European Commission

JRC TECHNICAL REPORTS

Development and Optimisation of the GM Oilseed Rape Event-Specific Pre-Spotted Plate (OSR-PSP)

EURL-SP-07/16

Francesco Gatto

2017

EUR 28749 EN

Contact information
Name: Francesco GATTO
Address: Via Enrico Fermi 2749 TP 201, I-21027 Ispra (VA) Italy
Email: Francesco.GATTO@ec.europa.eu
Tel.: +39 0332 786439

JRC Science Hub
https://ec.europa.eu/jrc

JRC106101

EUR 28749 EN

PDF ISBN 978-92-79-72286-8 ISSN 1831-9424 doi:10.2760/061914

Luxembourg: Publications Office of the European Union, 2017

How to cite this report: Gatto, F., *Development and Optimisation of the GM Oilseed Rape Event-Specific Pre-Spotted Plate (OSR-PSP): EURL-SP-07/16*, EUR 28749 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-72286-8, doi:10.2760/061914, JRC106101.

Contents

Acknowledgements

This work was partially funded by DG Health and Food Safety - European Commission through the Administrative Arrangement N° *SANTE/2016/S12.727764/Genetically Modified Organisms*

Author

Francesco Gatto

European Commission, Joint Research Centre

Health, Consumers and Reference Materials

Food & Feed Compliance Unit (F.5)

Via Enrico Fermi, 2749. TP 201

I-21027 Ispra (VA) Italy

e-mail: Francesco.GATTO@ec.europa.eu

Abstract

This report describes the development of the "GM oilseed rape event-specific pre-spotted plate (OSR-PSP)" as a ready-to-use tool for GMO detection and identification.

The OSR-PSP allows the detection of all GM oilseed rape events listed in the EU register of authorised GM events as of February 2017 and one non-authorised event. The plate includes a total of 12 methods, consisting of 11 event-specific methods and one taxon-specific method selected from those validated and approved by the EU Reference Laboratory for GM Food and Feed (EURL GMFF).

The reaction conditions were standardised and the performance of the methods, in terms of specificity and sensitivity, were re-assessed in a single-laboratory study.

The results confirm that the methods, under the new reaction conditions, fulfil the EU requirements for GMO testing and can be used for the detection of single and stacked oilseed rape GM events in food and feed samples.

1 Introduction

Pre-spotted plates (PSPs) consist of ready-to-use real-time PCR plates containing individual reaction wells that have been spotted with primers and probes targeting specific sequences. Their use provides the advantage of being time- and cost-efficient and they offer a straightforward tool for high-throughput testing needs. The first application of pre-spotted plates for GMO detection was developed by the JRC in 2009 (Querci et al. 2009).

The JRC has now developed a GM oilseed rape event-specific PSP (OSR-PSP) that allows the detection of the oilseed rape GM events for which the authorisation for the EU market has been granted or is pending as of February 2017 ([1]). In addition, a few GM events for which the EU authorisation period ended and which are subject to Commission Decisions on withdrawal from the market (European Commission 2016) and the unauthorised event OXY-235 (ACS-BNØ11-5), recently detected in a conventional seed lot in the EU (EURL 2016), can also be detected (Table 1). Stacked oilseed rape GM events obtained by commercial cross breeding of single GM lines may also be detected.

The plate allow to perform a total of 12 methods, consisting of 11 methods for oilseed rape GM events and one method for the oilseed rape reference gene (Figure 1). The OSR-PSP represents an updated and optimised version of the previous release (Gatto et al. 2013) and contains 3 additional tests for the detection of the GM events 73496 (DP-Ø73496-4), MON88302 (MON-883Ø2-9) and OXY-235 (ACS-BNØ11-5).

For the methods already used in the previous version of the PSP (Gatto et al. 2013), the reaction volume was scaled down from 50 µl to 25 µl. A bridging study is described here for the verification of the performance of all the validated methods under the new reaction conditions.

