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Report on the use of EU Reference Methods and JRC decision tools for GMO analysis

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Table of Content

Executive Summary

To ensure harmonised scientific and technical approaches for GMO detection the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) at the Joint Research Centre (JRC) has developed a freely accessible database, called "GMOMETHODS" providing a state-of-the-art catalogue of EU reference methods for GMO analysis. The EURL GMFF launched in 2015 a survey to assess the use of these EU reference methods by the official GMO control laboratories in the EU and to collect information on non-EU reference methods possibly employed for the same purpose. The survey aimed also to verify if, and to which extent, laboratories use two decision supporting tools, the JRC GMO-Matrix and Event-Finder which are available on the web site of the EURL GMFF. The survey was also directed to verify the types and frequencies of modifications possibly implemented in the protocols of the validated methods used by the official control laboratories.

Results from the survey indicate that almost all official control laboratories (98 %) are using event-specific EU reference methods for quantifying GMOs while a lower number of laboratories is using EU reference methods for qualitative analyses (55 % for element-specific methods and 40 % for construct-specific methods). The use of qualitative non-EU reference methods for screening purposes may reflect the laboratory needs when facing rapid alert emergencies of quickly implementing analytical strategies for detecting non-authorised GM events. Indeed genetically modified crops have continued to increase globally, both in terms of approval status and event/trait diversification. In those cases methods validated in collaborative studies and having the status of EU-reference methods are generally not yet available.

In the survey close to half of the respondents (41 %-47 %) declared also to employ to different extents the two JRC decision supporting tools, GMO-Matrix and Event-Finder.

Interestingly the survey shows that almost half of the protocols of the reference methods used by the laboratories are somewhat adapted to laboratory specific conditions, mainly with respect to the master mix and the reaction volume of the polymerase chain reactions (PCR) while the primers and probes are never modified. In all cases, the impact of these modifications had been verified by the control laboratory to ensure the equivalence between the adapted and the original protocols. Without such proof, the laboratory would lose its mandatory accreditation. Moreover, participants in Comparative Testing schemes have achieve generally high score performance using those adapted methods suggesting that the modifications implemented do not affect analytical sensitivity, trueness and precision of the original protocols.

The outcome of the 2015 survey reveals therefore that the combined efforts of the EURL GMFF and ENGL have been successful for enhancing harmonisation in quantitative GMO analysis by the adoption of scientific and technical approaches. This achievement allows the consistency of results for GM labelling and an equal-level playing field in the EU Member States.

1. Glossary

- 'Element-specific' methods (ELE) target DNA sequences (trait/gene/promoter) that are solely confined to one particular molecular entity (such as the CaMV 35S promoter or the coding region of the cry1Ab gene).
- 'Construct-specific' methods (CON) target DNA sequences that span two different types of molecular entities, such as a promoter sequence and a gene sequence within a single transgenic construct.
- 'Event-specific' methods (EVE) target a special type of 'construct-specific' DNA sequences which allow to uniquely identifying the presence of one particular transformation event. These DNA sequences typically contain part of the host genome flanked by the transgenic insert.
- 'Species-specific' methods (TAX) target DNA sequences that are confined to a particular crop species.
- 'Plant-specific' assays (PLN) target DNA sequences that can uniquely differentiate plant from non-plant organisms.

2. Introduction

EU legislative enforcement and inspection controls on GMOs necessitate validated methods to ensure reliability and consistency of results and an equal implementation in the EU Member States (MS). For that purpose and in accordance with Article 32 of Regulation (EC) No 882/2004([1]), the European Union Reference Laboratories (EURLs) for feed and food are responsible, amongst others, for "providing national reference laboratories with details of analytical methods, including reference methods". In this context the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) has developed a freely accessible database, called "GMOMETHODS" ([2]) to provide a technical state-of-the-art catalogue of EU reference methods for GMO analysis. These are typically DNA-based detection methods using polymerase chain reactions (PCR), which have been validated in collaborative trials according to the principles and requirements of the international standard ISO 5725 ([3]) and/or IUPAC (International Union of Pure and Applied Chemistry) ([4]) protocols. Methods validated by the EURL GMFF in the context of GMO authorisation under Regulation (EC) No 1829/2003 ([5]) or in compliance with an EU legislative act or adopted by international organisations (such as ISO, CEN, OECD) ([6;7]) are assumed to meet those requirements and are included automatically in the database. In addition reference methods have to meet pre-established performance standards that have been agreed with the ENGL (see the JRC Technical Report "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing" at http://gmo-crl.jrc.ec.europa.eu/doc/MPR%20Report%20Application%2020_10_2015.pdf). The GMOMETHODS database, available at http://gmo-crl.jrc.ec.europa.eu/gmomethods/ provides information, up to June 2017, on 172 different PCR reference methods allowing identification of 77 single GM events covering all the GMOs that have been authorized in the EU, or whose authorization is pending or expired. In addition the database presents analytical tests for 25 taxon-specific genes and 32 screening assays. These allow detection of 18 different single or combined genetic elements which are commonly used for the development of GMOs allowing covering also non-authorized GM events.

Typically, control laboratories examine the presence of GM targets in the samples by first testing genetic elements commonly present in GMOs ("screening") and proceed to the identification of the related GM events in case of possible positive results. However, the many known GMOs currently entering the global market, and the expected further acceleration of this process, render ineffective those monitoring approaches that are exclusively based on the sequential detection of different targets, followed by their identification. Screening strategies, using optimal sets of methods targeting sequences shared by many different GM events (such as the element- and construct-specific methods contained in the GMOMETHODS database) can help sustaining more efficient and effective GMO monitoring. These approaches require decision support systems for designing the strategies and interpreting the results ([8;9]). Currently, two sets of interactive tools, the JRC GMO-Matrix and Event Finder ([10]), have been made available online for this purpose by the EURL-GMFF at http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/.

The first tool allows the generation of customizable matrices for selecting the methods to be used in an optimized screening strategy. It presents the relation between the reference methods from the GMOMETHODS database and the GM event(s) they can detect, starting from a web-form where the user selects (a part of) the GMO "universe" (i.e., the rows of the matrix) and the reference methods of choice (the columns).

The second tool is a data interpretation tool, where GM events (and combinations thereof) are identified according to an observed pattern of positive and negative experimental results obtained by using the EU reference methods.

The GMOMETHODS database and the connected tools facilitate harmonisation and ultimately standardisation in the adoption of scientific-technical approaches for GMO analysis in the EU. Official control laboratories should use those methods to ensure that they are capable of providing data of the required quality. Comparative testing (CT) have highlighted that official laboratories adapt in a number of cases the standard operating procedures (SOP) of the methods validated by the EURL GMFF in the framework of Regulation (EC) No 1829/2003 to specific laboratory needs.

To monitor their use DG SANTE has requested the EURL GMFF to carry out a survey showing to which degree NRLs employ reference methods included in the GMOMETHODS database for official control and to what extent the laboratories routinely use the JRC GMO-Matrix and Event finder applications. To enlarge the pool of respondents the survey was extended to non-NRL laboratories of the ENGL. They were all invited to respond to an extensive questionnaire on the GMO-testing methods they were using and, in case they were implementing EU reference methods, to which extent, and how, they adapted them to their specific laboratory situation.

As a final objective, the survey aimed at identifying the taxon-specific modules and the non-EU reference methods (in-house developed, published in literature, not validated) employed by the laboratories.

This document provides an overview and a statistical analysis of the data collected and offers input in particular to the ENGL WG on update of methods aiming at providing guidelines on method adaptations.

3. Survey Set-up

3.1 Time Frame

- The survey was launched on the 19th of June 2015.
- The deadline for reply was first set to the 17th of July and later extended to the 11th of August 2015.

3.2 Layout

The survey was designed in three sections using the European Commission tool "EUSurvey". The first section covered the employment of EU reference methods by NRLs and other members of the ENGL, the modifications implemented and their extent of use. The second section was designed for collecting information on other methods used by the laboratories (in-house developed, published in literature, not validated). The third section addressed the use of the JRC GMO-Matrix and Event finder applications by official control laboratories.

a) EU Reference Methods

In the EU Reference Methods section questions on the methods were grouped according to the scope of the PCR, namely quantitative or qualitative and further subdivided in GMO- or taxon-specific assays. The latter allow identifying the presence of a species/ingredient in a product and are often used for the relative quantification of the GM content of that product. GMO-specific methods were listed according to their target specificity (i.e. element-, construct- or event-specificity), while the taxon-specific methods were subdivided in plant- or species-specific methods. Event-specific and species-specific methods were grouped by species.

In the "A - EU Reference Methods" section the participants were asked to specify if they were routinely employing (used/not used) a particular category of methods (i.e. event-, construct-, element-, species- and plant- specific methods) (see Figure 1). Selection of the "used" button in the event- and species- specific sections prompted the appearance of species sub-sections (see Figure 2), where the participants could specify the crop species for which they were employing (used/not used) EU reference methods. In all cases, further selection of the "used" button elicited the final visualisation of a check-box matrix table (see Figure 3) listing all related methods from the GMOMETHODS database. The respondents had to select from the table the methods employed in the laboratory, mark the adjacent "Modif" option if they had modified the originally validated method protocol and further specify the modification(s) implemented by selecting one or more of the offered options: reaction volume (Rx Vol.), annealing temperature (Ann. T°), master mix (MMix), primers and probe concentrations ([Primers] and [Probe]), cycle number (Cycle #), dynamic range and reference gene (Ref. Gene). A link to a PDF containing the description of the validated method protocol was provided for each method, facilitating the compilation of these responses.

Figure 1– Survey on EU Reference Methods: Quantitative/Qualitative and GMO-specific/Taxon-specific Sections

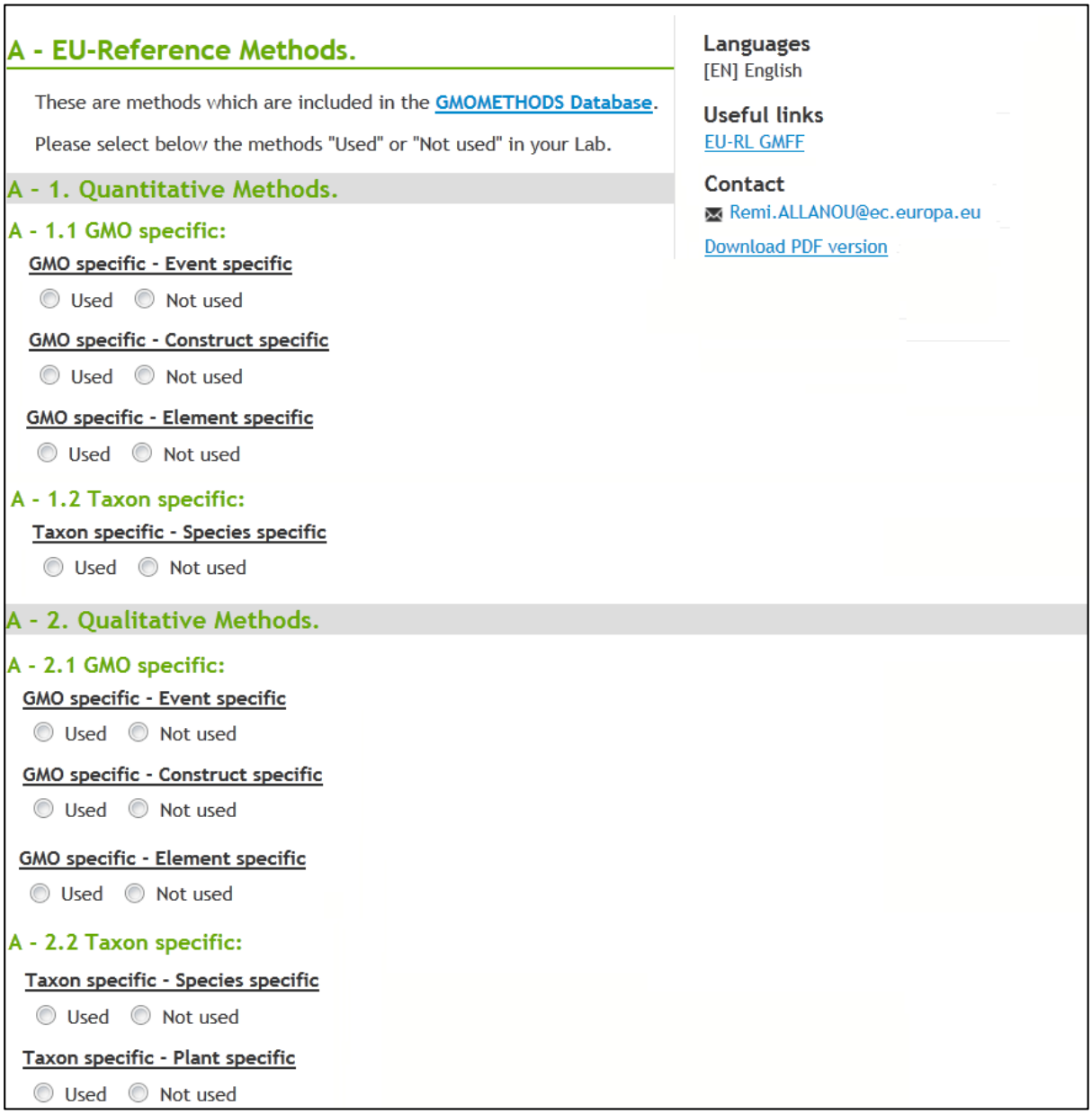


Figure 2 – Survey on EU Reference Methods: Selection of the "Used" Button in the Event-specific Section Prompted the Appearance of the Species Sub-sections

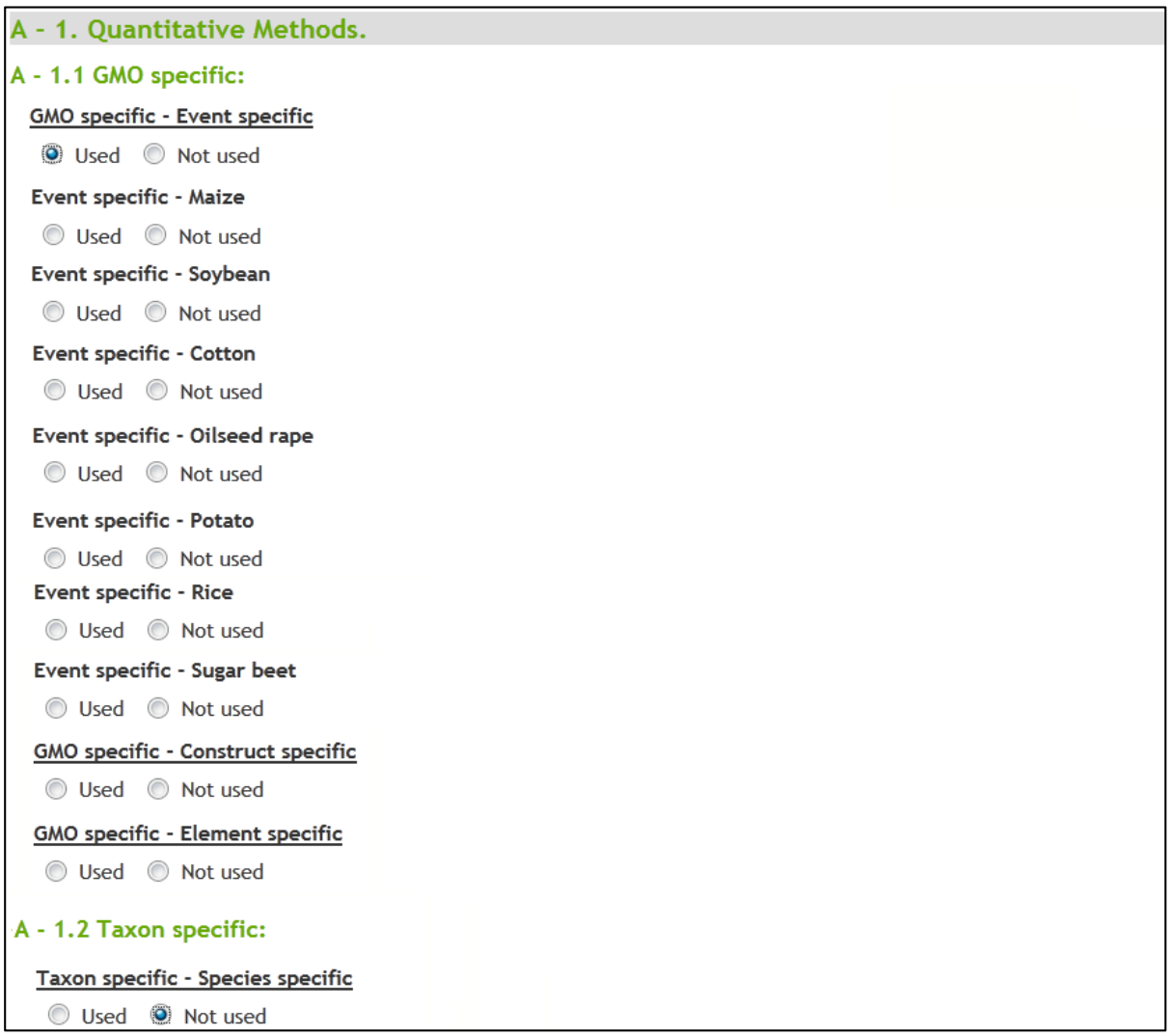
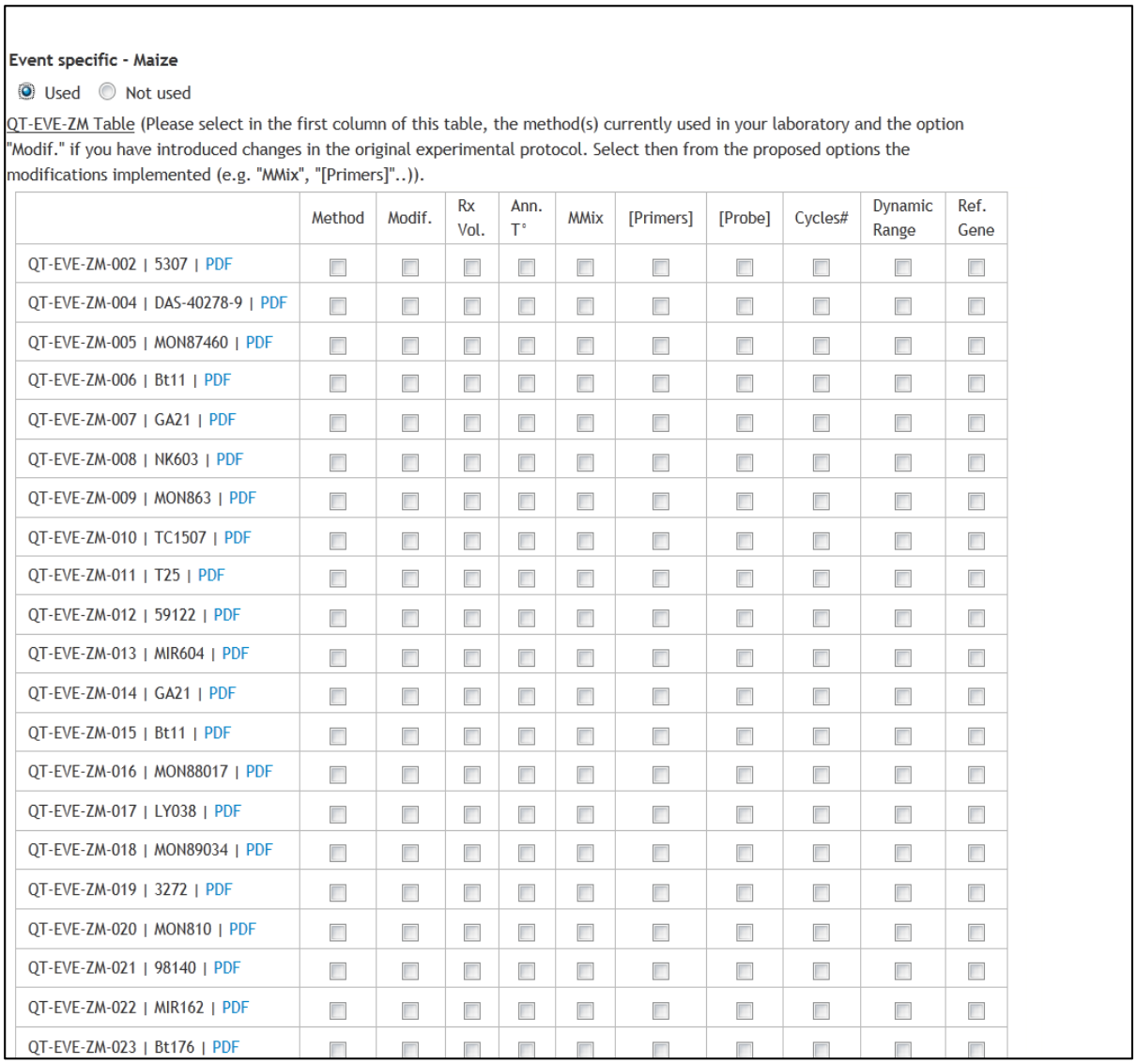


