phase is synchronous. However, there is no sexually compatible corn relatives reported as weeds in Indonesia (Waterhouse, 1993), because of spatial isolation the hybridisation with wild relatives is not possible.

II.4. Environmental Risk Communication

Socialization prior to CFT activities is carried out through risk communication activities, which must be carried out in conducting Environmental Risk Analysis (ERA). This risk communication aims to provide information to stakeholders and the community in the area where CFT is conducted, related to the implementation of ERA.

Risk communication activities are carried out by PT Syngenta Seed Indonesia in collaboration with four universities in the CFT area, as an independence institution to do the study. GM study at CFT is conducted in 4 (four) locations, namely: 1) Lampung in cooperation with Universitas Lampung, held on September 20, 2012; 2) Bogor in collaboration with Pusat Penelitian Sumber Daya Hayati dan Bioteknologi IPB, held on August 1, 2013; 3) Yogyakarta in collaboration with Fakultas Pertanian Universitas Gadjah Mada, held on April 24, 2013; 4) Malang in collaboration with Universitas Muhammadiyah Malang, held on July 26, 2012.

The material of environmental risk communication must include an explanation of the GM crop to be tested, the benefits of GM crop, risks that may arise in the environmental component due to GM planting, GM regulations, as well as procedures for conducting the CFT. Environmental risk communication was attended by lecturers and researchers from university, agricultural service officers and environmental management bodies, students, farmers, village officials and district government officials in the surrounding of CFT locations. The scope of activities carried out are the explanation about: 1) Agricultural innovation through biotechnology and its regulatory status in Indonesia; 2) Introduction of GA21 maize products owned by PT Syngenta Seed Indonesia; 3) Procedures / protocols and variables testing GM maize in CFT, followed by discussions with participants who attended the environmental risk communication.

The discussion on the environmental risk communication showed a positive response and the participants greatly appreciated the contribution of biotechnology in the assembly of good varieties with a note that all the activities are based on the precautionary principle.

II.5. Management and Monitoring plan

The management plan that will be carried out to prevent the emergence of resistant weeds is the implementation of a stewardship program to overcome the development of glyphosate resistant weeds.

To anticipate any possible impact, moonitoring after GM maize release will be carried out through routine and cases monitoring. In addition, validation will be carried out if there is a report on the impact of the environment and report the validation results validation to the BTT of genetically modified products in accordance to the applicable regulations.

If from routine or case reports it is proven that GA21 maize in the field has a negative impact on the environment, human and/or animal health, then PT Syngenta Seed Indonesia will take control and counter measures in accordance to the applicable regulation.

1. **Conclusion**

Based on the results of the assessment on GM crop information, genetic information, potential impacts on non-target organisms, weed potential, gene transfer potential, environmental risk communication, and management and monitoring plan of GM crops, the following conclusions are summarized:

* 1. *Mepsps* gene in GA21 maize are stable for several generations (3 successive generations). GA21 maize contains 5 copies of transgene that found at a single locus, and does not contain a backbone sequence from the transformation plasmid pDPG434
	2. GA21 maize does not have any potential negative impacts on non-target organisms, soil fauna, and soil microbes
	3. GA21 maize does not have potential as a weed
	4. There is no potential geneflow that caused by GA21 maize cultivation due to the absence of compatible wild relatives in Indonesia.
	5. GA21 maize is expected to help farmers in managing weeds better and more efficiently.

Based on the description of the genetic information of *cry1Ab* and *pat* inserted into Bt11 maize, genetic stability, impact on non-target organisms, potential as weeds and potential for gene transfer, BTT summarize that Bt11 maize can be declared environmentally safe. If new data and information are found that are not in accordance with the environmental safety data obtained to date, the environmentally safe status of Bt11 PRG corn will be reviewed. Should negative impact to human and animal health is found after the environment safety decision, the applicant must take control and countermeasures, and withdraw the GA21 maize from the market in accordance to the applicable regulations.

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