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**Risk assessment of information on the subcombination  
Bt11 x 1507 x GA21, related to the application of  
Syngenta (EFSA-GMO-DE-2011-99) for authorisation of  
food and feed containing, consisting and produced from  
genetically modified maize**

**Bt11 x 59122 x MIR604 x 1507 x GA21**

Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda de Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, et al.

► **To cite this version:**

Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda de Schrijver, Mikolaj Antoni Gralak, et al.. Risk assessment of information on the subcombination Bt11 x 1507 x GA21, related to the application of Syngenta (EFSA-GMO-DE-2011-99) for authorisation of food and feed containing, consisting and produced from genetically modified maize Bt11 x 59122 x MIR604 x 1507 x GA21. EFSA Journal, 2017, 15 (12), pp.1-8. 10.2903/j.efsa.2017.4745 . hal-01740354

**HAL Id: hal-01740354**

**<https://hal.science/hal-01740354>**

Submitted on 21 Mar 2018

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ADOPTED: 2 March 2017

doi: 10.2903/j.efsa.2017.4745

## **Risk assessment of information on the subcombination Bt11 × MIR162, related to the application of Syngenta (EFSA-GMO-DE-2009-66) for authorisation of food and feed containing, consisting and produced from genetically modified maize Bt11 × MIR162 × MIR604 × GA21**

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### **Abstract**

The EFSA Panel on Genetically Modified Organisms (GMO) has previously assessed the single events Bt11, MIR162, MIR604 and GA21 as well as different stacked events corresponding to combinations of these events. These maize events were found to be as safe as their conventional counterparts and other appropriate comparators with respect to potential effects on human and animal health and the environment. In its assessment of the four-event maize stack Bt11 × MIR162 × MIR604 × GA21, the GMO Panel also assessed all the subcombinations of these events not previously assessed, including some for which little or no experimental data were provided, including the two-stack maize event Bt11 × MIR162 (application EFSA-GMO-DE-2009-66). On 8 November 2016, the European Commission received from Syngenta information on the subcombination Bt11 × MIR162. On 14 December 2016, the European Commission tasked EFSA to analyse these data and to indicate whether they have an impact on the previously issued opinion on the four-event stack Bt11 × MIR162 × MIR604 × GA21 and its subcombinations. The GMO Panel used the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants to analyse the provided data. The levels of the newly expressed proteins Cry1Ab, PAT, Vip3Aa20 and PMI are similar between the two-event stack Bt11 × MIR162 and the corresponding single events Bt11 and MIR162. Based on this analysis, there is no indication of an interaction between the events combined in maize Bt11 × MIR162 that would affect the levels of the newly expressed proteins. Thus, the GMO Panel considers that the new information for maize Bt11 × MIR162 does not alter the conclusions of the scientific opinion on application EFSA-GMO-DE-2009-66.

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**Keywords:** GMO, maize (*Zea mays*), subcombination Bt11 × MIR162, four-event maize stack Bt11 × MIR162 × MIR604 × GA21, Regulation (EC) No 1829/2003

**Requestor:** European Commission

**Question number:** EFSA-Q-2016-00730

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**Acknowledgements:** The Panel wishes to thank the following for the support provided to this scientific output: the members of the Working Group on Molecular Characterisation.

**Suggested citation:** EFSA Panel on genetically modified organisms (GMO), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M and Paraskevopoulos K, 2017. Statement on the risk assessment of information on the subcombination Bt11 × MIR162, related to the application of Syngenta (EFSA-GMO-DE-2009-66) for authorisation of food and feed containing, consisting and produced from genetically modified maize Bt11 × MIR162 × MIR604 × GA21. *EFSA Journal* 2017;15(3):4745, 8 pp. doi:10.2903/j.efsa.2017.4745

**ISSN:** 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



## Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
2.2.1. New information for the subcombination maize Bt11 × MIR162 submitted as part of the current mandate ...	5
3. Assessment.....	5
4. Conclusions.....	5
Documentation provided to EFSA.....	5
References.....	6
Abbreviations.....	6
Annex A.....	7