Figure 3 – Survey on EU Reference Methods: Selection of the "Used" Button in the Event-specific Maize Sub-section Prompted the Appearance of a Matrix Table Listing All Related Methods from the GMOMETHODS Database and Check Box Options



Event specific - Maize

(•) Used ( ) Not used

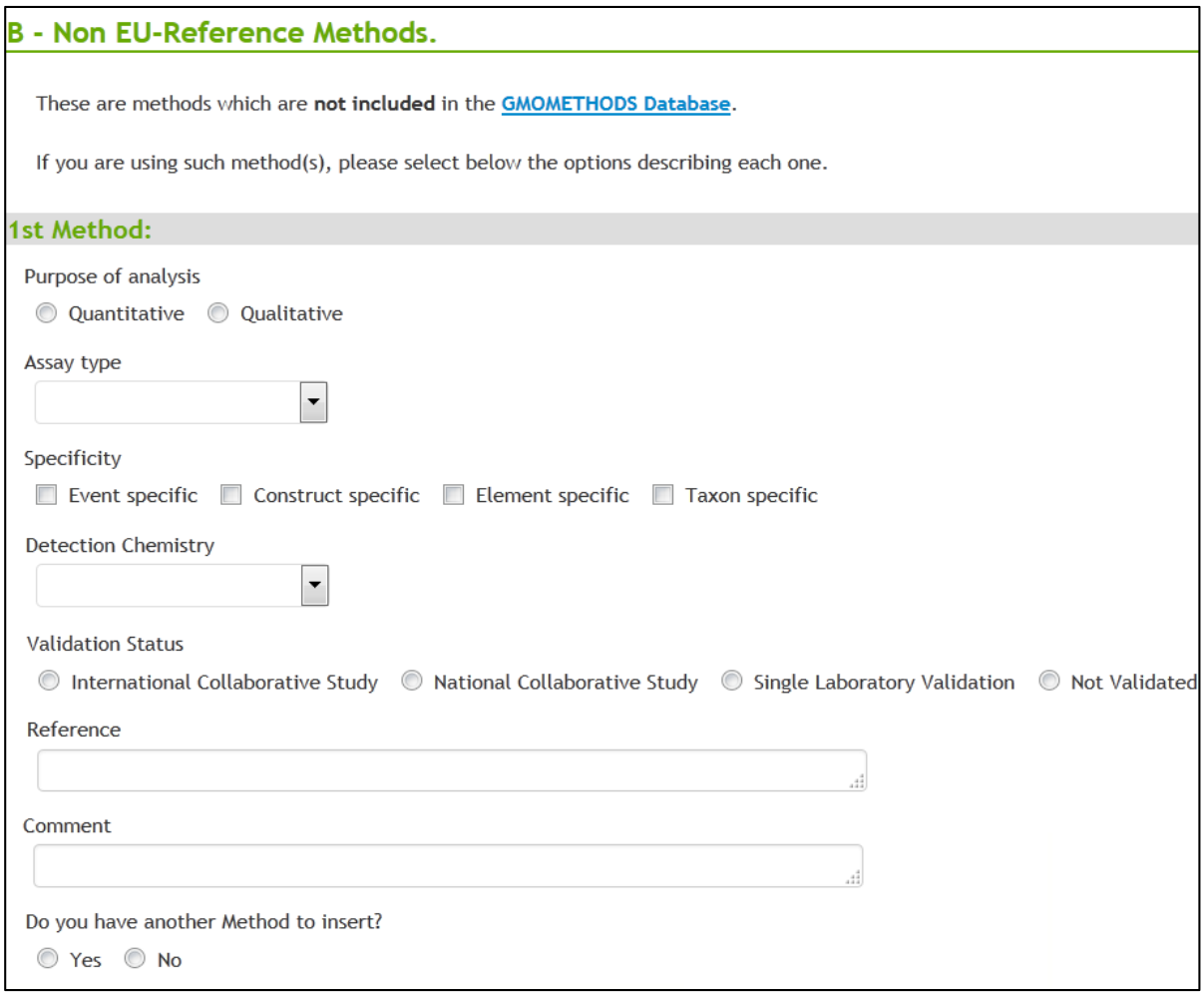
QT-EVE-ZM Table (Please select in the first column of this table, the method(s) currently used in your laboratory and the option "Modif." if you have introduced changes in the original experimental protocol. Select then from the proposed options the modifications implemented (e.g. "MMix", "[Primers]"..)).

	Method	Modif.	Rx Vol.	Ann. T°	MMix	[Primers]	[Probe]	Cycles#	Dynamic Range	Ref. Gene
QT-EVE-ZM-002 | 5307 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-004 | DAS-40278-9 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-005 | MON87460 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-006 | Bt11 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-007 | GA21 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-008 | NK603 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-009 | MON863 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-010 | TC1507 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-011 | T25 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-012 | 59122 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-013 | MIR604 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-014 | GA21 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-015 | Bt11 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-016 | MON88017 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-017 | LY038 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-018 | MON89034 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-019 | 3272 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-020 | MON810 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-021 | 98140 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-022 | MIR162 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
QT-EVE-ZM-023 | Bt176 | PDF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

b) Non-EU Reference Methods

In the section on non-EU reference methods the respondents (see Figure 4) had to specify if the other methods used in the laboratory had a quantitative or qualitative purpose. Then they had to select from drop down menus the assay type, the detection chemistry, the specificity and the validation status of those methods. Finally they were asked to provide, if available, a reference to a publication describing the method in detail. Further information could be inserted in a "Comment" space. Specific data for up to four non-EU reference methods could be provided by each respondent.

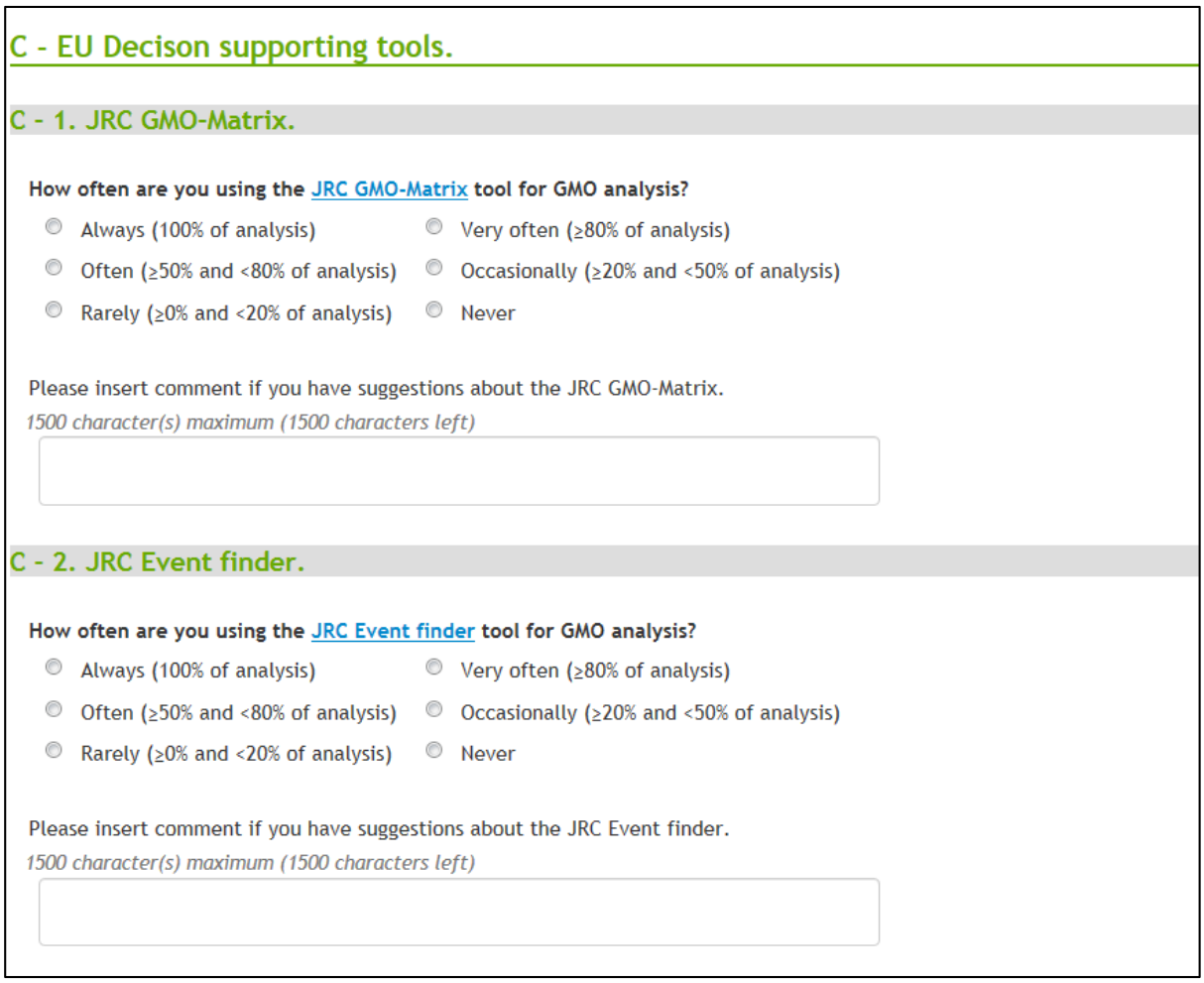
Figure 4 – Survey on Non-EU Reference Methods: Method Description Section



c) Use of JRC Tools

In the third section (see Figure 5) the respondents were asked to provide information about their extent of use of the JRC GMO-Matrix and Event-finder decision support tools, which are offered on line since December 2014 at http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/.

Figure 5 - Survey on EU Decision Supporting Tools: JRC GMO-Matrix and Event Finder Sections



3.3 Data Source

The data outlined in Table 1, provide an overview on the methods included in the GMOMETHODS database up to June 2015 and presented to respondents in the EU Reference Methods section of the survey. A total of 96 quantitative and 51 qualitative methods were offered for selection.

Table 1 - GMO and Taxon-specific Methods Included in the EU Reference Methods Section of the Survey According to Specificity and Purpose (Qualitative/Quantitative)

Assay Type	Specificity	Quantitative	Qualitative	Total
GMO	Event-specific	59	4	63
GMO	Construct-specific	8	12	20
GMO	Element-specific	4	22	26
Taxon	Species-specific	25	12	37
Taxon	Plant-specific	0	1	1
	Total methods	**96**	**51**	**147**

The species and crop names detected by the EU reference methods and included in the survey are listed in Table 2. The methods offered detection for 8 crop species and 19 taxon-specific genes covering the main GM crops available on the market ([11]).

Table 2 - Species Detected by the EU Reference Methods

N	*Species*	Crop name
1	*Beta vulgaris*	sugar beet
2	*Brassica napus*	oilseed rape
3	*Glycine max*	soybean

N	*Species*	Crop name
4	*Gossypium hirsutum*	cotton
5	*Oryza sativa*	rice
6	*Solanum lycopersicum*	tomato
7	*Solanum tuberosum*	potato
8	*Zea mays*	maize

3.4 Data Analysis

The "EUsurvey" tool used for designing the survey did not offer the option of defining as mandatory certain selections (i.e. for the check boxes in the matrix tables represented in Figure 3). Not all respondents followed the instructions provided. As a result an incoherent selection was observed between the "Used"/"Not used" buttons and the "Methods", the "Modif" and other related options in the linked matrix tables. Therefore, to normalise the results, the following assumptions were systematically applied to the data collected.

1st Assumption: if the "Used" button was chosen without selection of a method in the linked matrix table, the "Used" selection was changed to "Not used".

2nd Assumption: if the "Used" button was chosen and only the "Modif" option was marked in the linked matrix table also the related method was considered as selected.

3rd Assumption: if no choice was made between the "Used"/"Not used" buttons but some selections were made in the linked matrix table the "Used" button was considered as selected.

4th Assumption: if the "Not used" button was chosen but some options were marked in the linked matrix table those were considered as not selected.

The resulting normalised data was used for further analysis of the survey.

3.5 Invited Laboratories

The survey was launched on the 19th of June 2015 and was extended to non-NRL laboratories of the ENGL to widen as much as possible the monitoring of GMO analysis performed in the EU MS. The invitation was submitted to 94 laboratories. Their list is displayed in Table 3.

Table 3 - List of the Invited Laboratories (Ordered by Country Name)

Invited Laboratories (N= 94)	Country (N= 31)
AGES -Austrian Agency for Health and Food Safety	Austria (AT)
Environment Agency Austria	Austria (AT)
Institute for Agricultural and Fisheries Research	Belgium (BE)
Scientific Institute of Public Health	Belgium (BE)
Walloon Agricultural Research Centre - Department Valorization des productions (D4) - Unit 16 - Authentication and traceability	Belgium (BE)
GMO Laboratory of the Executive Environmental Agency	Bulgaria (BG)
National Center of Public Health and Analyses (NCPHA), GMO Unit	Bulgaria (BG)
Biotechnological Analyses Division Seed Testing Laboratory Institute for Seed and Seedlings Croatian Centre for Agriculture, Food and Rural Affairs	Croatia (HR)
Croatian National Institute for Public Health - GMO Quantification and risk assessment Unit	Croatia (HR)
Agricultural Research Institute	Cyprus (CY)
State General Laboratory	Cyprus (CY)
Crop Research Institute - Reference Laboratory for GMO Detection and DNA fingerprinting	Czech Republic (CZ)
Institute of Chemical Technology Prague	Czech Republic (CZ)
National Institute of Public Health, Laboratory for Molecular Biological Methods, Centre for Health, Nutrition and Food.	Czech Republic (CZ)
State Veterinary Institute Jihlava	Czech Republic (CZ)
Danish Veterinary and Food Administration, Laboratory for Diagnostics in Plants, Seed, and Feed	Denmark (DK)

Invited Laboratories (N= 94)	Country (N= 31)
Agricultural Research Centre	Estonia (EE)
Laboratory of DNA analysis - Department of Gene Technology - Tallinn University of Technology	Estonia (EE)
Finnish Customs Laboratory	Finland (FI)
Finnish Food Safety Authority Evira	Finland (FI)
BioGEVES - Groupement d'Intérêt Public – Groupe d'Etude et de contrôle des Variétés et des Semences	France (FR)
Plant Health Laboratory	France (FR)
Service commun des laboratoires du ministère de l'économie et des finances- Etablissement de Strasbourg	France (FR)
Bavarian Health and Food Safety Authority	Germany (DE)
Berlin-Brandenburg State Laboratory	Germany (DE)
Center for Agricultural Technology Augustenberg	Germany (DE)
Chemical and Veterinary Analytical Institute Münsterland - Emscher - Lippe	Germany (DE)
Environmental Protection Agency of Saxony-Anhalt; Surveillance Laboratory for Genetic Engineering – Halle	Germany (DE)
Federal Office of Consumer Protection and Food Safety- Berlin	Germany (DE)
Federal State Agency of Analysis and Diagnosis for Rhineland-Palatinate - Institute of Food Chemistry Trier	Germany (DE)
Hessian State Laboratory- Kassel	Germany (DE)
Institute for Consumer Protection, Department 3 - Food Safety - Halle	Germany (DE)
Institute for Hygiene and Environment- Hamburg	Germany (DE)
Laboratory for the Detection of GMO in Food - Bad Langensalza	Germany (DE)
Landeslabor Schleswig-Holstein - Food, Veterinary and Environmental Diagnostic Institute	Germany (DE)
Lower Saxony State Office for Consumer Protection and Food Safety, State Food Laboratory Braunschweig	Germany (DE)
Lower Saxony trade and inspection agency, Hildesheim	Germany (DE)
LUFA Speyer	Germany (DE)
Office for Consumer Protection of the German Federal State Saarland-Saarbrücken	Germany (DE)
Saxon State Company of Environmental and Agriculture - Radebeul, Business Division Laboratories Agriculture	Germany (DE)
State Institute of Chemical and Veterinarian Analysis - Freiburg	Germany (DE)
State Institute of Chemical and Veterinarian Analysis Eastwestphalia-Lippe-Detmold	Germany (DE)
State Institute of Chemical and Veterinarian Analysis Rhine-Ruhr-Wupper Krefeld	Germany (DE)
State Office for Agriculture, Food safety and Fisheries - Mecklenburg Western Pomerania- Rostock	Germany (DE)
State Veterinarian Analysis Office Arnsberg	Germany (DE)
Thüringer State Office for Agriculture Dep. 200 – Jena	Germany (DE)
Thuringian State Authority for Food Safety and Consumer Protection. Dept. 3 Genetic Engineering Surveillance	Germany (DE)
Hellenic Agricultural Organisation _Demetra	Greece (GR)
Ministry of Finance, Secretariat General for Public Revenue, General Chemical State Laboratory (GCSL), Food Division	Greece (GR)
BIOMI Ltd	Hungary (HU)
Central Agricultural Office, Food and Feed Safety Directorate, Feed Investigation National Reference Laboratory	Hungary (HU)
National Food Chain Safety Office, Food and Feed Safety Directorate, GMO Laboratory	Hungary (HU)
Plant Health Laboratory, Seed Certification Division, Backweston Agri-labs	Ireland (IE)
Public Analyst Laboratory	Ireland (IE)
CRA-SCS Sede di Tavazzano – Laboratorio	Italy (IT)
Italian National Institute for Health - Department of Veterinary Public Health and Food Safety - Unit GMOs and Mycotoxins	Italy (IT)
Veterinary Public Health Institute for Lazio and Toscana Regions; National Reference Centre for GMO Analysis	Italy (IT)