Molecular specificity was assessed *in silico* and experimentally. Finally, the sensitivity of the modified methods was re-assessed by evaluating the limit of detection (LOD) and the probability of detection (POD) under the new reaction conditions.

Table 1. List of single line GM events.

GM Event	Unique Identifier	EU authorisation status
73496	DP-Ø73496-4	Pending
GT73	MON-ØØØ73-7	Authorised
MON88302	MON-883Ø2-9	Authorised
Ms1	ACS-BNØØ4-7	Withdrawn
Ms8	ACS-BNØØ5-8	Authorised
OXY-235	ACS-BNØ11-5	Not Auth.
Rf1	ACS-BNØØ1-4	Withdrawn
Rf2	ACS-BNØØ2-5	Withdrawn
Rf3	ACS-BNØØ3-6	Authorised
Topas 19/2	ACS-BNØØ7-1	Withdrawn
T45	ACS-BNØØ8-2	Authorised

[1] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Figure 1. Layout of the GM oilseed rape event-specific pre-spotted plate (OSR-PSP).

	1	2	3	4	5	6	7	8	9	10	11	12
A	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
B	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
C	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
D	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
E	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
F	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
G	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45
H	CruA	73496	GT73	MON 88302	Ms1	Ms8	OXY 235	Rf1	Rf2	Rf3	Topas 19/2	T45

2 Materials and methods

2.1 Materials

Certified reference materials (CRM) from the Joint Research Centre (JRC, Geel, Belgium)[2] and from the American Oil Chemists' Society (AOCS, Urbana, IL, USA) at the highest nominal GM mass fraction available were used in this study. In addition, OXY-235 DNA was provided to the EURL GMFF by the company that developed this GMO. Seeds of wheat, rice and cotton were purchased on the local market. The complete list of materials is shown in Annex 1.

Seeds and grains were ground to a fine powder using Grindomix GM 200 (Retsch GmbH, Haan, Germany).

DNA from plant material constituted of oilseed rape, potato, rice, soybean and wheat was extracted using a CTAB-based extraction procedure (ISO, 2005); the Nucleospin Food kit (Macherey-Nagel GmbH, Düren, Germany) was used to extract DNA from maize plant material and the Foodproof GMO Sample Preparation kit (Biotecon Diagnostics GmbH, Postdam, Germany) was used for cotton.

Each sample was extracted in duplicate and quantified by fluorescence detection using PicoGreen® ds DNA quantitation kit (Invitrogen, Molecular Probes, Eugene, OR, USA). All extracts were examined on agarose gel to verify the DNA integrity and tested for the absence of PCR inhibitors by running an inhibition test with a taxon-specific reference gene (ENGL, 2011). DNA samples derived or constituted by CRMs at the nominal level of 100 % in mass fraction were diluted in order to get a GM target concentration of at least 2500 copies per reaction. The estimation of the GM copies took into consideration the haploid genome size ([3]) (Bennet 2005) and the zygosity for oilseed rape, maize and cotton. DNA extracted from conventional plant material was loaded at the final content higher than 5000 copies per reaction.

A multi-target plasmid control sample (PCS) containing the amplicon sequences of the 12 methods was purchased from Eurogentec SA (Belgium) and used for the assessment of the limit of detection (LOD) and the probability of detection (POD) of the methods (see methods).

[2] Former 'Institute for Reference Materials and Measurements (IRMM)'
[3] http://data.kew.org/cvalues/

2.2 Methods

2.2.1 qPCR methods

Methods were selected among those listed in the EU Database of Reference Methods for GMO Analysis "GMOMETHODS" ([4]) (Bonfini et al. 2012) except for the method targeting the event OXY-235, that was derived from the one validated by the EURL GMFF (EURL GMFF 2016).

Primer and probe sequences are those described in the original protocols available in the above mentioned database and corresponding to the Method ID reported in Table 2.

Reaction conditions were standardised to a volume of 25 µL containing 1× TaqMan® Universal PCR Master Mix no UNG (Applied Biosystems), and primers and probes were used at a final concentration of 900 nM and 250 nM, respectively.