## 1. Introduction

The EFSA Panel on Genetically Modified Organisms (GMO) has previously assessed the single events Bt11, MIR162, MIR604 and GA21 as well as different stacked events corresponding to combinations of these events (Table 1). In the framework of the risk assessment of the four-event stack Bt11 × MIR162 × MIR604 × GA21, the GMO Panel also assessed all the subcombinations of this stacked event including those not previously assessed such as the two-stack maize event Bt11 × MIR162 (application EFSA-GMO-DE-2009-66, EFSA GMO Panel, 2015). This subcombination, the two-stack maize event Bt11 × MIR162, was obtained by conventional crossing and data on the levels of the newly expressed proteins were produced and provided by the applicant. The GMO Panel assessed this information to determine whether it has an impact on the opinion previously issued by the European Food Safety Authority (EFSA) on the four-event stack Bt11 × MIR162 × MIR604 × GA21 and its subcombinations.

**Table 1:** GMO Panel scientific opinions on the single maize events Bt11, MIR162, MIR604 GA21 and stacks containing these single events.

Event	Application	EFSA Scientific Opinions
<b>Bt11</b>	C/F/96/05.10 EFSA-GMO-RX-Bt11 EFSA-M-2012-0232(a)	EFSA (2005), EFSA (2009a), EFSA GMO Panel (2012b)
<b>MIR162</b>	EFSA-GMO-DE-2010-82	EFSA GMO Panel (2012a)
<b>MIR604</b>	EFSA-GMO-UK-2005-11	EFSA (2009b)
<b>GA21</b>	EFSA-GMO-UK-2005-19 EFSA-GMO-RX-GA21	EFSA (2007b)
<b>Bt11 × GA21</b>	EFSA-GMO-UK-2007-49	EFSA GMO Panel (2009)
<b>MIR604 × GA21</b>	EFSA-GMO-UK-2007-48	EFSA GMO Panel (2010a)
<b>Bt11 × MIR604</b>	EFSA-GMO-UK-2007-50	EFSA GMO Panel (2010b)
<b>Bt11 × GA21 × MIR604</b>	EFSA-GMO-UK-2008-56	EFSA GMO Panel (2010c)
<b>Bt11 × MIR162 × MIR604 × GA21</b>	EFSA-GMO-DE-2009-66	EFSA GMO Panel (2015)

### 1.1. Background and Terms of Reference as provided by the requestor

The completion of the risk assessment of the four-event stack maize event Bt11 × MIR162 × MIR604 × GA21 and subcombinations independently of their origin (application EFSA-GMO-DE-2009-66 of Syngenta), was followed by the decision of the European Commission authorising the food and feed containing, consisting and produced from GM maize Bt11 × MIR162 × MIR604 × GA21 and subcombinations under Regulation (EC) 1829/2003 (Commission implementing decision 2016/1685/EU). As laid down in Article 5 of this decision, the authorisation holder is requested to provide information on the expression levels of the newly expressed proteins for some of the subcombinations if these were to be created by targeted breeding.

On 8 November 2016, Syngenta informed the European Commission that the subcombination Bt11 × MIR162 will be produced by targeted breeding for commercialisation. Furthermore, on the basis of Article 5 of the Commission implementing decision 2016/1685/EU, Syngenta sent information to the European Commission on this subcombination relating to application EFSA-GMO-DE-2009-66. On 14 December 2016, the European Commission requested EFSA to assess this information and indicate whether it has an impact on the opinion for this application adopted by EFSA. Subsequently, the GMO Panel has evaluated the data and methodology provided and considered this information in the context of its previous conclusions on application EFSA-GMO-DE-2009-66 of Syngenta.

## 2. Data and methodologies

### 2.1. Data

The applicant provided data in line with the GMO Panel guidelines (EFSA, 2007a and EFSA GMO Panel, 2011) on the expression levels of the newly expressed proteins in the two-event stack Bt11 × MIR162.