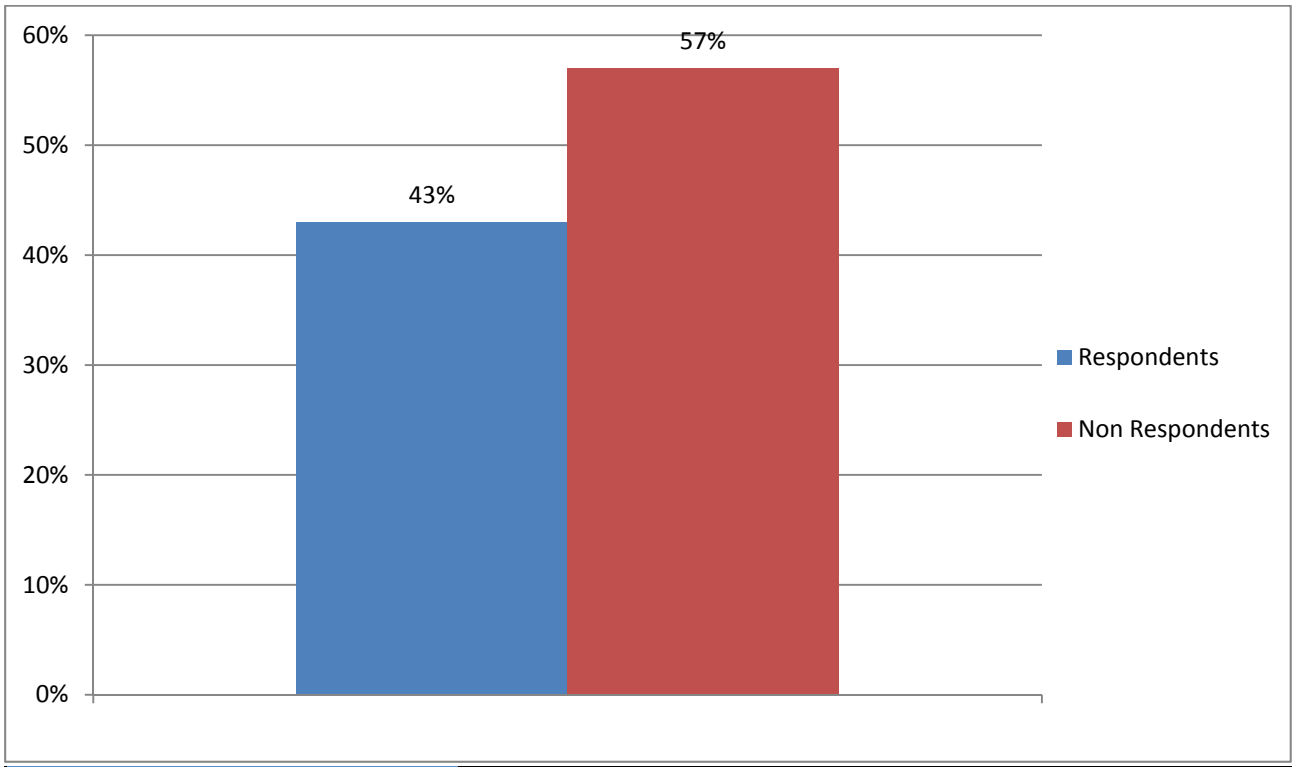
Invited Laboratories (N= 94)	Country (N= 31)
Institute of Food Safety, Animal Health and Environment BIOR	Latvia (LV)
National Phytosanitary Laboratory	Latvia (LV)
National Food and Veterinary Risk Assessment Institute Molecular Biology and GMO Department	Lithuania (LT)
National Health Laboratory, Food Control Department	Luxembourg (LU)
Public Health Laboratory	Malta (MT)
Nederlandse Algemene Keuringsdienst	Netherlands (NL)
RIKILT Wageningen UR	Netherlands (NL)
Test organisation zzzbb	Netherlands (NL)
The Netherlands Food and Consumer Product Safety Authority	Netherlands (NL)
Norwegian Food Safety Authority	Norway (NO)
Norwegian Veterinary Institute	Norway (NO)
Institute of Biochemistry and Biophysics Polish Academy of Sciences, Genetic Modifications Analysis Laboratory	Poland (PL)
National Research Institute of Animal Production, National Feed Laboratory	Poland (PL)
National Veterinary Research Institute in Pulawy, Department of Hygiene of Animal Feeding stuff	Poland (PL)
Plant Breeding and Acclimatization Institute – National Research Institute, GMO Controlling Laboratory	Poland (PL)
State Sanitary and Epidemiological Station, Regional Laboratory of Genetically Modified Food	Poland (PL)
INIAV-Instituto Nacional de Investigação Agrária e Veterinária - Laboratório de OGM	Portugal (PT)
Institute for Diagnosis and Animal Health, Molecular Biology and GMOs Unit - National Reference Laboratory for GMOs in food and feed	Romania (RO)
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, GMO Reference Laboratory	Romania (RO)
Central Control and Testing Institute of Agriculture	Slovakia (SK)
State Veterinary and Food Institute Dolny Kubin	Slovakia (SK)
Agricultural Institute of Slovenia	Slovenia (SI)
National Institute of Biology	Slovenia (SI)
Biological Quantitative Analyses Service Center for Research in Agricultural Genomics CSIC-IRTA-UAB	Spain (ES)
Laboratorio Arbitral Agroalimentario	Spain (ES)
National Centre for Food, Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN)	Spain (ES)
National Food Agency, Science Department	Sweden (SE)
Federal Department of Economic Affairs, Education and Research EAER Institute for Livestock Sciences ILS, Agroscope	Switzerland (CH)
Federal Food Safety and Veterinary Office Risk Assessment Division - Laboratories Sector	Switzerland (CH)
Federal Office for Agriculture	Switzerland (CH)
Ankara Provincial Control Laboratory	Turkey (TR)
National Food Reference Laboratory	Turkey (TR)
Food and Environment Research Agency	United Kingdom (UK)
LGC Limited	United Kingdom (UK)
Science and Advice for Scottish Agriculture	United Kingdom (UK)
UK Food Standard Agency	United Kingdom (UK)
Worcestershire County Council Scientific Services	United Kingdom (UK)

4. Participation

Of 94 invited laboratories, 40 participated to the survey. The resulting response rate (43 %) (see Figure 6) indicates that the data collected is sufficiently representative for the ENGL members.

4.1 Response Rate

Figure 6 - Response Rate of the Survey

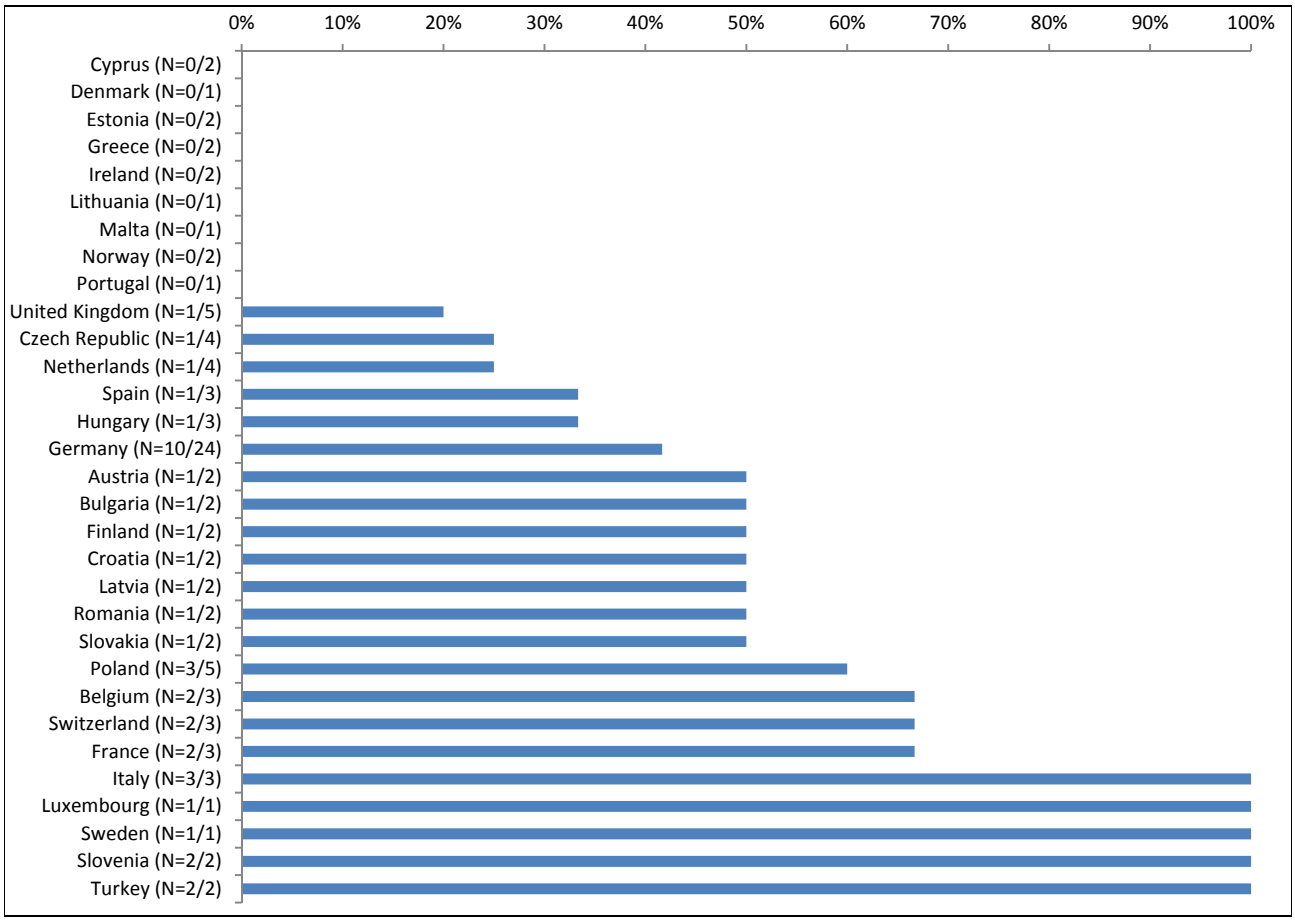


Invited Laboratories	Respondents	Non Respondents
94	40	54

4.2 Response Rate/Country

Laboratories from 31 different countries were invited to participate to the survey. Figure 7 indicates, for each country, the number of respondents (R) over the invited (I) laboratories (N = R/I). The graphics show that the respondents were representative of 22 countries and were therefore widely distributed geographically across Europe.

Figure 7 - Response Rate/Country

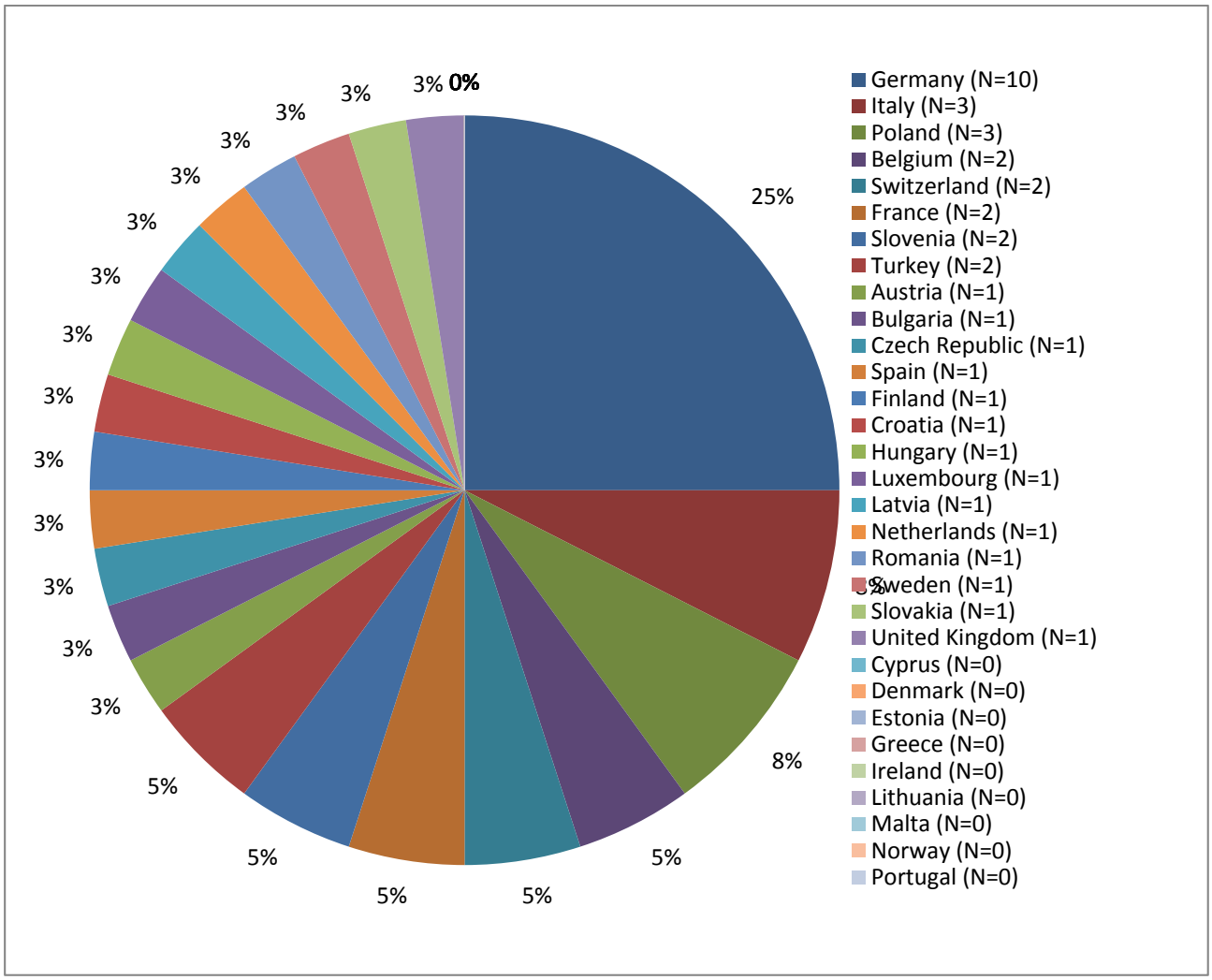


Legend: (N = R/I) = Number of respondent laboratories (R) over invited laboratories (I)

4.3 Country Representation of Respondents

Further analyses on country representation (Figure 8) indicate that 25 % of the respondents were laboratories from Germany while laboratories from each other country contributed in absolute numbers with a minor percentage (3 %-8 %).

Figure 8 - Country Representations of Respondents

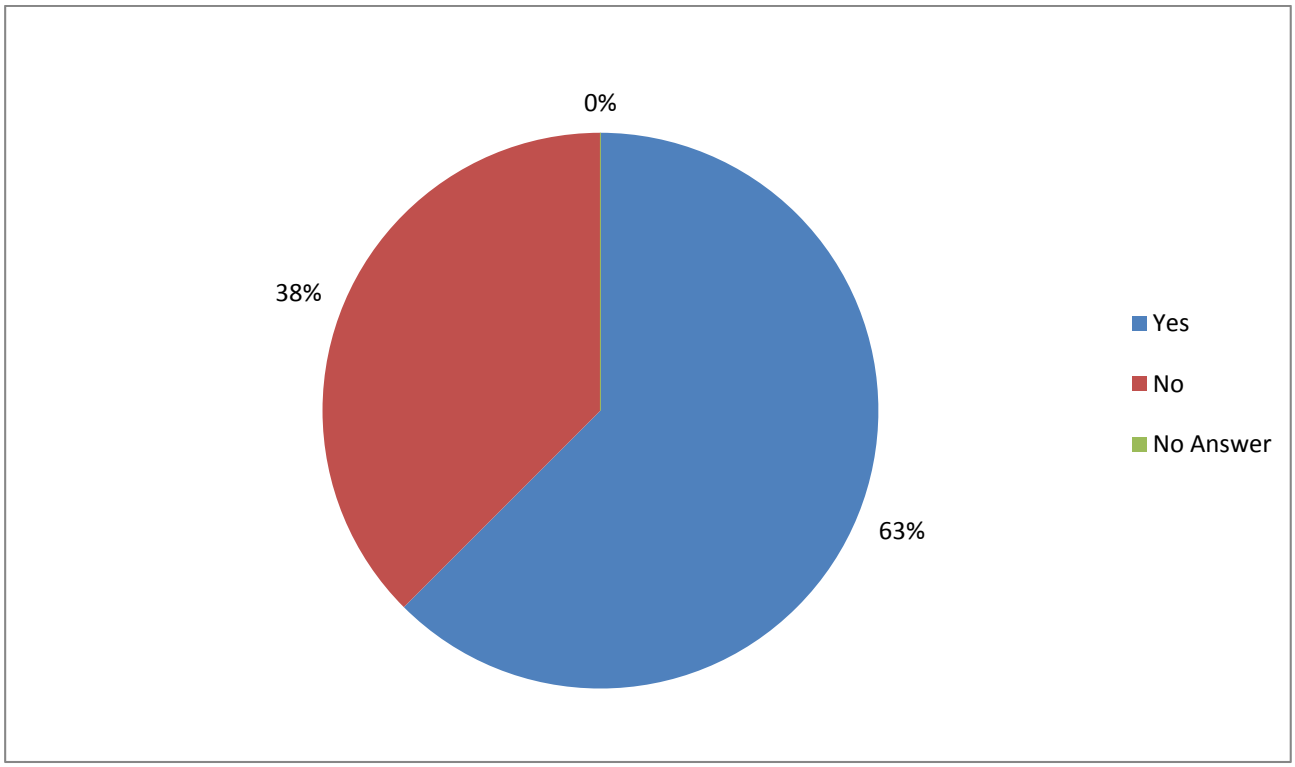


Legend: N= Number of respondent laboratories

4.4 882/2004 NRL Respondents

The laboratories were asked to specify, if they were a NRL operating under Reg. (EC) No 882/2004. As shown in Figure 9 the majority of the respondents (63 %) declared to be appointed under that Regulation.

Figure 9 - Percentages of NRLs Respondents

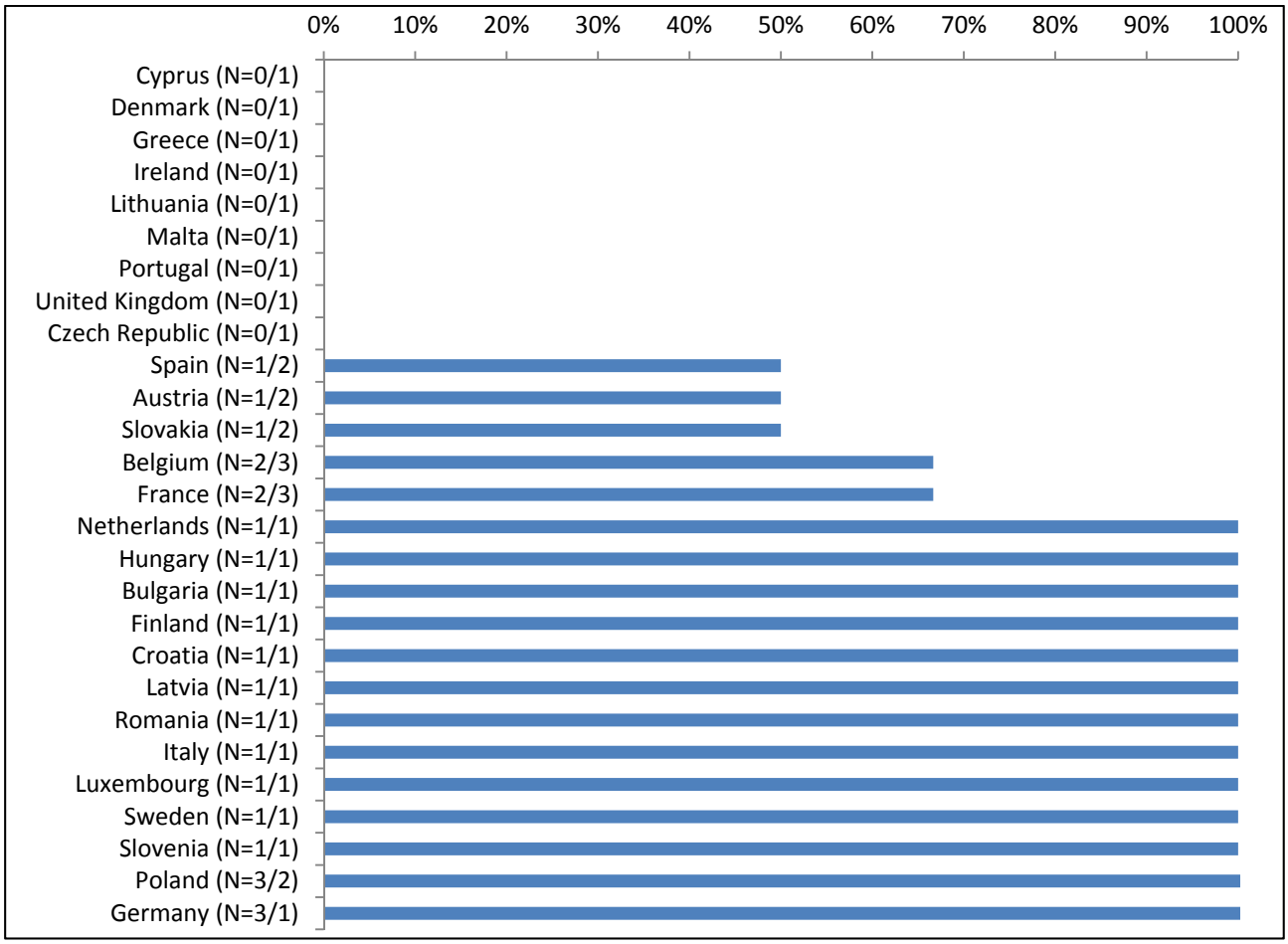


Legend: Yes= NRL laboratory, No= Non NRL laboratory

4.5 882/2004 NRLs Response Rate/Country

NRLs from 27 different countries were invited to participate to the survey. Figure 10 indicates, for each country, the number of respondents (R) over the invited (I) NRLs (N = R/I). The graphics show that the responding NRLs were representing 18 countries and were therefore widely distributed geographically across Europe. It can be noticed that in some cases (Poland and Germany) more laboratories than actually appointed declared to operate as NRLs under Reg. (EC) No 882/2004. With those corrections it can be concluded that 60 % of the invited NRLs participated to the survey.

Figure 10 NRLs Response Rate/Country



Legend: (N = R/I) = Number of respondents (R) over invited (I) NRLs

5. Results about EU Reference Methods

The results on EU reference methods have been organised in the report to reflect the main objectives of the survey. Statistical analyses on the use of EU reference methods for quantitative and qualitative purposes are presented first in section 8.1. The following 8.2 section provides an overview on the rate of modification (number of modified methods over the total number of methods) for event-, construct-, element- and taxon- specific assays. In section 8.3 the type and frequency of modifications implemented by laboratories are displayed for the same methods categories. To facilitate the analysis only the charts representing a general overview of the results have been included in the report. The charts regarding restricted categories of methods or single methods that could support particular activities of the ENGL WGs have been included in Annex 1 and Annex 2. The original values corresponding to the number of respondents using the listed methods are displayed in the tables below the charts.