The thermal profile used was: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Data acquisition was set on the 60 °C step.

Table 2. Methods included in the OSR-PSP. "Method ID" refers to the GMOMETHODS database identification code.

Target	Method ID
CruA	qt-tax-bn-012
73496	qt-eve-bn-009
GT73	qt-eve-bn-004
MON 88302	qt-eve-bn-010
Ms1	qt-eve-bn-005
Ms8	qt-eve-bn-002
Oxy-235	EURL-EM-01/15VR[5]
Rf1	qt-eve-bn-006
Rf2	qt-eve-bn-007
Rf3	qt-eve-bn-003
Topas 19/2	qt-eve-bn-008
T45	qt-eve-bn-001

2.2.2 Molecular specificity

2.2.2.1 In silico specificity

In silico specificity of the methods was assessed against the sequences of the other GMO events present in the Central Core Sequence Information System (Patak 2011) of the JRC, as well as the whole genomes of more than 100 plants using the e-PCR prediction tool (NCBI) (Rotmistrovsky, Jang, and Schuler 2004)

2.2.2.2 Experimental specificity

Each method was tested in duplicate at the PSP conditions against the DNA extracts from CRMs or RMs containing GM oilseed rape (GT73, MON88302, 73496, Ms1, Ms8, Rf1, Rf2, Rf3, T45, Topas 19/2, OXY-235) and wild-type samples (oilseed rape, potato, rice, soybean, sugar beet, wheat, maize, cotton). qPCR methods were tested at the reaction conditions described above on a minimum of 2500 copies of GM target or 100 ng of

[4] http://gmo-crl.jrc.ec.europa.eu/gmomethods/
[5] http://gmo-crl.jrc.ec.europa.eu/Oilseed-Rape-Oxy-235.htm

genomic DNA (gDNA) per reaction. Runs were performed on a 7900 or 7500 Real-Time PCR System (Life Technologies).

Additionally, the event-specific methods for the GM events 73496, MON88302 and OXY-235 were tested in duplicate at the PSP reaction condition against DNA samples from GM soybean events (A2704, A5547, CV127, 68416, 44406, 81419, 305423, 356043, FG72, 40-3-2, MON87701, MON87705, MON87708, MON87769, MON89788), GM cotton events (281x3006, COT102, GHB119, GHB614, LL25, MON1445, MON15985, MON531, MON88913, T304), GM maize events (3272, 59122, 98140, Bt11, Bt176, 40278, GA21, MIR162, MIR604, MON810, MON863, MON87460, MON88017, MON89034, NK603, 1507, T25) and the GM rice event LLRice62.

2.2.3 Limit of detection (LOD) and probability of detection (POD).

The sensitivity of the methods was evaluated in terms of limit of detection (LOD) and probability of detection (POD) following the experimental design described in BVL (2016), using the multi-target PCS (see '2.1 Materials').

First, the concentration of the PCS was estimated by droplet digital PCR (ddPCR) by quantifying two targets (*CruA* and T45). 4 vials were randomly selected and analysed in quadruplicate in 20 µL of reaction mixture containing 2X QX200™ ddPCR SuperMix for Probes (no dUTP) (Biorad 186-3023), 600 nM of each primer, 200 nM of the fluorescently-labelled probe and 2 µL of the PCS solution. The thermocycling consisted of 10 minutes at 95 °C, followed by 40 cycles of 2 steps of 30 s at 94 °C and 1 min at 60 °C, and a final step at 98 °C for 10 min. Droplet PCRs were run on the Biorad QX200™ Droplet Digital PCR System and data analysed using the associated QuantaSoft Software.

Then, the PCS was diluted to a final concentration of 4, 2, 1, 0.6, 0.4, 0.3 and 0.02 copies/µl. Each dilution level was analysed with the 12 methods in 12 PCR replicates using 5 µL per reaction, resulting in 20, 10, 5, 3, 2, 1 and 0.1 copies per reaction.