Two studies were provided which included data on the levels of the newly expressed proteins, Cry1Ab, PAT, PMI and Vip3Aa20 in the two-event stack and the corresponding singles Bt11 and MIR162 (Bednarcik, 2014, 2015).<sup>1</sup> Bednarcik (2014) contains data derived from plants grown in one location in one season and is therefore in line with the previous GMO Panel guidelines for the risk assessment of genetically modified (GM) plants containing stacked transformation events (EFSA, 2007a). The other study, Bednarcik (2015) contains data derived from plants grown in three locations in one season and is therefore in line with the latest GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011) and also with the requirements as laid down in the Implementing Regulation (EU) No 503/2013.<sup>2</sup> Although both studies were considered by the GMO Panel, the risk assessment was based on the Bednarcik (2015) study which follows the latest requirements (EFSA GMO Panel, 2011 and Implementing Regulation (EU) No 503/2013).

## 2.2. Methodologies

In delivering this statement, the GMO Panel took into account the information provided by the applicant and carried out its scientific risk assessment based on the appropriate principles described in its guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011 and Implementing Regulation (EU) No 503/2013).

### 2.2.1. New information for the subcombination maize Bt11 × MIR162 submitted as part of the current mandate

Plants were grown under field conditions in Brazil in 2013–2014 (one location, five replicate plots, Bednarcik (2014)) and in USA in 2014 (three locations, five replicate plots, Bednarcik (2015)). In both studies, the levels of Cry1Ab, PAT, PMI and Vip3Aa20 proteins in the two-event stack maize and the two single events were quantified by enzyme-linked immunosorbent assay (ELISA). Protein levels were determined in leaves (V6 and R1 stages), roots (V6 and R1 stages), whole plants (V6 and R1 stages), pollen (R1 stage) and in grain (R6 and senescence stages) (Annex A) (Bednarcik, 2015).

## 3. Assessment

The GMO Panel assessed the levels of the newly expressed proteins in the two-event stack and the respective singles. The levels of the newly expressed proteins Cry1Ab, PAT, Vip3Aa20 and PMI are similar between the two-event stack Bt11 × MIR162 and the corresponding single events Bt11 and MIR162. Based on these data, there is no indication of an interaction between the events that would affect the levels of the newly expressed proteins in this two-event stack maize Bt11 × MIR162. This is consistent with the conclusions of the GMO Panel for application EFSA-GMO-DE-2009-66.

## 4. Conclusions

Based on the analysis of the data provided, there is no indication of an interaction between the events combined in maize Bt11 × MIR162 that would affect the levels of the newly expressed proteins. Thus, the GMO Panel considers that the new information for maize Bt11 × MIR162 does not alter the conclusions of the scientific opinion on application EFSA-GMO-DE-2009-66 (EFSA GMO Panel, 2015).

## Documentation provided to EFSA

Letter from the European Commission, received on 14 December 2016, concerning a request to analyse additional information related to the application for authorisation of food and feed containing, consisting and produced from genetically modified maize Bt11 × MIR162 × MIR604 × GA21 and genetically modified maize combining two or three of the events.

<sup>1</sup> Bednarcik, 2014 (Report No: TK0215384) and Bednarcik, 2015 (Report No: TK0224852).

<sup>2</sup> Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

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## Abbreviations

ELISA	enzyme-linked immunosorbent assay
GM	genetically modified
GMO	EFSA Panel on Genetically Modified Organisms
LOD	limit of detection

**Annex A – Means, standard deviations and ranges (n = 15) of protein levels in plant tissues from different developmental stages ( $\mu\text{g/g}$  dry weight) from maizes Bt11 × MIR162, Bt11 and MIR162**