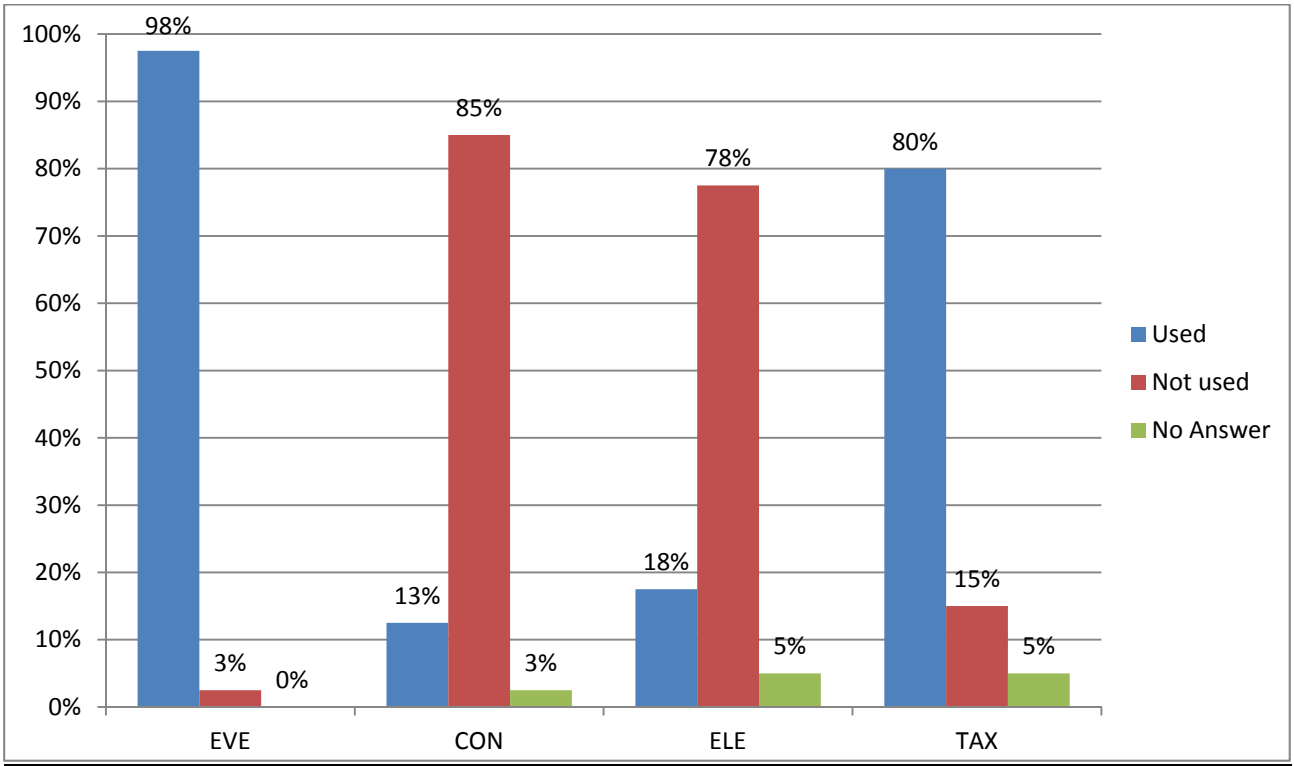
5.1 Use of EU Reference Methods

The charts in the following two chapters provide data collected from the section "A - EU Reference Methods" (Figure 1) and regard the "use/not use" of the EU reference methods according to specificity. The bars in the charts represent the percentage of use, not use or no reply by respondents for each method's specificity section. The tables beneath the charts report the related values collected from the survey. The results are divided in two chapters according to the quantitative or qualitative purpose of the analysis.

a) Quantitative EU Reference Methods

The data collected reveal that for quantitative analysis (see Figure 11) almost all respondents (98 %) were using EU reference methods for event-specific detection of GMOs. A large majority (80 %) was also employing taxon-specific reference methods. For quantitative purposes only a small number of laboratories were using construct-specific (13 %) and element-specific (18 %) EU reference methods. Considering the way the survey has been designed, it is worth noting that the "not used" replies in the "A – EU-Reference Methods" section include both those respondents who do not use quantitative methods at all under the respective specificity section and those who use alternative methods to EU reference methods in the respective specificity section. Therefore the low figures for quantitative construct-specific and element-specific EU-reference methods do not necessarily indicate the use of alternative methods, but may reflect the limited use of quantitative analysis for non-specific genetic targets as confirmed later in the section regarding the use of non-EU reference methods.

Figure 11 - Percentage of Respondents using Quantitative EU Reference Methods According to Specificity



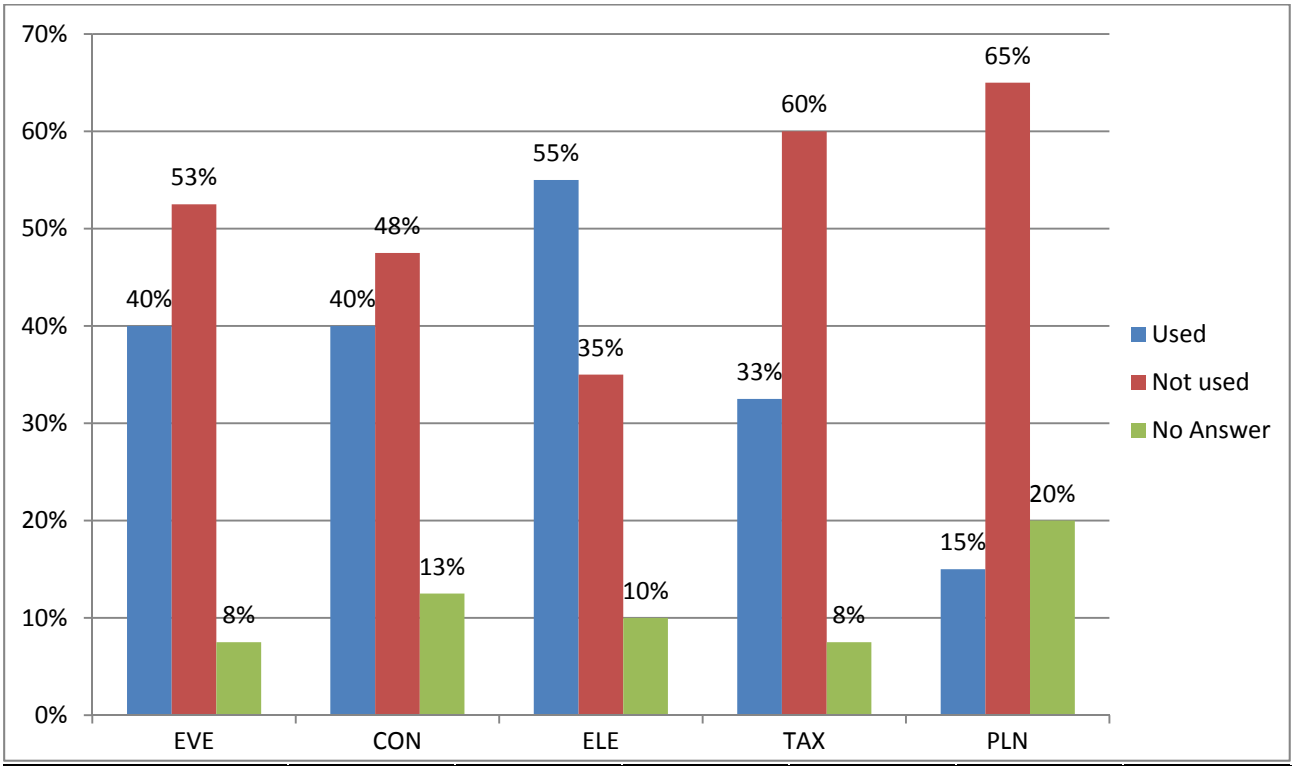
Answers QT Methods	EVE	CON	ELE	TAX	TOT
Used	39	5	7	32	83
Not used	1	34	31	6	72
No Answer	0	1	2	2	5
Total Answers	40	40	40	40	160

Legend: QT = Quantitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; TOT = Total quantitative methods

b) Qualitative EU Reference Methods

An opposite picture is observed for qualitative analysis (Figure 12). The majority of respondents (55 %) reported to use for qualitative purposes element-specific reference methods while a smaller proportion (40 %) declared to employ construct-specific or event-specific reference methods. Few laboratories (33 %) indicated using taxon-specific EU reference methods and an even lower number (15 %) plant-specific assay. As mentioned before for quantitative methods, these figures do not necessarily indicate a preferential use of non-EU reference methods but may reflect the limited use of qualitative analyses, for certain specificity sections (i.e. plant-, taxon- and event-specificity). Indeed laboratories tend to perform qualitative screening tests with general targets rather than event-specific targets to cover a maximum number of possible GM events. Similar trends are observed in the section regarding the use of non-EU reference methods.

Figure 12 - Percentage of Respondents using Qualitative EU Reference Methods According to Specificity



Answers QL Methods	EVE	CON	ELE	TAX	PLN	TOT
Used	16	16	22	13	6	73
Not used	21	19	14	24	26	104
No Answer	3	5	4	3	8	23
Total Answers	40	40	40	40	40	200

Legend: QL = Qualitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; PLN = Plant-specific methods; TOT = Total qualitative methods

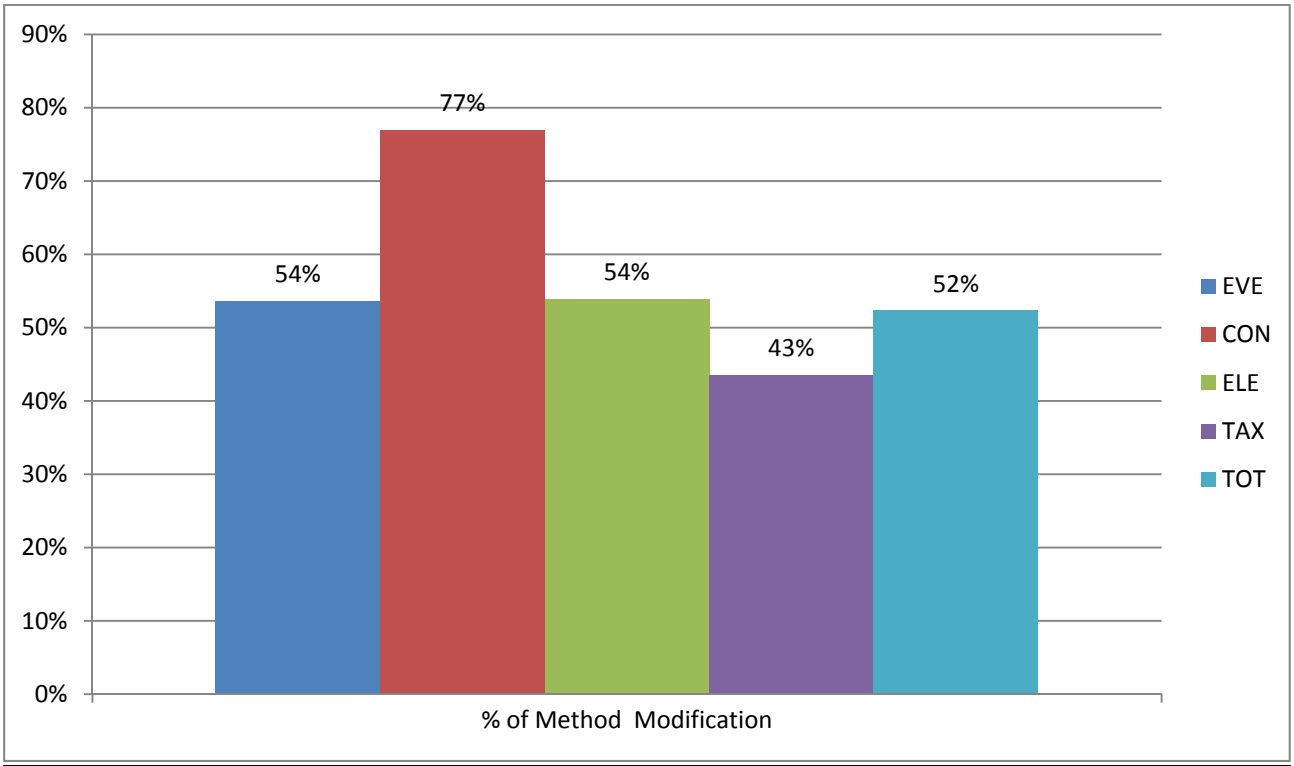
5.2 Modification of EU Reference Methods

The charts in the following two chapters present data regarding the selection of EU reference methods and the adjacent check box option "Modif" in the matrix tables (Figure 3) of the "A - EU Reference Methods" section. The bars in the column charts below represent the ratio of methods modified over the total number of methods used for each specificity section. The tables below the column charts report the related values collected from the survey.

a) Quantitative EU Reference Methods

The data indicate that about half of the protocols for quantitative GMOs analysis are modified by the respondent laboratories. As shown in Figure 13 the average rate of modification for all quantitative assays corresponds to 52 %. Close to half of the event-specific (54 %), element-specific (54 %) and taxon-specific (43 %) EU reference methods are altered by respondents while the construct-specific methods show an even higher degree of variation (77 %). The latter however represent only 1 % of the total methods used for quantitative purposes. The large majority (84 %) of the methods employed for quantitative analysis are indeed event-specific. This may reflect the laboratories needs of providing specific quantitative confirmation of a GM event presence above the legal threshold but also it may reflect the number of quantitative event-specific methods actually available in the GMOMETHODS database. Up to June 2015 the database included in the quantitative section 59 event-specific and only 8 construct-specific methods (see Table 1).

Figure 13 – Rate of Modification for Quantitative EU Reference Methods According to Specificity



QT Methods	EVE	CON	ELE	TAX	TOT
EU Methods Used*	1364	13	13	237	1627
Methods Modified	731	10	7	103	851
Rate of Modification	54 %	77 %	54 %	43 %	52 %
% of Total Used Methods	84 %	1 %	1 %	15 %	100 %

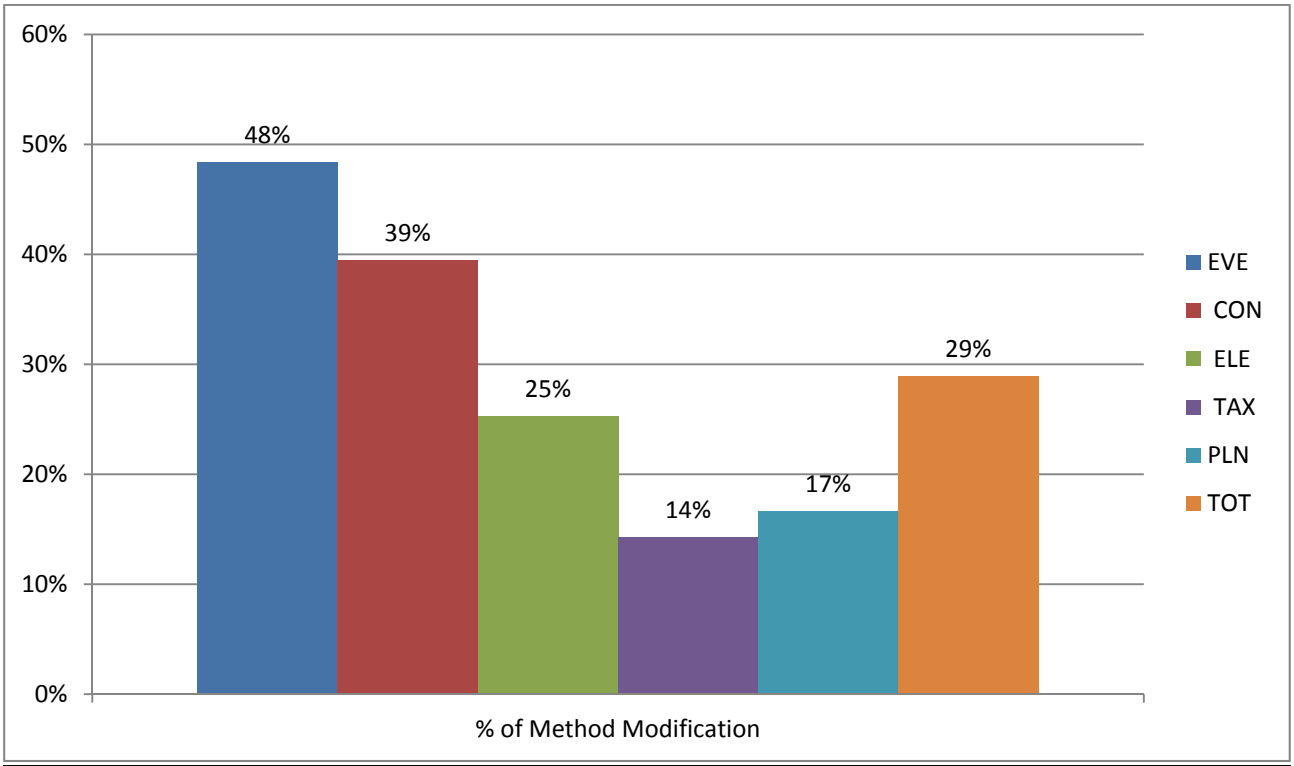
Legend: QT = Quantitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; TOT = Total used quantitative methods

* Each laboratory uses several EU methods for each specificity section

b) Qualitative EU Reference Methods

Qualitative EU reference methods show a higher variability of modifications according to the specificity of the analysis (See Figure 14). The event-, construct- and element- specific methods revealed alteration rates of 48 %, 39 %, and 25 %, respectively, while taxon-specific and plant-specific assays showed lower rates of modification (14 % and 17 %, respectively). The average for all types of qualitative EU reference methods corresponds to a modification rate of 29 %, quite lower than for the quantitative EU reference methods. It can be noted that the element-specific, plant-specific and taxon-specific assays cover the large majority of the methods used for qualitative purposes (total 67 %). This may reflect the laboratories' needs of performing qualitative screening tests with general targets for covering a maximal number of possible GM events, but it may also reflect the number of qualitative element-specific methods available at the time of the survey in the GMOMETHODS database. Up to June 2015 the database included 22 element-specific methods and only 4 event-specific assays (see Table 1) in the qualitative section.

Figure 14 - Rate of Modification for Qualitative EU Reference Methods According to Specificity



QL Methods	EVE	CON	ELE	TAX	PLN	TOT
EU Methods Used*	31	38	87	42	6	204
Methods Modified	15	15	22	6	1	59
Rate of Modification	48 %	39 %	25 %	14 %	17 %	29 %
% of Total Used Methods	15 %	19 %	43 %	21 %	3 %	100 %

Legend: Legend: QL = Qualitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; PLN = Plant-specific methods; TOT = Total used qualitative methods
* Each laboratory uses several EU methods for each specificity section

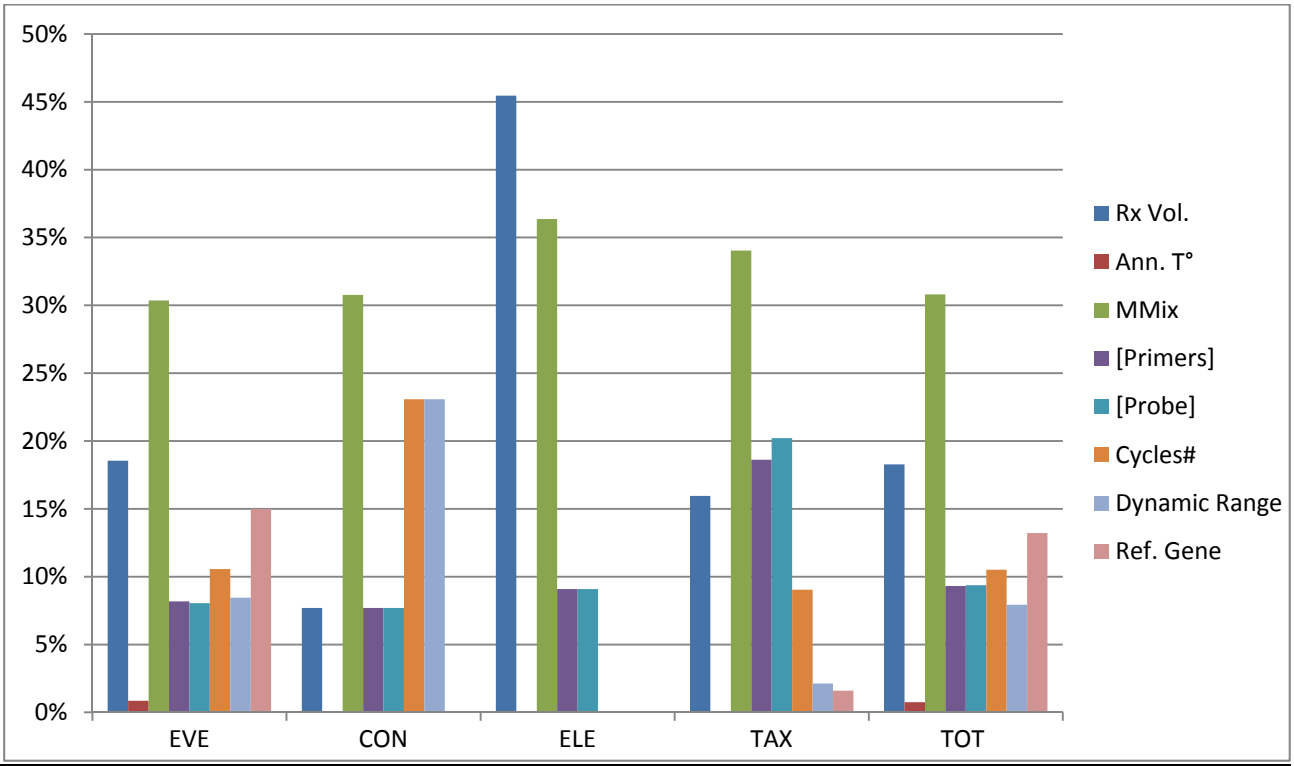
5.3 Type and Frequency of Modifications for EU Reference Methods

The charts in the following two chapters present data regarding the types of modification options selected in the matrix tables (Figure 3) of the "A - EU Reference Methods" section. The bars in the column charts represent the relative contribution of each type of modification to the total number of modifications used for each specificity section. The tables below the column charts report the relative values collected from the survey.

a) Quantitative EU Reference Methods

The data collected from the survey indicate that more than 30 % of the modifications implemented in the protocols regard the master mix of the PCR reaction (see Figure 15). For event-specific, construct-specific and taxon-specific methods this is the parameter most frequently altered. For element-specific methods the reaction volume has an even higher rate of modification (45 %). This parameter has the second highest value (19 %) for event-specific methods and on average (18 %) for all quantitative assays. The concentrations of primers and probes of the PCR reaction mix are changed for approximately 20 % of the taxon-specific protocols, while the reference gene is changed in 15 % of the event-specific analysis.