The LOD was determined as the last dilution level at which all 12 replicates provided positive amplification signals.

The POD was determined from the same data set used for the LOD according to the methodology described in Uhlig et al. (2015) and calculated using the web service for "Validation of qualitative PCR methods within a single laboratory" ([6]).

[6] https://quodata.de/content/validation-qualitative-pcr-methods-single-laboratory

3 Results

The final layout of the oilseed rape event-specific PSP (Figure 1) includes the oilseed rape taxon-specific method (*CruA*) and 11 GM oilseed rape event-specific methods, thus permitting the detection of all events from this crop currently listed in the EU register (*i.e.* authorised, pending and withdrawn) and one non-authorised GM event. The methods were derived from those validated by the EURL GMFF used in a qualitative way for the detection and identification of the GM events**.**

In order to be used on PSPs, modifications of the PCR reaction conditions were needed in terms of oligonucleotide concentration, volume of reaction, and reaction mixture composition. Therefore, additional experiments were performed to confirm that the modifications did not affect the method performance in terms of specificity and sensitivity.

3.1 Molecular specificity

3.1.1 *In silico* specificity

In silico specificity analysis confirmed that no cross-reactivity was to be expected between the chosen methods and other GM events or plant genomes.

The event-specific methods did not display potential amplification when the simulation was performed against the whole genomes of more than 100 plants, including those from the plant genera *Brassica*, *Glycine*, *Oryza*, *Solanum*, *Zea*, *Gossypium*, *Beta* and *Triticum*. The simulation only identified the corresponding GM event, with the exception of the method for OXY-235. The latter method showed potential amplification with the GM carnation FLO-40685-2, although the experimental verification performed on the original protocol did not show any amplification signal with GM carnation (EURL GMFF 2016).

As expected, the taxon-specific method showed potential amplification with whole genomes from the genus *Brassica* and no potential cross-reactivity with any of the GM events.

3.1.2 Experimental specificity

Molecular specificity was confirmed under the PSP reaction and cycling conditions for each method. Amplification was observed for all expected positive samples and no cross-reaction occurred (Table 3), confirming that the modifications introduced to the original PCR reaction conditions did not affect the performance of the methods in terms of specificity.

Unexpected weak amplification signals (Cq > 37) were observed in samples from conventional oilseed rape and MON88302 when tested with the method for GT73. Further investigations using the EURL GMFF validated protocol confirmed the contamination of these materials with the targeted GM event.

Similarly, the methods for the GM events 73496, MON88302 and OXY-235 did not show any false amplification signal when tested against GM events from the other crops. Tests were conducted on GM events from Cotton (281x3006, COT102, GHB119, GHB614, LL25, MON1445, MON15985, MON531, MON88913, T304), Maize (3272, 59122, 98140, Bt11, Bt176, 40278, GA21, MIR162, MIR604, MON810, MON863, MON87460, MON88017, MON89034, NK603, T25, 1507), Soybean (A2704, A5547, CV127, 44406, 68416, 81419, 305423, 356043, FG72, 40-3-2, MON87701, MON87705, MON87708, MON87769, MON89788) and Rice (LLRice62).

Table 3. Summary of the specificity assessment. Rows refer to the methods and columns to the samples. Positive and negative results are shown in green and blue, respectively.

	Wild-type samples								Oilseed rape GM events										
Method	Maize	Soy	Oilseed rape	Cotton	Rice	Potato	Sugarbeet	Wheat	73496	GT73	MON 88302	Ms1	Ms8	Oxy-235	Rf1	Rf2	Rf3	Topas 19/2	T45
CruA	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
73496	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
GT73	-	-	-*	-	-	-	-	-	-	+	-*	-	-	-	-	-	-	-	-
MON 88302	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Ms1	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Ms8	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Oxy-235	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Rf1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Rf2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Rf3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Topas 19/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
T45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

* Weak positive amplification signals observed

3.2 Limit of detection (LOD) and probability of detection (POD)

The sensitivity of the methods was evaluated through the determination of the LOD assessed using the multi-target plasmid control sample (PCS). Results are displayed in Table 4.