Tissue	Stage	Hybrid	Protein			
			Cry1Ab	PAT	Vip3Aa20	PMI
Leaves	V6	Bt11 × MIR162	195 <sup>(a)</sup> ± 149 <sup>(b)</sup>	0.784 ± 0.677	187 ± 102	13.4 ± 7.95
			(95.9–280) <sup>(c)</sup>	(0.219–1.15)	(138–282)	(7.18–17.7)
		Bt11	176 ± 149	0.721 ± 0.677	---	---
			(72.8–239)	(0.167–1.16)		
		MIR162	---	---	228 ± 102	11.1 ± 7.95
					(142–339)	(5.37–16.7)
	R1	Bt11 × MIR162	73.1 ± 99.8	0.788 ± 0.586	198 ± 73.7	13.4 ± 5.8
(13.2–171)			(0.263–1.14)	(137–243)	(9.24–17)	
Bt11		48.3 ± 99.8	0.858 ± 0.586	---	---	
		(7.01–123)	(0.372–1.18)			
	MIR162	---	---	190 ± 73.7	12.4 ± 5.8	
				(87.5–303)	(8.99–19.7)	
Roots	V6	Bt11 × MIR162	65.5 ± 16.1	0.915 ± 0.344	91.2 ± 61.7	8.45 ± 5.23
			(53.2–78.3)	(0.563–1.26)	(55.7–126)	(6.26–14.5)
		Bt11	62.4 ± 16.1	0.929 ± 0.344	---	---
		(48.3–72.2)	(0.547–1.63)			
	MIR162	---	---	94.4 ± 61.7	8.17 ± 5.23	
				(47.4–122)	(5.14–13.5)	
Roots	R1	Bt11 × MIR162	26.2 ± 17.5	0.840 ± 1.13	40.4 ± 26.7	3.18 ± 1.55
			(14.3–39.2)	(0.103–1.63)	(11.6–64.3)	(1.32–4.2)
		Bt11	22.8 ± 17.5	0.783 ± 1.13	---	---
		(14.2–37.4)	(0.0988–1.34)			
	MIR162	---	---	40.2 ± 26.7	3.32 ± 1.55	
				(16.9–65.2)	(1.87–4.89)	
Whole Plant	V6	Bt11 × MIR162	150 ± 109	0.944 ± 0.516	250 ± 150	11.1 ± 5.7
			(80.4–242)	(0.463–1.21)	(163–318)	(6.28–15.1)
		Bt11	129 ± 109	0.900 ± 0.516	---	---
		(69–201)	(0.358–1.27)			
	MIR162	---	---	229 ± 150	9.44 ± 5.7	
				(137–388)	(5.03–13.3)	
Whole Plant	R1	Bt11 × MIR162	36.0 ± 46.3	0.978 ± 0.197	119 ± 57.9	7.07 ± 2.64
			(7.34–69.2)	(0.716–1.21)	(76.2–195)	(4.79–9.57)
		Bt11	28.5 ± 46.3	0.939 ± 0.197	---	---
		(6.1–60.8)	(0.749–1.11)			
	MIR162	---	---	152 ± 57.9	7.62 ± 2.64	
				(120–176)	(5.41–9.15)	
Kernels	R6	Bt11 × MIR162	5.02 ± 2.08	–	99.9 ± 18.1	2.4 ± 0.94
			(2.98–7.3)	(< LOD <sup>(d)</sup> –0.0446)	(65.8–138)	(1.6–3.55)
		Bt11	4.72 ± 2.08	–	---	---
		(3.19–6.72)	(< LOD–0.0400)			
	MIR162	---	---	115 ± 18.1	2.66 ± 0.94	
				(84.5–132)	(1.91–3.73)	



Tissue	Stage	Hybrid	Protein			
			Cry1Ab	PAT	Vip3Aa20	PMI
Kernels	Senescence	Bt11 × MIR162	5.21 ± 2.7	–	76.5 ± 40	1.75 ± 0.49
			(2.9–7.61)	(< LOD–0.0574)	(45.2–105)	(1.33–2.12)
		Bt11	4.79 ± 2.7	–	---	---
			(3.17–7.21)	(< LOD–0.0505)		
		MIR162	---	---	83.1 ± 40	1.84 ± 0.49
					(50.3–106)	(1.35–2.35)
Pollen	R1	Bt11 × MIR162	0.203 ± 0.0802	–	54.5 ± 27.5	3.47 ± 1.98
			(0.153–0.289)	< LOD	(40.2–82.2)	(2.91–4.52)
		Bt11	0.162 ± 0.0802	–	---	---
			(0.11 – 0.21)	< LOD		
		MIR162	---	---	48.6 ± 27.5	3.77 ± 1.98
					(41.3–60.8)	(2.67–9.23)

(a): Mean.

(b): Range.

(c): Standard deviation.

(d): LOD for PAT in kernel and pollen = 0.025 µg/g.

–: Not applicable.

---: Not assayed.