Figure 15 - Type and Frequency of Modifications for Quantitative EU Reference Methods According to Specificity



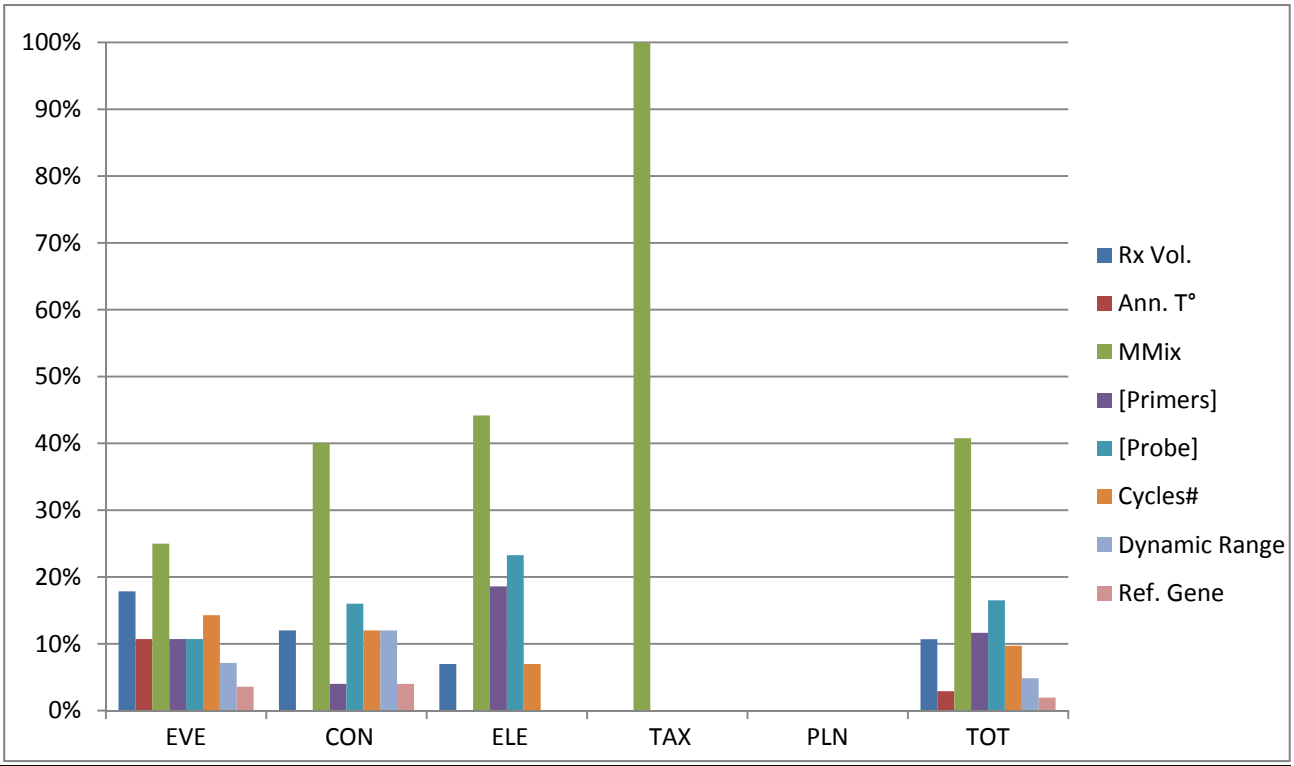
QT Modification	EVE #	CON #	ELE #	TAX #	TOT #
Rx Vol.	281	2	5	30	318
Ann. T°	13	0	0	0	13
MMix	460	8	4	64	536
[Primers]	124	2	1	35	162
[Probe]	122	2	1	38	163
Cycles#	160	6	0	17	183
Dynamic Range	128	6	0	4	138
Ref. Gene	227	0	0	3	230
Total Modifications	1515	26	11	188	1740
QT Modification	**EVE %**	**CON %**	**ELE %**	**TAX %**	**TOT %**
Rx Vol.	19 %	8 %	45 %	16 %	18 %
Ann. T°	1 %	0 %	0 %	0 %	1 %
MMix	30 %	31 %	36 %	34 %	31 %
[Primers]	8 %	8 %	9 %	19 %	9 %
[Probe]	8 %	8 %	9 %	20 %	9 %
Cycles#	11 %	23 %	0 %	9 %	11 %
Dynamic Range	8 %	23 %	0 %	2 %	8 %
Ref. Gene	15 %	0 %	0 %	2 %	13 %
Total Modifications	100 %	100 %	100 %	100 %	100 %

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific methods; QT = Quantitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; TOT = Total used quantitative methods

b) Qualitative EU Reference Methods

The data in Figure 16 indicate that the master mix of the PCR reaction is the parameter most frequently altered also for qualitative analysis and the only one changed for taxon-specific assays. However, the data are not really representative since only a few respondents declared to modify the protocols of qualitative methods.

Figure 16 - Type and Frequency of Modifications for Qualitative EU Reference Methods According to Specificity



QL Modification	EVE #	CON #	ELE #	TAX #	PLN #	TOT #
Rx Vol.	5	3	3	0	0	11
Ann. T°	3	0	0	0	0	3
MMix	7	10	19	6	0	42
[Primers]	3	1	8	0	0	12
[Probe]	3	4	10	0	0	17
Cycles#	4	3	3	0	0	10
Dynamic Range	2	3	0	0	0	5
Ref. Gene	1	1	0	0	0	2
Total Modifications	28	25	43	6	1	103
QL Modification	**EVE %**	**CON %**	**ELE %**	**TAX %**	**PLN %**	**TOT %**
Rx Vol.	18 %	12 %	7 %	0 %	0 %	11 %
Ann. T°	11 %	0 %	0 %	0 %	0 %	3 %
MMix	25 %	40 %	44 %	100 %	0 %	41 %
[Primers]	11 %	4 %	19 %	0 %	0 %	12 %
[Probe]	11 %	16 %	23 %	0 %	0 %	17 %
Cycles#	14 %	12 %	7 %	0 %	0 %	10 %
Dynamic Range	7 %	12 %	0 %	0 %	0 %	5 %
Ref. Gene	4 %	4 %	0 %	0 %	0 %	2 %
Total Modifications	100 %	100 %	100 %	100 %	100 %	100 %

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; QL = Qualitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; PLN = Plant-specific methods; TOT = Total used qualitative methods

6. Results about non-EU Reference Methods

The charts in the following chapters provide data collected from the section "B - non-EU Reference Methods" (Figure 4). The results have been organised in a way to facilitate a comparison with those of the "A - EU reference methods" section. Statistical analyses on the use of the non-EU reference methods for quantitative and qualitative purposes are presented first in section 9.1. Charts representing their validation status are provided in section 9.2, while a schematic summary on the numbers and novelty of methods reported by respondents is offered in section 9.3. A complete overview of the information provided in the survey and more detailed aspects of the methods (targets, and detection chemistry) are displayed in Annex 2.

6.1 Use of non-EU Reference Methods

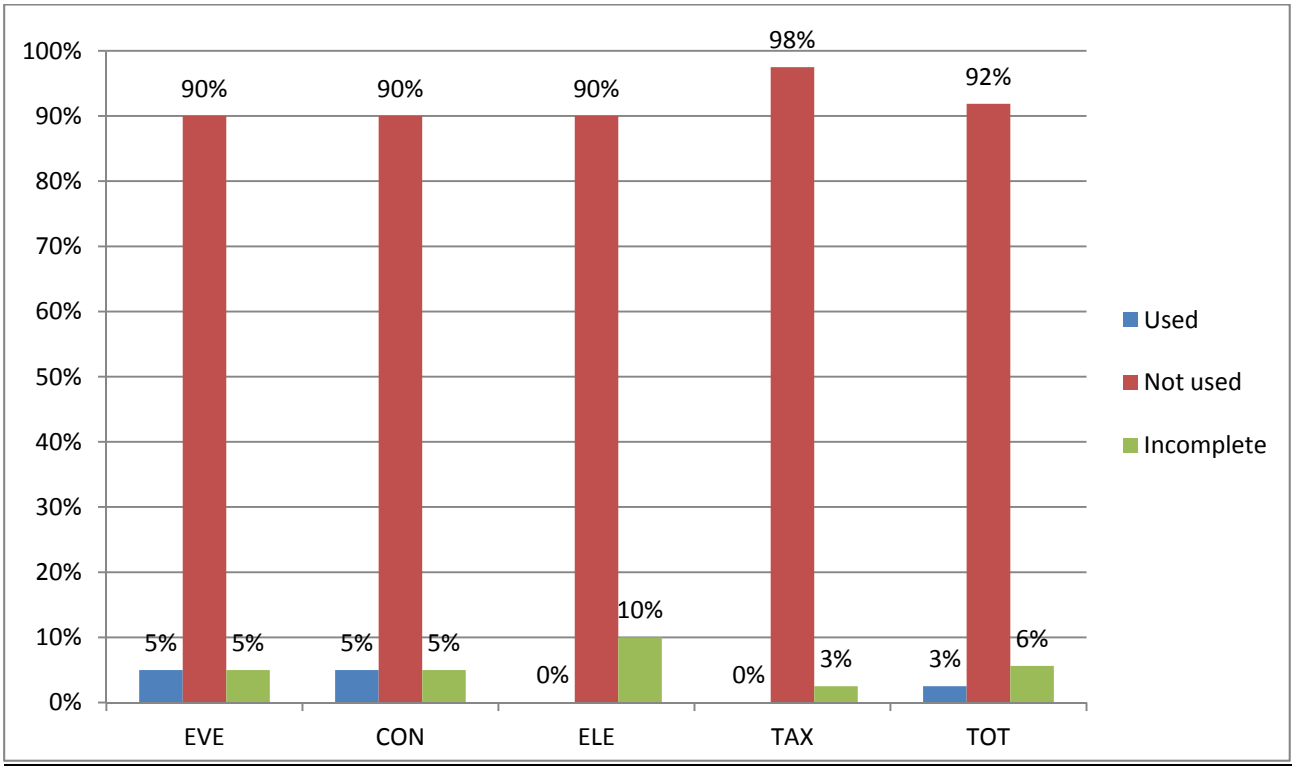
The charts in the following two chapters regard the "use/not use" of non-EU reference methods by respondents according to specificity. The tables beneath the charts report the related numbers collected from the survey. The results are divided in two chapters according to the quantitative or qualitative purpose of the analysis.

The bars in the charts represent the percentage of use, not use or incomplete reply by respondents for each method's specificity section. A reply was considered incomplete when the purpose of the assay (qualitative/quantitative) had not been selected by the respondent and/or when the specificity of the assay, if not indicated, could not be inferred from the genetic targets and the references provided. When none of the main parameters (purpose, specificity, target, reference and validation status) had been indicated by the respondent the answer was considered equivalent to a "not use" selection in the statistics. Eight methods reported in this section resulted to be included in the GMOMETHODS database. For three other methods the respondents indicated an inconsistent purpose as well as inconsistent specificity, genetic target(s) and validation status not corresponding to the reference provided. All these eleven methods were not included in the statistics.

a) Quantitative non-EU Reference Methods

The data collected reveal that only 5 % of the respondents (see Figure 17) were using non-EU reference methods for event-specific quantification of GM events. For quantitative purposes only a small number of laboratories (5 %) were using construct-specific while none (0 %) was employing element-specific or taxon-specific non-EU reference methods.

Figure 17 - Percentage of Respondents using Quantitative non-EU Reference Methods According to Specificity



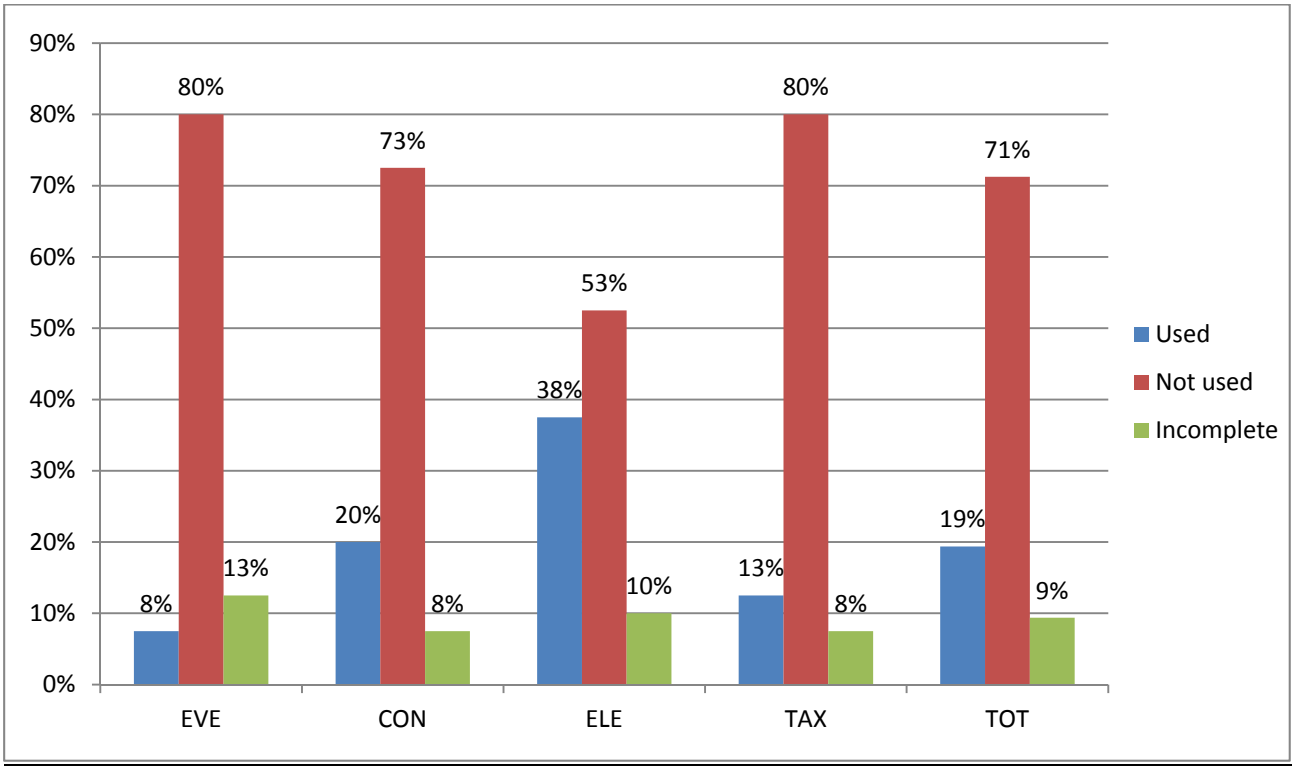
Answers QT Methods	EVE	CON	ELE	TAX	TOT
Used	2	2	0	0	4
No Answer	36	36	36	39	147
Incomplete Answers	2	2	4	1	9
Total Answers	40	40	40	40	160

Legend: QT = Quantitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element specific methods; TAX = Taxon-specific methods; TOT = Total quantitative non-EU reference methods

b) Qualitative non-EU Reference Methods

A different picture is observed for qualitative analysis (Figure 18). A significant number of respondents (38 %) reported to use element-specific non-EU reference methods for qualitative purposes while a smaller proportion (20 %) declared to employ construct-specific non-EU reference methods. Few laboratories indicated using qualitative taxon-specific (13 %) or event-specific (8 %) non-EU reference methods.

Figure 18 - Percentage of Respondents using Qualitative non-EU Reference Methods According to Specificity



Answers QL Methods	EVE	CON	ELE	TAX	TOT
Used	3	8	15	5	31
No Answer	32	29	21	32	114
Incomplete Answers	5	3	4	3	15
Total Answers	40	40	40	40	160

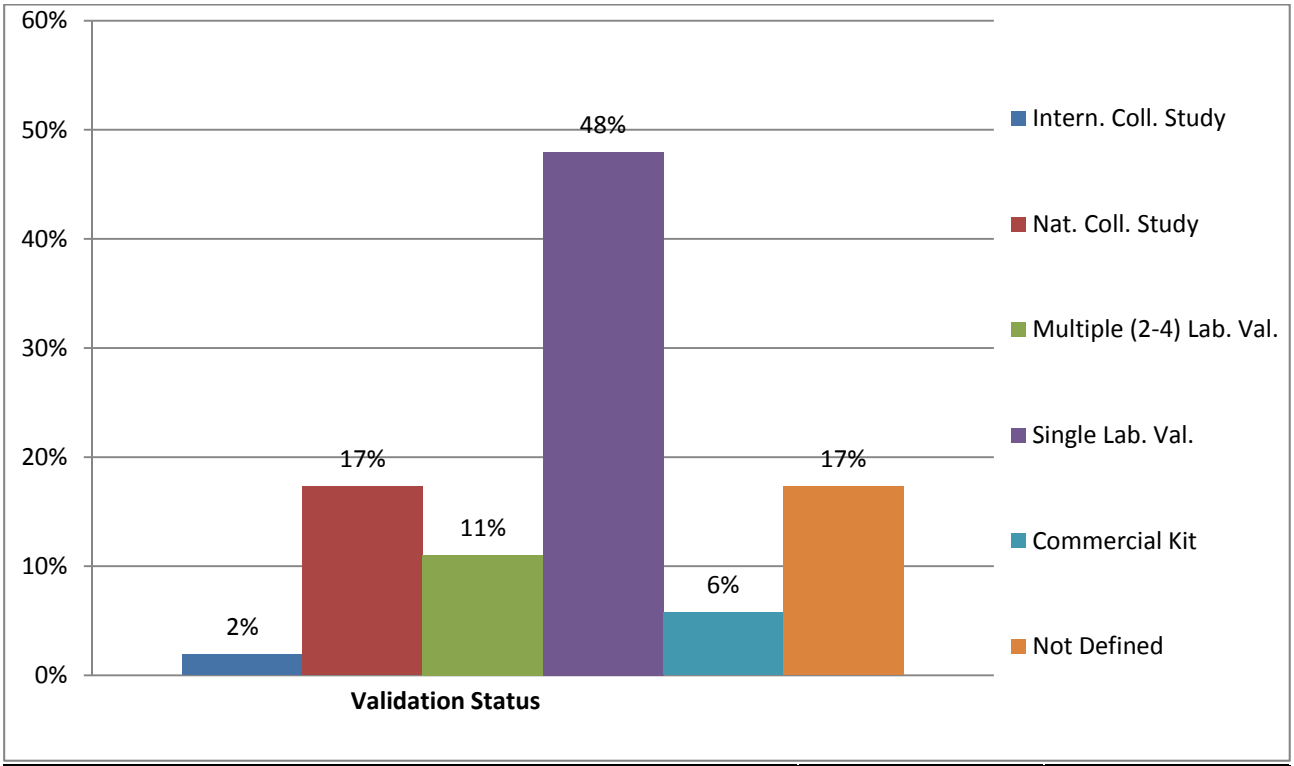
Legend: QL = Qualitative; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element specific methods; TAX = Taxon-specific methods; TOT = Total qualitative non-EU reference methods

6.2 Validation Status of non-EU Reference Methods

The chart below indicates the validation status of the non-EU reference methods reported by the laboratories. When not indicated by the respondent, in some cases the validation status had been inferred from the related reference or corrected if inconsistent.