The LOD of all methods at PSP reaction conditions was between 5 and 20 copies per reaction, thus below 25 copies per reaction which is the performance requirement defined by the European Network of GMO Laboratories (ENGL) (ENGL 2015)

From the same data set, the probability of detection (POD) for each method was then calculated according to Uhlig et al. (2015). This analysis allows the calculation of the $LOD_{95\%}$ and the number of copies of the target sequence required to ensure 95 % probability of detection with the 95% confidence interval.

The experimental LODs fall in the 95% confidence interval of the probability of detection of the respective methods, with deviations for Ms1 and Rf2, however, the LOD is there the more conservative estimate. Results show that the two ways of assessing the data of the dilutions series are appreciably consistent.

Table 4. LOD and POD expressed in copy number per reaction (cp/rcn). POD column shows the calculated $LOD_{95\%}$ and the minimum and maximum of the confidence interval.

Method	LOD (cp/rcn)	LOD Mean Cq ± SD	POD (cp/rcn)
CruA	10	37.8 ± 1.0	13.3 [9.3, 19.1]
73496	5	37.8 ± 0.8	6.4 [4.5, 9.2]
GT73	5	36.9 ± 1.3	4.1 [2.8, 5.9]
MON88302	10	37.2 ± 0.6	10.0 [7.0, 14.2]
Ms1	10	37.7 ± 0.3	6.7 [4.7, 9.6]
Ms8	5	37.7 ± 0.9	4.2 [2.9, 6.1]
Oxy-235	10	37.2 ± 1.0	7.0 [4.9,10.1]
Rf1	20	37.1 ± 0.5	19.9 [13.8, 20.0]
Rf2	10	36.0 ± 0.5	3.8 [2.6, 5.5]
Rf3	10	37.8 ± 0.6	10.2 [7.1, 14.6]
Topas 19/2	5	37.7 ± 0.8	5.4 [3.7, 7.8]
T45	5	37.5 ± 0.8	5.6 [3.9, 8.2]

4 Conclusions

The modifications to the methods made for adapting them to the PSP conditions did not affect the specificity and sensitivity of the methods. Indeed, *in silico* and experimental specificity were confirmed for all methods and the modified methods displayed an adequate sensitivity.

In conclusion, the methods included in this PSP version are in line with the minimum performance requirements as established by the ENGL (ENGL 2015).

The OSR-PSP can therefore be used for the detection and identification of GM oilseed rape events for regulatory control of food and feed.

5 References

Bennet, M. D. 2005. "Nuclear DNA Amounts in Angiosperms: Progress, Problems and Prospects." *Annals of Botany* 95(1): 45–90.

Bonfini, Laura et al. 2012. "GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis." *Journal of AOAC International* 95(6): 1713–19.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL). 2016. *Guidelines for the Single-Laboratory Validation of Qualitative Real-Time PCR Methods*. Braunschweig. http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/Guidelines for the single laboratory.pdf?__blob=publicationFile&v=2.

European Commission. 2016. 342/34 *COMMISSION IMPLEMENTING DECISION (EU) 2016/2268 of 14 December 2016 Amending Decisions 2007/305/EC, 2007/306/EC and 2007/307/EC as Regards the Tolerance Period for Traces of Ms1×Rf1 (ACS-BNØØ4-7×ACS-BNØØ1-4) Hybrid Oilseed Rape, Ms1×Rf2 (ACS-BNØØ4-7×ACS-B*.

European Network of GMO Laboratories (ENGL). 2011. *Verification of Analytical Methods for GMO Testing When Implementing Interlaboratory Validated Methods*. http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm.

European Network of GMO Laboratories (ENGL). 2015. *Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing*. http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm.

European Union Reference Laboratory for GM Food and Feed (EURL GM FF). 2016. *Event-Specific Method for the Detection of Oilseed European Union Reference Laboratory for*. http://gmo-crl.jrc.ec.europa.eu/doc/EM0115_Oxy235_validation_report Final.pdf.

Gatto, Francesco, Sabrina Francesca Rosa, Maddalena Querci, and Joachim Kreysa. 2013. *Development of the New Version of the Ready-to-Use and Multi-Target System for Detection of GMOs Based on Event-Specific Assays. JRC87491*. Available upon request.

ISO, International Organization for Standardization. *ISO 21571:2005 Foodstuffs -- Methods of Analysis for the Detection of Genetically Modified Organisms and Derived Products -- Nucleic Acid Extraction*.

Patak, Alex. 2011. "CCSIS Specialist EMBnet Node: AGM2011 Report." *EMBnet.journal*: 13–14. http://journal.embnet.org/index.php/embnetjournal/article/view/226/547.

Querci, Maddalena et al. 2009. "Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection." *Food Analytical Methods* 2: 325–36.

Rotmistrovsky, Kirill, Wonhee Jang, and Gregory D Schuler. 2004. "A Web Server for Performing Electronic PCR." *Nucleic Acids Research* 32(Web Server): W108–12.

Uhlig, Steffen et al. 2015. "Validation of Qualitative PCR Methods on the Basis of Mathematical–statistical Modelling of the Probability of Detection." *Accreditation and Quality Assurance* 20(2).

List of abbreviations and definitions

AOCS: American Oil Chemists' Society

EURL GMFF: European Union Reference Laboratory for GM Food and Feed

GMO: Genetically Modified Organism(s)