A verification of the supporting references revealed that eight methods reported in this section were included in the GMOMETHODS database. For another three methods respondents indicated an inconsistent purpose, as well as inconsistent specificity, genetic target(s) and validation status not corresponding to the reference provided. These eleven methods are not included in the charts. Other methods reported to be validated in international ring trials were actually described in the corresponding publications as single- or three-laboratories validation or were based on a commercial kit from Eurofins. On the other hand, a method designated as national standard was described in the supporting reference as internationally validated. The corrected validation status of the reported non-EU reference methods is displayed in
Figure 19. The chart shows that non-EU reference methods are mostly (48 %) validated in a single laboratory. A smaller proportion of the methods (17 %) had been validated in national collaborative studies or tested in a two- to four-laboratory trial (11 %). Commercial kits represent 6 % of the non-EU reference methods employed by laboratories. Finally only one reported method (2 %) had been validated in an international collaborative study.

Figure 19 - Validation Status of non-EU Reference Methods



Validation Status	TOT #	TOT %
Intern. Collaborative Study	1	2 %
Nat. Collaborative Study	9	17 %
Multiple (2-4) Laboratories Validation	6	11 %
Single Laboratory Validation	26	48 %
Commercial Kit	3	6 %
Validation Status not Defined	9	17 %
Total Methods	54	100 %

Legend: Intern. = International; Coll. = Collaborative; Nat. = National; Lab. = Laboratory; Val=Validation

6.3 Novelty of non-EU Reference Methods

A summary on the non-EU reference methods reported by respondents is provided in Table 4. A more detailed description of these methods can be found in Table 5 of Annex 2. The table below provides information on the number of non-EU reference methods that were reported for a qualitative or quantitative analytical purpose grouped according to their year of publication (before or after 2013). The methods for which the respondent did not supply the information as requested are listed under the category "Not Provided". The table does not contain eight reported methods that were found to be included in the GMOMETHODS database and another three methods for which the described analytical purpose, specificity, targets and references were fully inconsistent. It can be noticed that qualitative methods represent the majority (65 %) of the methods reported in the "B - non-EU reference" section. Eleven of these were described in articles issued before 2013 while another nine were recently published. Quantitative methods represent only 9 % of the methods reported and were all published before 2013. For a considerable number of methods (26 %) the respondents did not provide the related analytical purpose.

Table 4 – Novelty of non-EU Reference Methods

Purpose	Number of Methods Provided	Year of Publication	% QL/QT/NP	% TOT
Qualitative	11	< 2013	31 %	20 %
	9	≥ 2013	26 %	17 %
	13	Not provided	37 %	24 %
	2	Commercial kit	6 %	4 %
Total Qualitative	35			65 %
Quantitative	4	< 2013	80 %	7 %
	0	≥ 2013	0 %	0 %
	0	Not provided	0 %	0 %
	1	Commercial kit	20 %	2 %
Total Quantitative	5			9 %
Not Provided	12	< 2013	86 %	22 %
	2	≥ 2013	14 %	4 %
Total Not Provided	14			26 %
Total Methods	54			100%

Legend: % QL/QT/NP = Percentages of methods described in the single sections "Qualitative", "Quantitative" and "Not Provided"; % TOT = Percentages of methods described in Total in all three sections

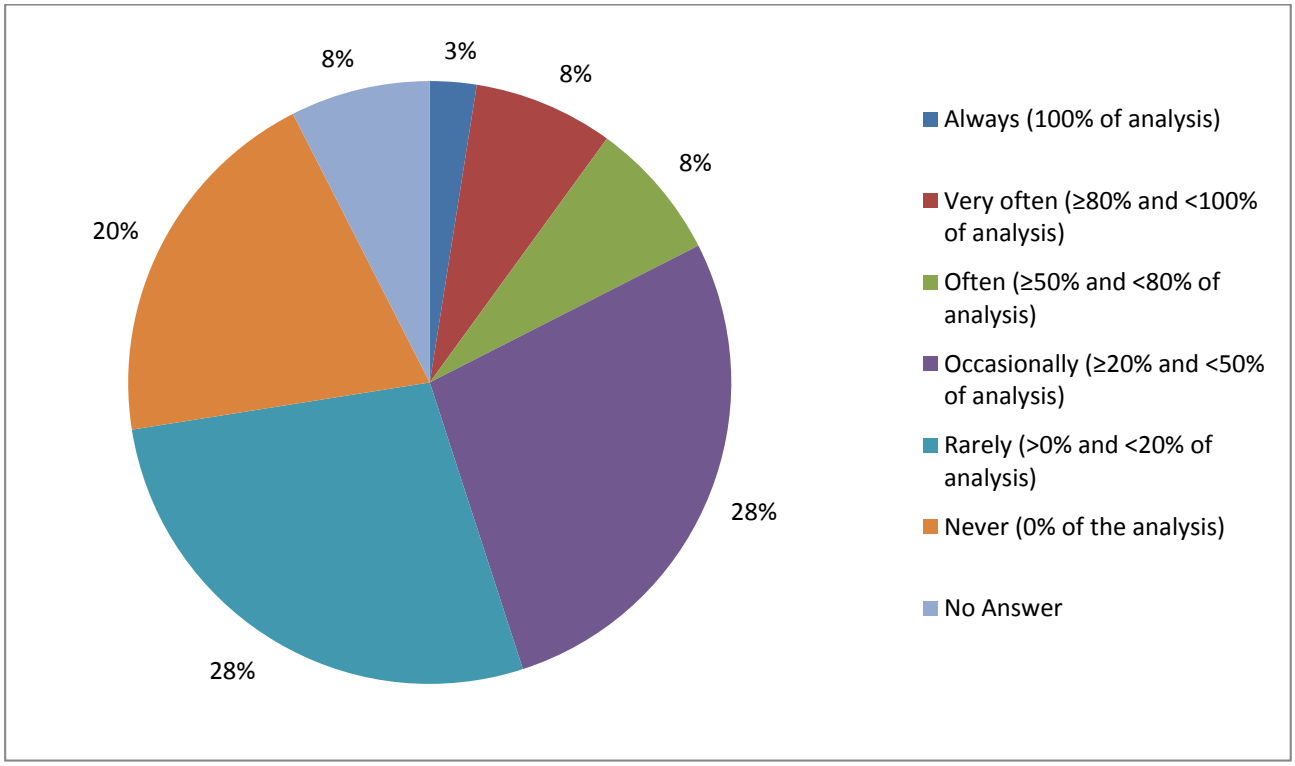
7. Results about EU Decision Supporting Tools

The pie charts below present data regarding the extent of use of the JRC GMO-Matrix and Event-finder selected in the section "C - EU Decision Supporting Tools" (Figure 5).

7.1 JRC GMO-Matrix

Figure 20 indicates that close to half of the respondents (47 %) use the JRC GMO-Matrix tool. In particular, a significant number of laboratories (28 %) declared to employ it occasionally for their analyses while a smaller number (19 %) selected the "always", "very often" or "often" options of use.

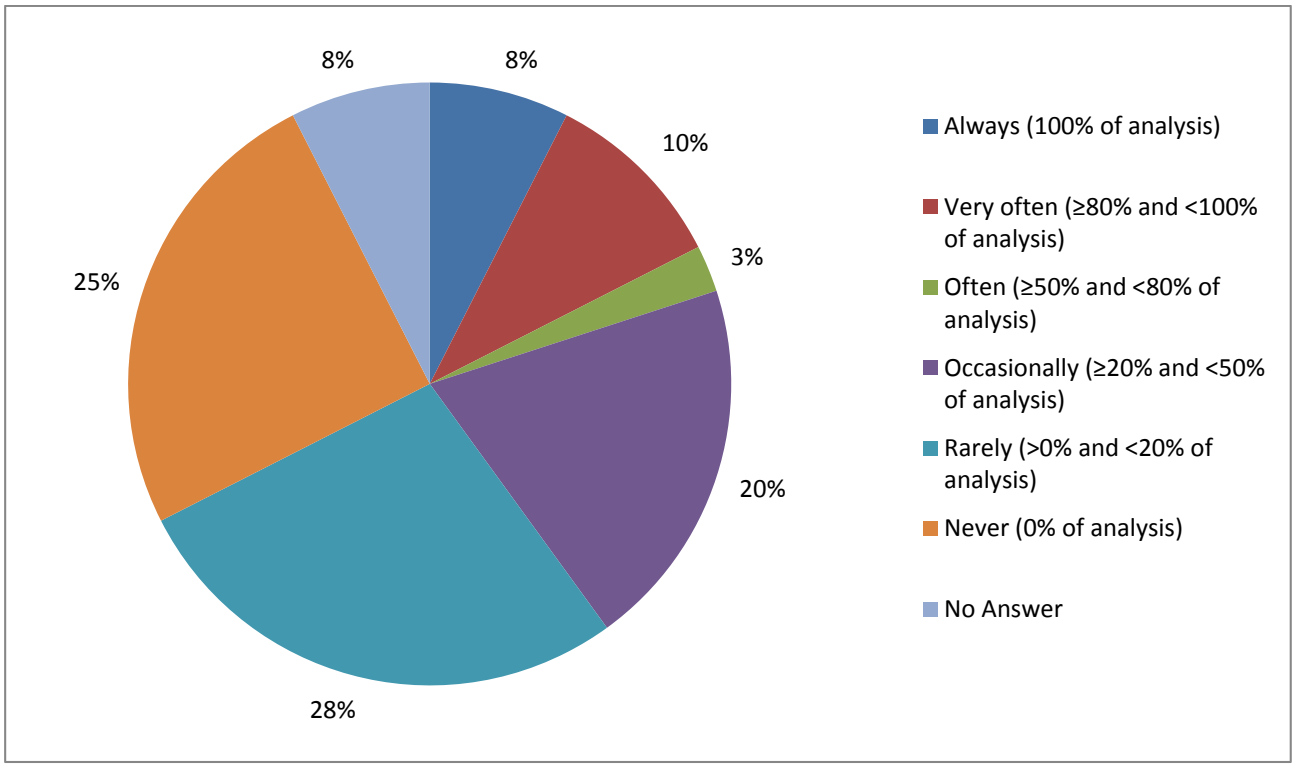
Figure 20 –Extent of Use of the JRC GMO-Matrix Tool



7.2 JRC Event Finder

Similar results are portrayed in Figure 21 for the use of the JRC Event finder tool. A significant number of laboratories (20 %) declared to employ it occasionally while an almost equal number (21 %) selected the "always", "very often" or "often" options of use.

Figure 21 –Extent of Use of the JRC Event Finder Tool



8. Conclusions

One of the most remarkable results of the survey is that almost all official control laboratories (98 %) are using event-specific EU reference methods for performing quantitative analysis of GMOs. A similar survey performed in 2005 ([12]) revealed that at that time only about half of the methods used for detection of soybean event GTS 40-3-2 and maize events Bt176, Bt11, T25 and GA21 were validated in a collaborative study and that testing for many other GM events then authorized in the EU (i.e., oilseed rape GT73, MS8, RF3, Falcon GS/40/90pHoe6/Ac, Cotton MON1445, MON531 and maize TC1507) was performed exclusively with methods validated *in-house*. It should be noticed that Regulation (EC) 1829/2003 entered into force in 2004 and introduced the requirement for the applicant to provide a quantitative event-specific method to be validated by the EURL GMFF with the support of the ENGL. For quantitative purposes only a small number of laboratories were using construct-specific (13 %) and element-specific (18 %) EU reference methods. These low numbers do not necessarily indicate the use of alternative methods, but may be due to a limited use of non-specific genetic targets for quantitative analysis. The data collected about non-EU reference methods confirms these findings. Indeed none or 5 % of the respondents declared to employ non-EU reference methods for element- or construct-specific quantitative analysis, respectively. The outcome of the 2015 survey reveals therefore that the combined efforts of the EURL GMFF and ENGL have been successful for enhancing harmonisation in quantitative GMO analysis by the adoption of scientific and technical approaches. This achievement allows the consistency of results for GM labelling and an equal-level playing field in the EU.

For qualitative assays the survey indicates that a significant but lower number of respondents (55 % and 40 %) use element-specific and construct-specific EU reference methods, respectively. This data is consistent with the findings about non-EU reference methods where a significant number of respondents (38 %) reported to use element-specific non-EU reference methods for qualitative purposes and a smaller proportion (20 %) declared to employ construct-specific non-EU reference methods. Those methods are generally applied for initial screening purposes and are mainly validated *in-house* and to a lesser extent in national collaborative studies.
This may reflect the needs of laboratories facing rapid alert emergencies of quickly implementing analytical strategies for detecting non-authorised GM events. Indeed genetically modified crops have continued to increase globally, both in terms of approval status and event/trait diversification. Detection methods for those new genetic elements are generally not yet validated in collaborative studies and therefore not available as EU-reference methods.

Current efforts by the EURL GMFF in providing ready-to-use multi-target tools, both for screening and identification by means of pre-spotted plates (PSPs) should support the desirable harmonisation also for GMO screening, given that all methods enabled by the plates are:

- EU reference methods included in the GMOMETHODS database
- Running in accordance to the same, not modifyable protocol
- Ensuring that also the screening strategies, i.e. the set of methods regularly employed for screening, are the same if the same plates are used.

Only one of the non-EU reference methods used by the respondents was found to be internationally validated in a collaborative study. This suggests that the GMOMETHODS database is providing a quite comprehensive and updated catalogue of methods that have been validated according to international standards.

Survey data regarding the extent of use of the JRC GMO-Matrix and Event-finder decision supporting tools indicate that close to half of the respondents employ them at different levels for GMO analysis. A significant part of the laboratories (19 %-21 %) even declared to apply them often, very often or always. Given the fact that the JRC GMO-Matrix and Event finder tools had been made available by the EURL GMFF just a few months before launching of the survey and that the hit rate of the internet applications are quite high, it can be assumed that these figures have already increased until today. The tools are particularly useful if employed in connection with the JRC pre-spotted plates screening (PSPs) and their use may remarkably grow once official control laboratories start employing them for their GMO analysis.

The survey also confirms that the majority of the protocols for quantitative GMO detection are adapted by the laboratories to their laboratory needs. A significant level of alteration is even observed for qualitative protocols although to a variable extent depending on the specificity of the analysis.

More than 30 % of the modifications implemented in the protocols concern the master mix of the PCR reaction, presumably for minimising the number of different master mixes used by a laboratory. The reaction volume has the second highest alteration rate for all quantitative assays, probably for adapting older methods to the technological developments of the PCR equipment or for managing limited DNA availability. Also other reported alterations can be attributed to the increasing number of GMOs entering the global market and forcing control laboratories to streamline their analytical approaches. Therefore, master mixes, reaction volumes and amplification reaction conditions have been harmonised, in some cases, for all the analyses performed in the same laboratory.

A similar approach has been followed by the EURL GMFF for developing its pre-spotted-plates where the reaction conditions of all the validated methods combined on one plate have been fully harmonised. This is possible provided that the method performance remains within the boundaries defined in the document "ENGL Method Performance Requirements" (see http://gmo-crl.jrc.ec.europa.eu/doc/MPR%20Report%20Application%2020_10_2015.pdf). In the EU, official control laboratories are accredited under ISO/IEC 17025 for the methods they are using. This accreditation requires demonstrating the capacity to correctly apply the accredited methods. In other words the method, as applied by the laboratory, must meet the Method Performance Requirements (MPR) established by the ENGL. Hence, it can be concluded that the method adaptations carried out by the laboratories remain at a level where the proper functioning of the methods is not jeopardised. However, as a consequence of the survey, and in order to provide guidance to the laboratory for correctly adapting methods to their laboratory-specific situation and/or technological progress, the ENGL has set-up a working group on updating methods and another one on supporting the transfer of methods from real-time PCR to digital PCR platforms. Both groups have taken note and benefited from the results of this survey.

The survey also suggests that a wide use of standardised pre-spotted plates in routine control would further contribute to harmonisation of GMO testing throughout the EU, particularly with regard to screening.

9. Acknowledgments

We are grateful to Mrs. Ilaria Ciabatti and Mr. Hendrik Emons for carefully reading the manuscript and for helpful comments. We thank also the members of the laboratories that participated to the survey and made possible the analysis at the EU level on the use of EU Reference Methods and JRC decision tools.

10. References

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11. Annex 1: EU Reference Methods

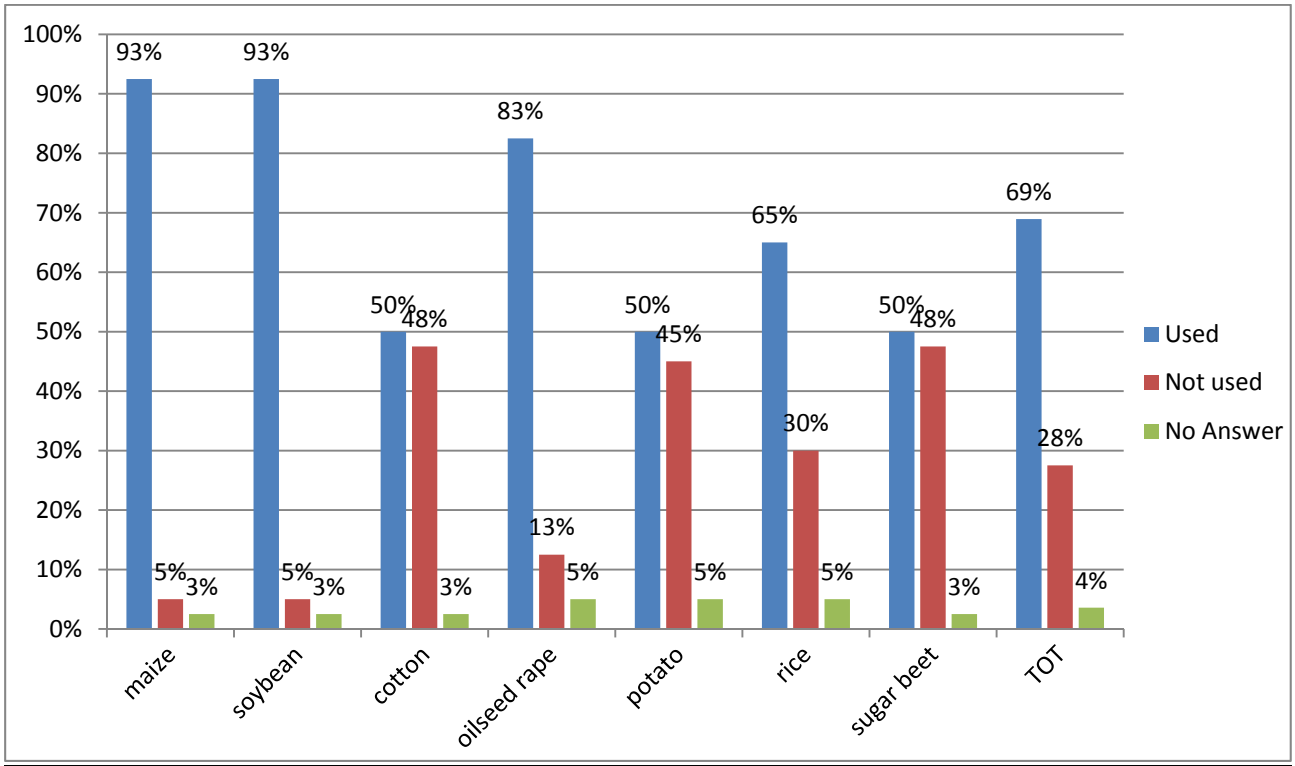
Results on EU Reference Methods have been arranged for reflecting the main objectives of the survey. The first section presents charts with statistical analyses on the use of EU reference methods for quantitative and qualitative purposes. The following section provides an overview on the rate of modification (number of modified methods over the total number of methods). In the last section the type and frequency of modifications implemented by laboratories are displayed for the same method categories. The absolute values corresponding to the number of respondents using the listed methods are displayed in the tables below the charts. Data collected for quantitative and qualitative analyses are divided as in the survey in event-specific, construct-specific, element-specific, taxon-specific and plant-specific parts. Event-specific and taxon- specific section are further subdivided in species parts.