JRC: Joint Research Centre

LOD: Limit of detection

PCS: Plasmid control sample

POD: Probability of detection

PSP: Pre-spotted plate

OSR-PSP: Oilseed rape GM event-specific pre-spotted plate

List of figures and tables

Annexes

Annex 1. List and origin of plant materials used

Event/Plant material	Provider	ID	Event/Plant material	Provider	ID
73496 (DP-073496-4)	JRC	ERM-BF434e	MON863 (MON-00863-5)	JRC	ERM-BF416d
GT73 (MON-00073-7)	AOCS	AOCS 0304-B	MON87460 (MON-87460-4)	AOCS	AOCS 0709-A
MON88302 (MON-88302-9)	AOCS	AOCS 1011-A	MON88017 (MON-88017-3)	AOCS	AOCS 0406-D
Ms1 (ACS-BN004-7)	AOCS	AOCS 0711-A2	MON89034 (MON-89034-3)	AOCS	AOCS 0906-E
Ms8 (ACS-BN005-8)	AOCS	AOCS 0306-F6	NK603 (MON-00603-6)	JRC	ERM-BF415f
OXY-235 (ACS-BNØ11-5)	EURL GMFF	EM-01/15	T25 (ACS-ZM003-2)	AOCS	AOCS 0306-H6
Rf1 (ACS-BN001-4)	AOCS	AOCS 0711-B2	1507 (DAS-01507-1)	JRC	ERM-BF418d
Rf2 (ACS-BN002-5)	AOCS	AOCS 0711-C2	A2704 (ACS-GM005-3)	AOCS	AOCS 0707-B4
Rf3 (ACS-BN003-6)	AOCS	AOCS 0306-G5	A5547 (ACS-GM006-4)	AOCS	AOCS 0707-C3
T45 (ACS-BN008-2)	AOCS	AOCS 0208-A5	CV127 (BPS-CV127-9)	AOCS	AOCS 0911-C
Topas 19/2 (ACS-BN007-1)	AOCS	AOCS 0711-D3	44406 (DAS-44406-6)	JRC	ERM-BF436e
281X3006 (DAS-24236-5 x DAS-21023-5)	JRC	ERM-BF422b	68416 (DAS-68416-4)	JRC	ERM-BF432d
COT102 (SYN-IR102-7)	AOCS	AOCS 1012-C	81419 (DAS-81419-2)	JRC	ERM-BF437e
GHB119 (BCS-GH005-8)	JRC	ERM-BF428c	305423 (DP-305423-1)	JRC	ERM-BF426d
GHB614 (BCS-GH-002-5)	AOCS	AOCS 1108 A5	356043 (DP-356043-5)	JRC	ERM-BF425d
LLCotton25 (ACS-GH001-3)	AOCS	AOCS 0306 E2	FG72 (MST-FG072-2	AOCS	AOCS 0610-A2
MON1445 (MON-01445-2)	AOCS	AOCS 0804-B	40-3-2 (MON-04032-6)	JRC	ERM-BF410gk
MON15985 (MON-15985-7)	AOCS	AOCS 0804-D	MON87701 (MON-87701-2)	AOCS	AOCS 0809-A
MON531 (MON-00531-6)	AOCS	AOCS 0804-C	MON87705 (MON-87705-6)	AOCS	AOCS 0210-A
MON88913 (MON-88913-8)	AOCS	AOCS 0906-D	MON87708 (MON-87708-9)	AOCS	AOCS 0311-A
T304-40 (BCS-GH004-7)	JRC	ERM-BF429c	MON87769 (MON-87769-7)	AOCS	AOCS 0809-B
3272 (SYN-E3272-5)	JRC	ERM-BF420c	MON89788 (MON-89788-1)	AOCS	AOCS 0906-B
98140 (DP-098140-6)	JRC	ERM-BF427d	LLRice62 (ACS-OS002-5)	AOCS	AOCS 0306-I4
Bt11 (SYN-BT011-1)	JRC	ERM-BF412f	Cotton (Conventional)	JRC	ERM-BF429a
Bt176 (SYN-EV176-9)	JRC	ERM-BF411f	Maize (Conventional)	JRC	ERM-BF427a
DAS 40278 (DAS-40278-9)	JRC	ERM-BF433d	Potato (Conventional)	JRC	ERM-BF431a
DAS 59122 (DAS-59122-7)	JRC	ERM-BF424d	Oilseed rape (Conventional)	AOCS	AOCS 0304-A
GA21 (MON-00021-9)	JRC	ERM-BF414f	Rice (Conventional)	Retailer	
MIR162 (SYN-IR162-4)	AOCS	AOCS 1208-A	Soybean (Conventional)	JRC	ERM-BF425a
MIR604 (SYN-IR604-5)	JRC	ERM-BF423d	Sugar beet (Conventional)	JRC	ERM-BF419a
MON810 (MON-00810-6)	JRC	ERM-BF413gk	Wheat (Conventional)	Retailer	

Europe Direct is a service to help you find answers to your questions about the European Union.

Freephone number (*)**:**

00 800 6 7 8 9 10 11

(*) The information given is free, as are most calls (though some operators, phone boxes or hotels may charge you).

More information on the European Union is available on the internet (http://europa.eu).

HOW TO OBTAIN EU PUBLICATIONS

Free publications:

- one copy:
  via EU Bookshop (http://bookshop.europa.eu);
- more than one copy or posters/maps:
  from the European Union's representations (http://ec.europa.eu/represent_en.htm);
  from the delegations in non-EU countries (http://eeas.europa.eu/delegations/index_en.htm);
  by contacting the Europe Direct service (http://europa.eu/europedirect/index_en.htm) or
  calling 00 800 6 7 8 9 10 11 (freephone number from anywhere in the EU) (*).

  (*) The information given is free, as are most calls (though some operators, phone boxes or hotels may charge you).

Priced publications:

- via EU Bookshop (http://bookshop.europa.eu).

KJ-NA-28749-EN-N

JRC Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

EU Science Hub
ec.europa.eu/jrc

@EU_ScienceHub

EU Science Hub - Joint Research Centre

Joint Research Centre

EU Science Hub

Publications Office

doi:10.2760/061914

ISBN 978-92-79-72286-8