11.1 Use of EU Reference Methods

The charts in this section provide data regarding the "use/not use" of the EU reference methods according to GM species, GM events, genetic targets or crops. The bars in the charts represent the percentage of use, not use or no reply by respondents for each method's classification.

a) Quantitative Event-specific Methods

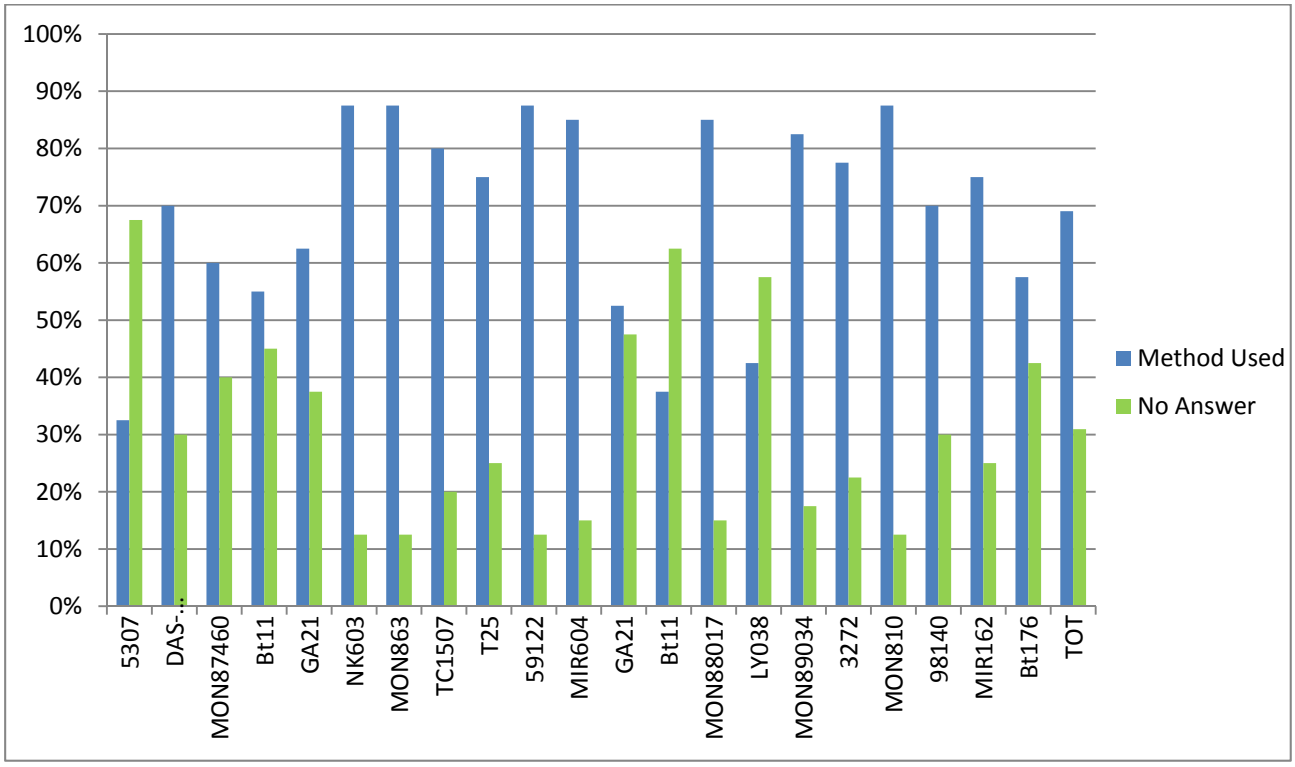
Figure 22 - Percentage of Respondents using Quantitative Event-specific Methods According to GM Species



QT EVE Methods	maize	soybean	cotton	oilseed rape	potato	rice	sugar beet	TOT
Used	37	37	20	33	20	26	20	193
Not used	2	2	19	5	18	12	19	77
No Answer	1	1	1	2	2	2	1	10
TOT Answer	40	40	40	40	40	40	40	280

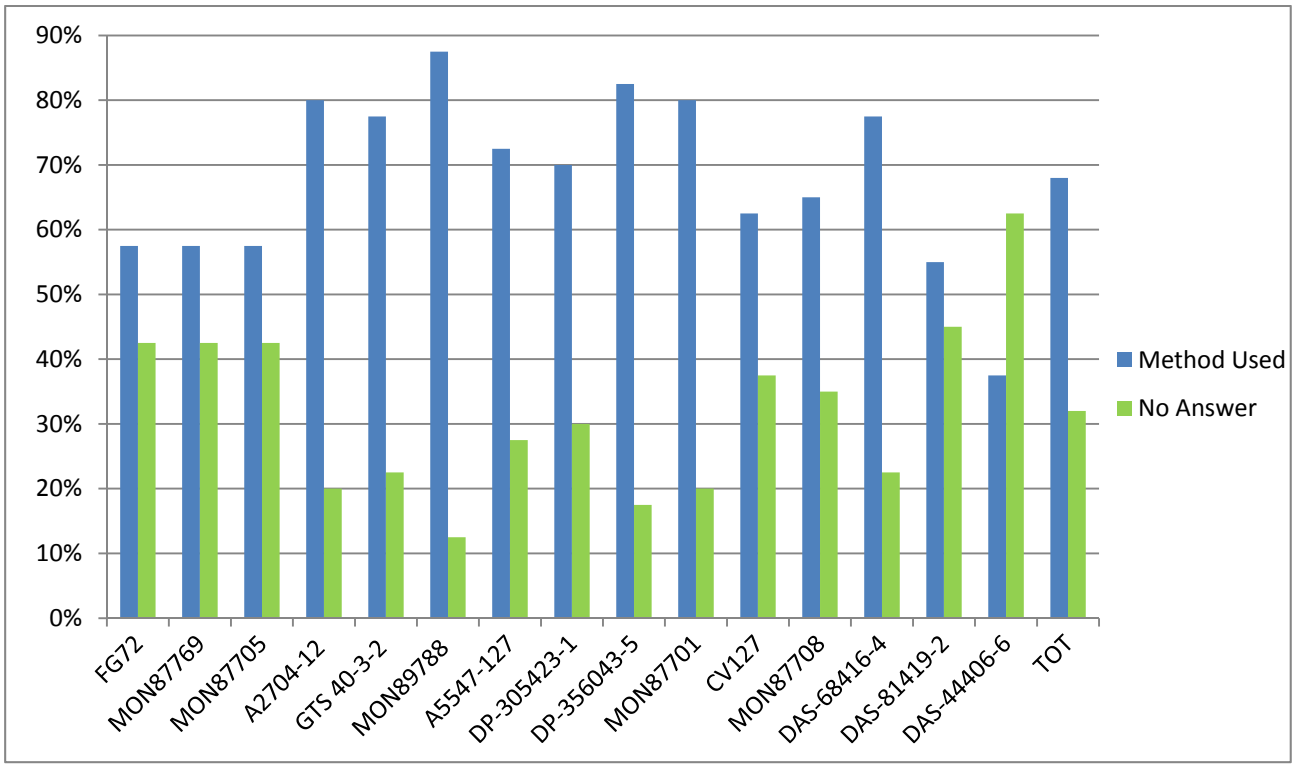
Legend: QT = Quantitative; EVE = Event-specific methods; TOT = Percentages for all quantitative event-specific methods

Figure 23 - Percentage of Respondents using Quantitative Event-specific Methods According to Maize GM lines



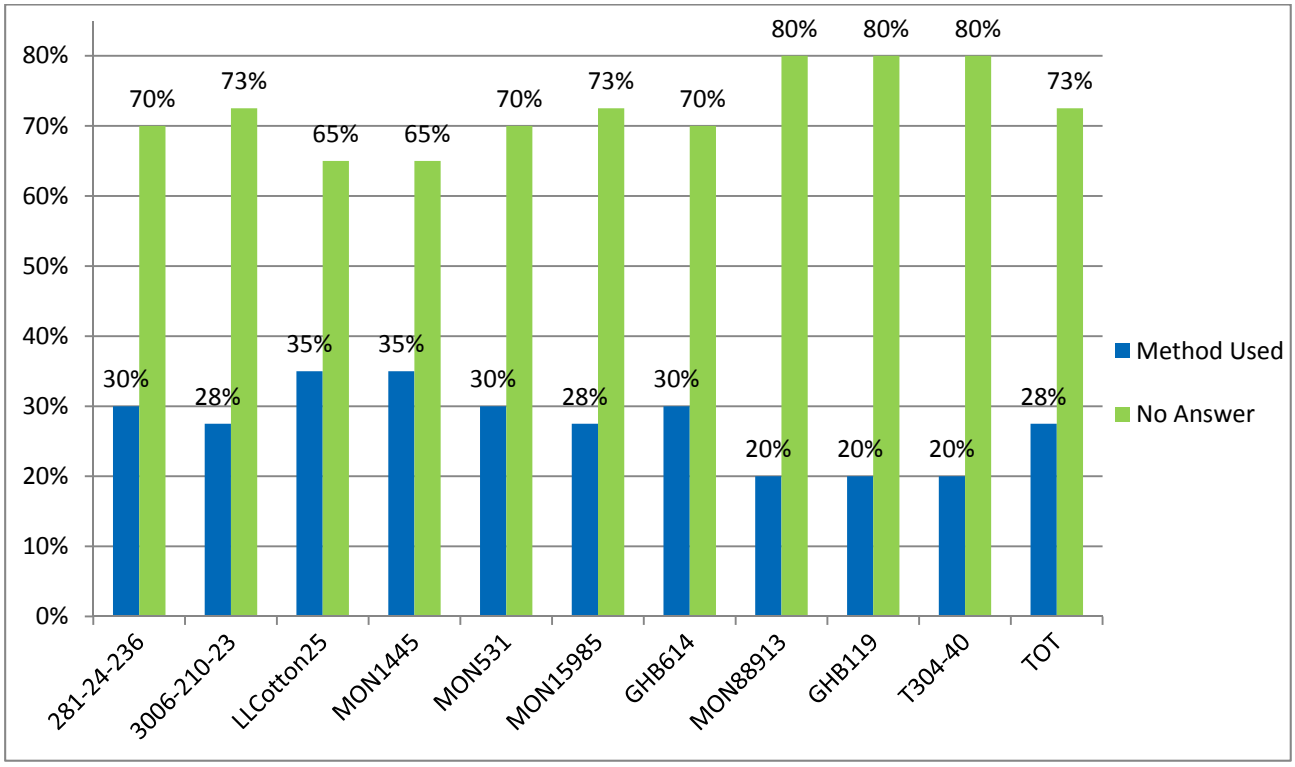
Legend: TOT = Percentage of all quantitative event-specific maize methods

Figure 24 - Percentage of Respondents using Quantitative Event-specific Methods According to Soybean GM lines



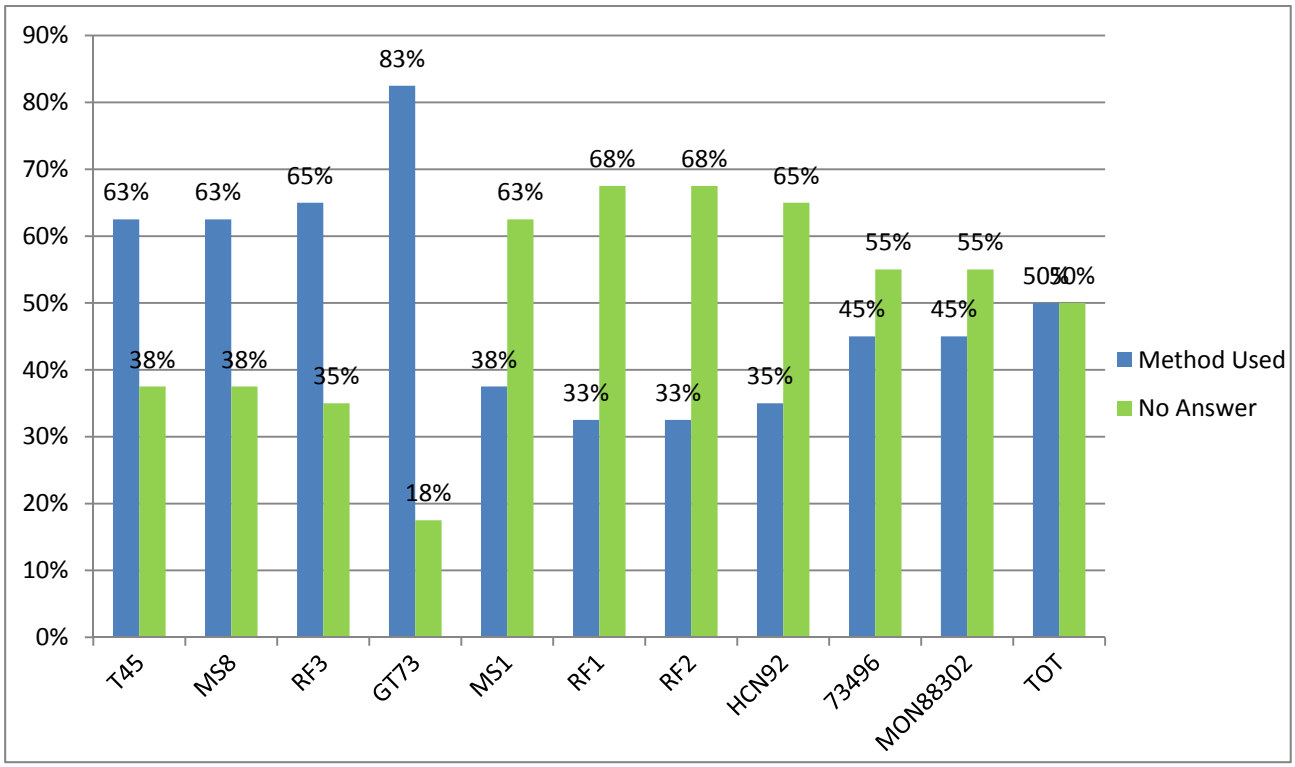
Legend: TOT = Percentages for all quantitative event-specific soybean methods

Figure 25 - Percentage of Respondents using Quantitative Event-specific Methods According to Cotton GM lines



Legend: TOT = Percentages for all quantitative event-specific cotton methods

Figure 26 - Percentage of Respondents using Quantitative Event-specific Methods According to Oilseed Rape GM lines



Legend: TOT = Percentages for all quantitative event-specific oilseed rape methods

Figure 27 - Percentage of Respondents using Quantitative Event-specific Methods According to Potato GM lines

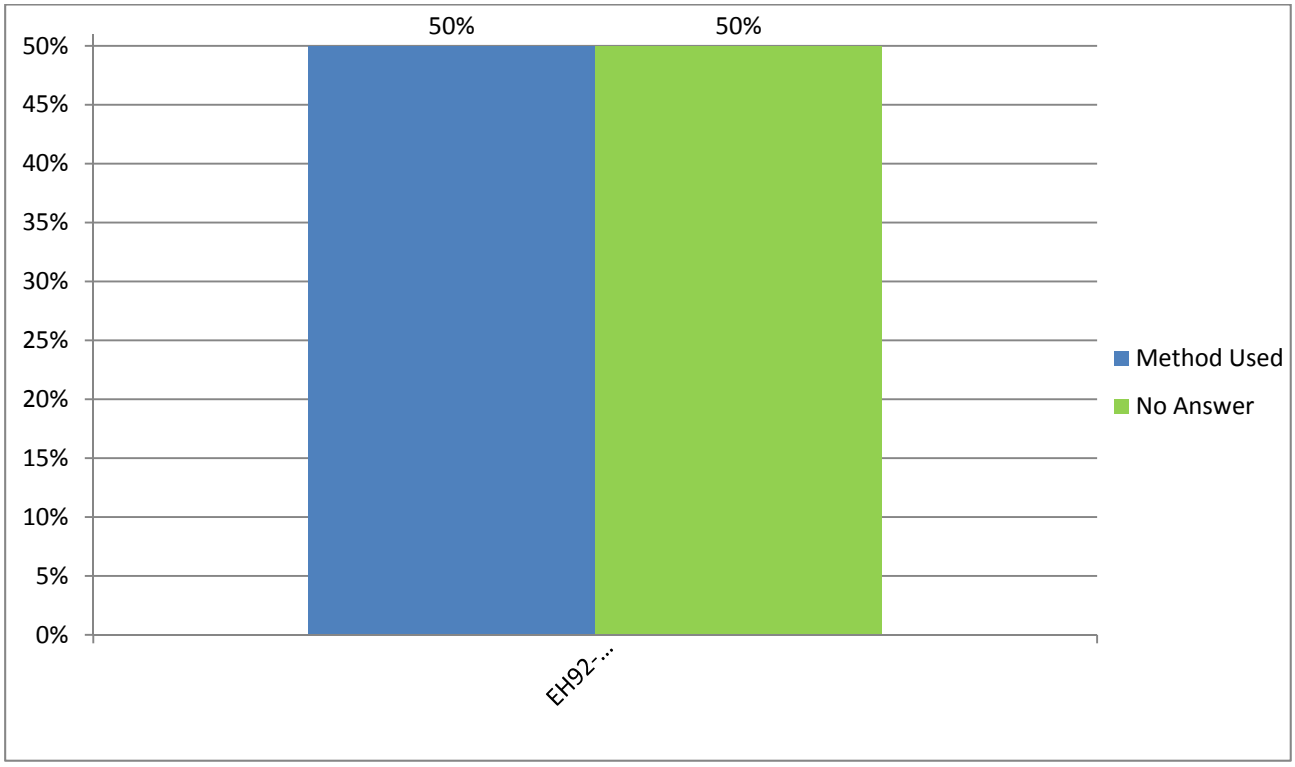


Figure 28 - Percentage of Respondents using Quantitative Event-specific Methods According to Rice GM lines

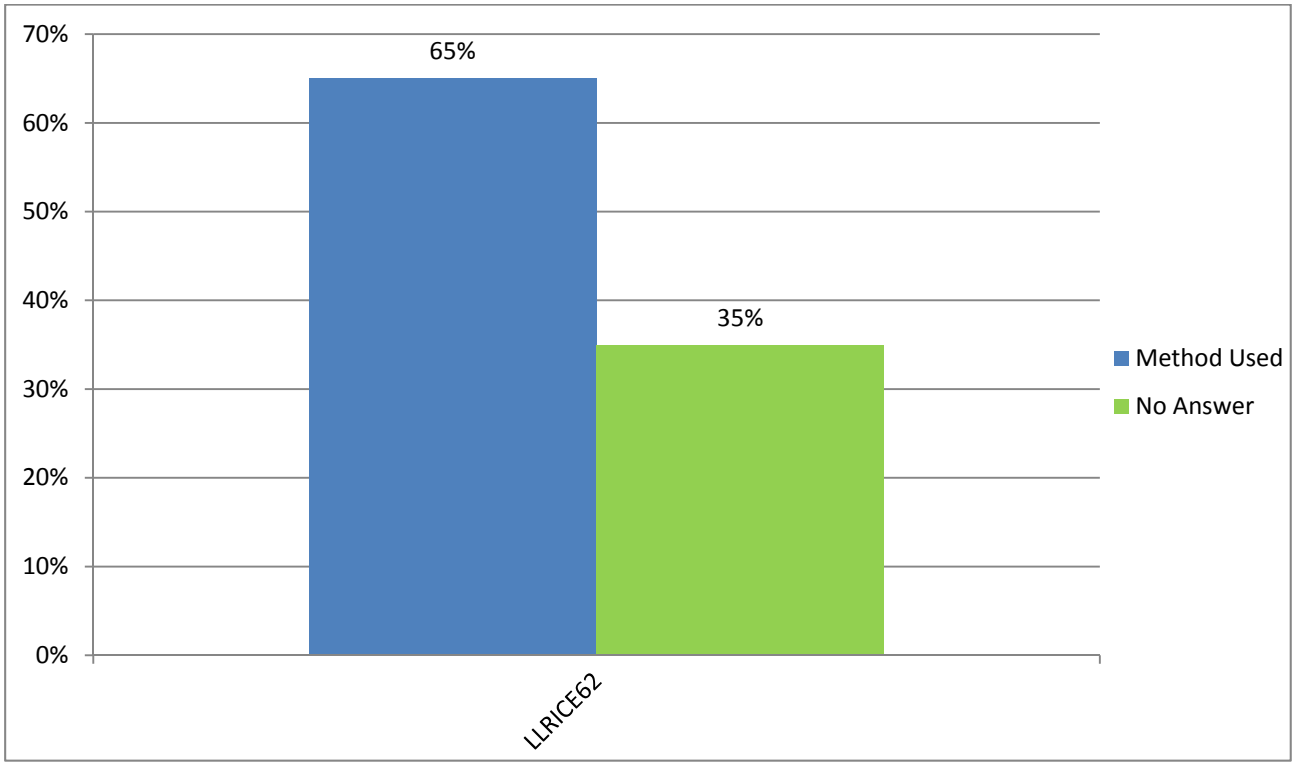
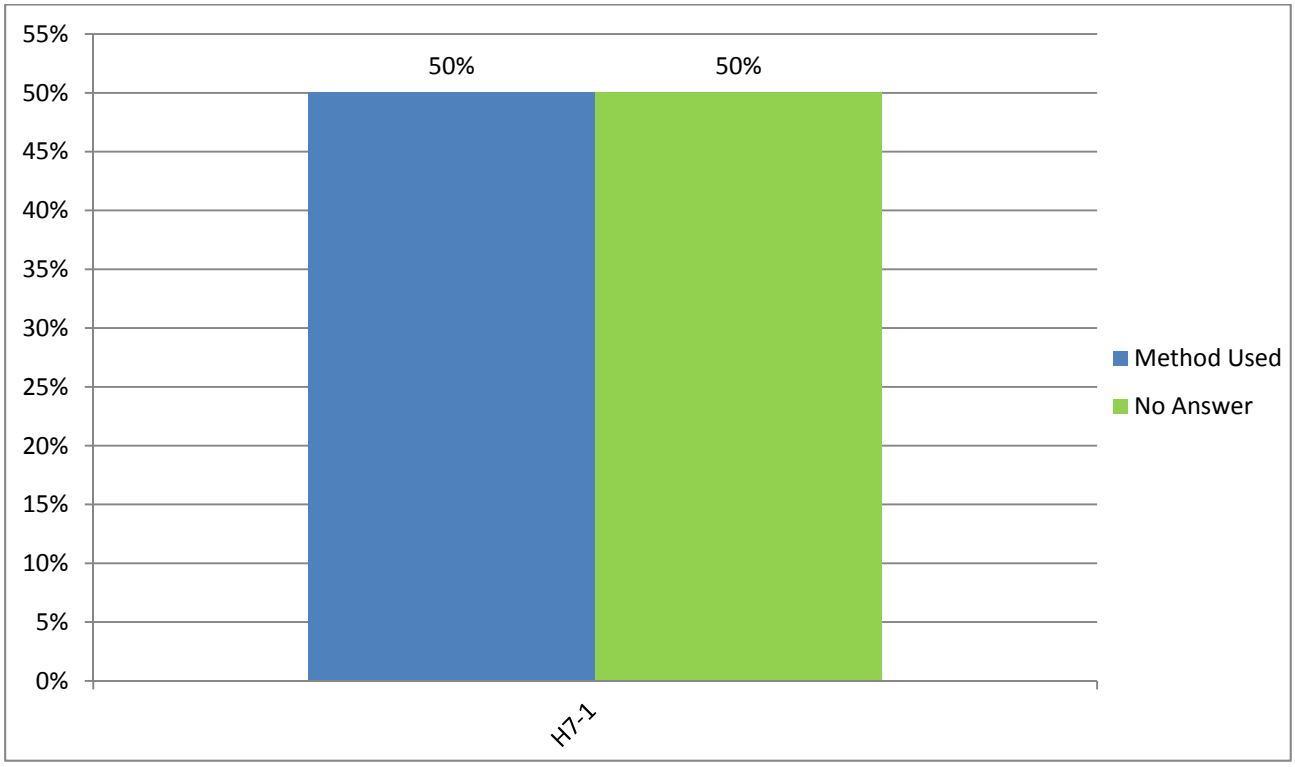
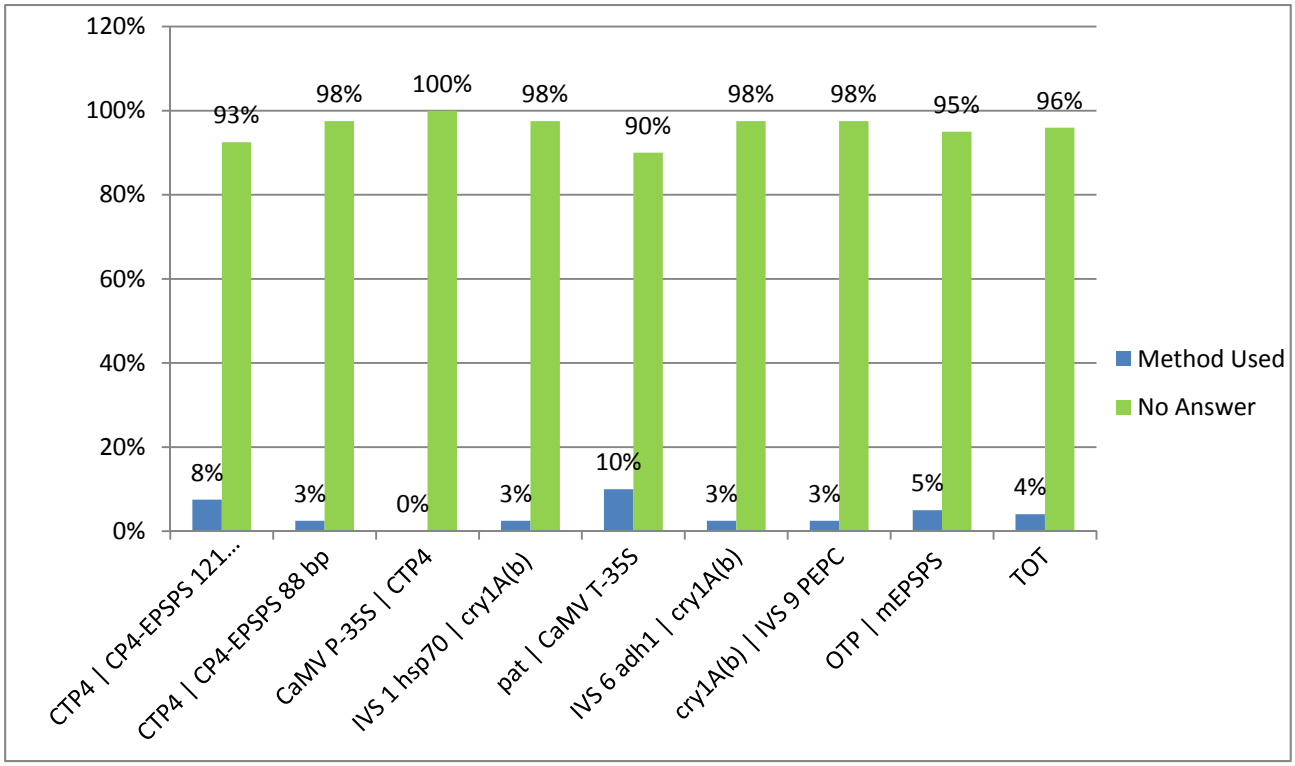


Figure 29 - Percentage of Respondents using Quantitative Event-specific Methods According to Sugar Beet GM lines



b) Quantitative Construct-specific Methods

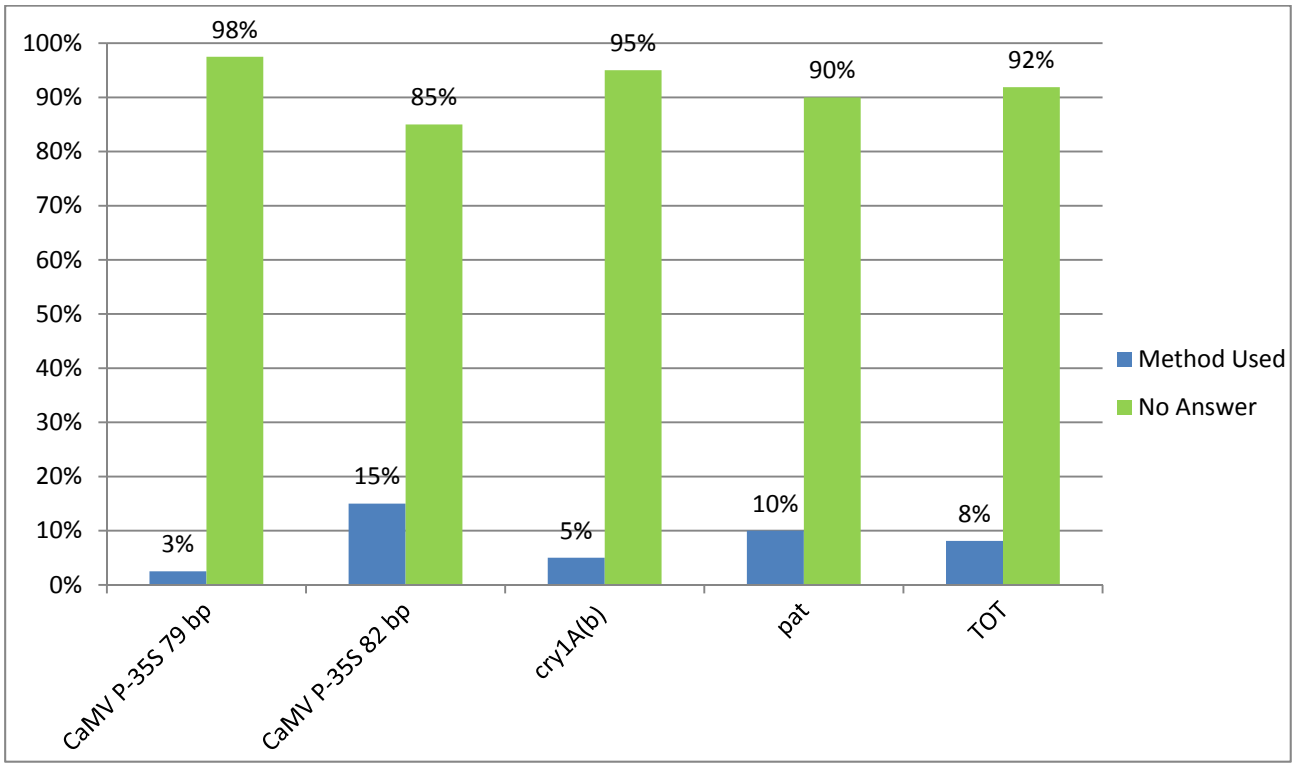
Figure 30 - Percentage of Respondents using Quantitative Construct-specific Methods According to Genetic Targets



Legend: CTP4 = Chloplast transit peptide coding sequence from *Petunia hybrida*; CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; IVS 1 hsp70 = Intervening sequence 1 from *Zea mays hsp70* gene; cry1A(b) = *cry1Ab* delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes*; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; IVS 6 adh1 = Intervening sequence 6 from *Zea mays* alcohol dehydrogenase 1 gene; IVS 9 PEPC = Intervening sequence 9 from the phospho-enol-pyruvate carboxylase gene; OTP = Optimized transit peptide sequence from *Zea mays*; mEPSPS = Point mutated epsps gene from *Zea mays*; TOT = Percentages for all quantitative construct-specific methods

c) Quantitative Element-specific Methods

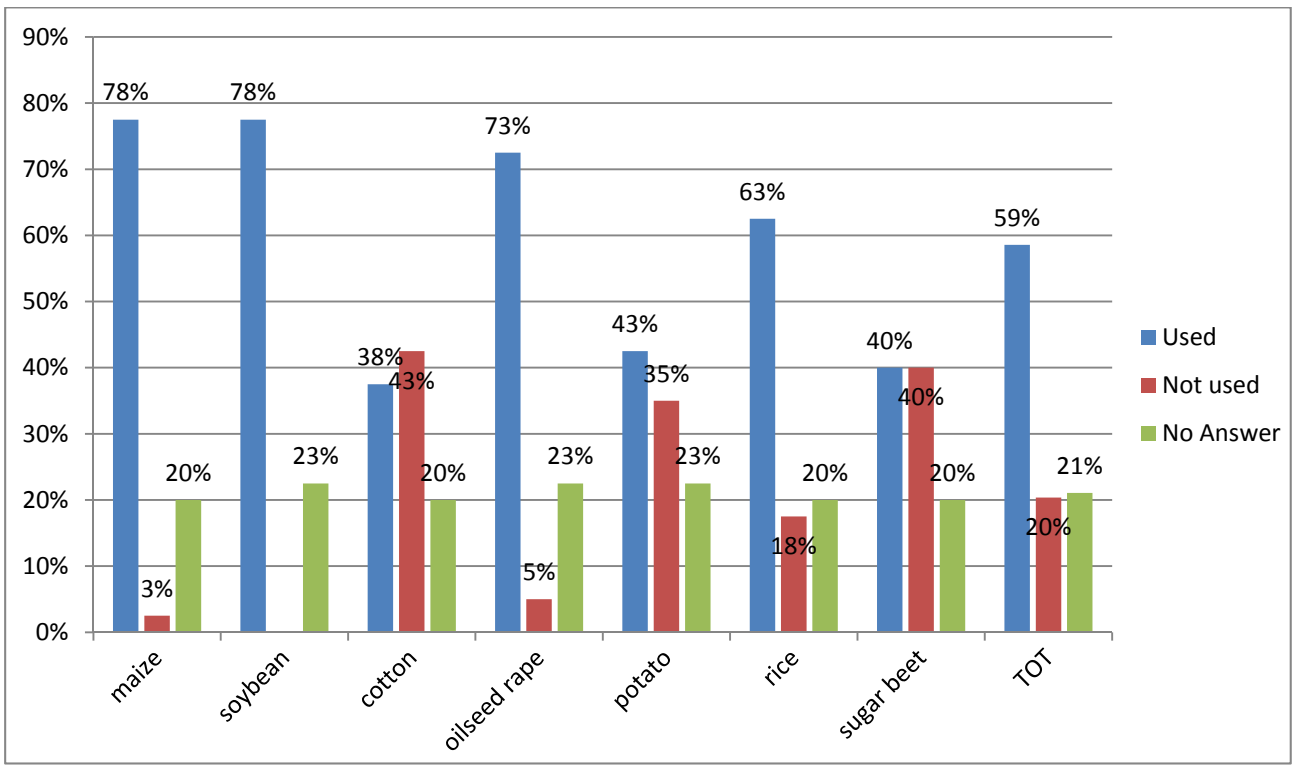
Figure 31 - Percentage of Respondents using Quantitative Element-specific Methods According to Genetic Targets



Legend: CaMV P-35S = *Cauliflower Mosaic Virus* 35S Promoter; cry1A (b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes;* TOT = Percentages for all quantitative element-specific methods

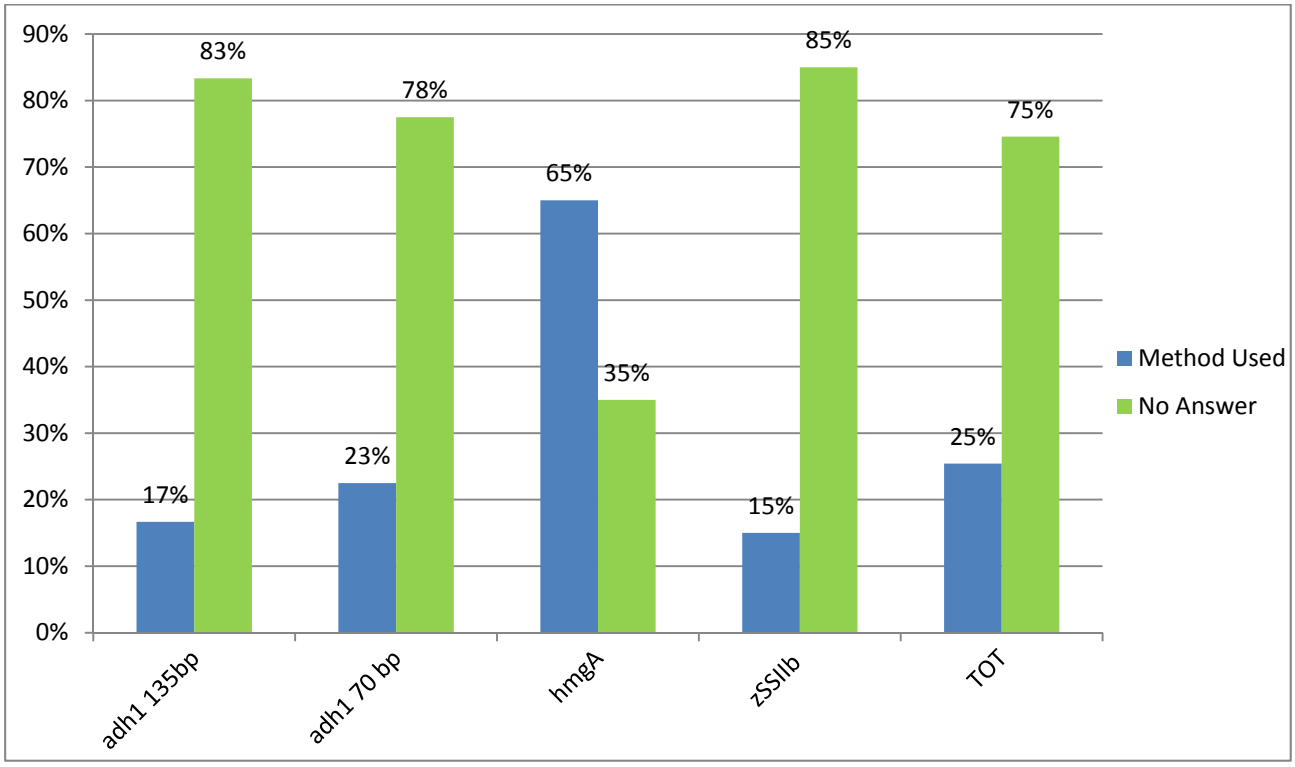
d) Quantitative Taxon-specific Methods

Figure 32 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Species



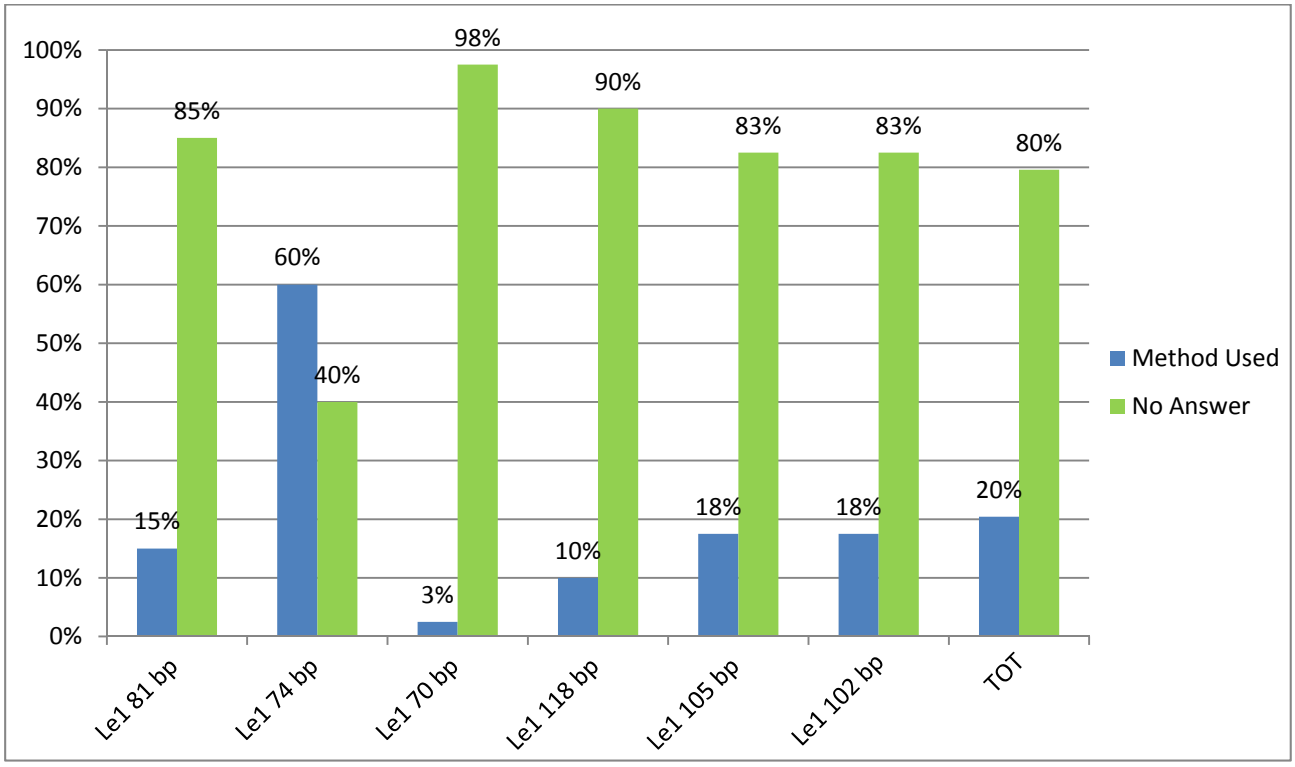
Legend: TOT = Percentages for all quantitative taxon-specific methods

Figure 33 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Maize Reference Genes



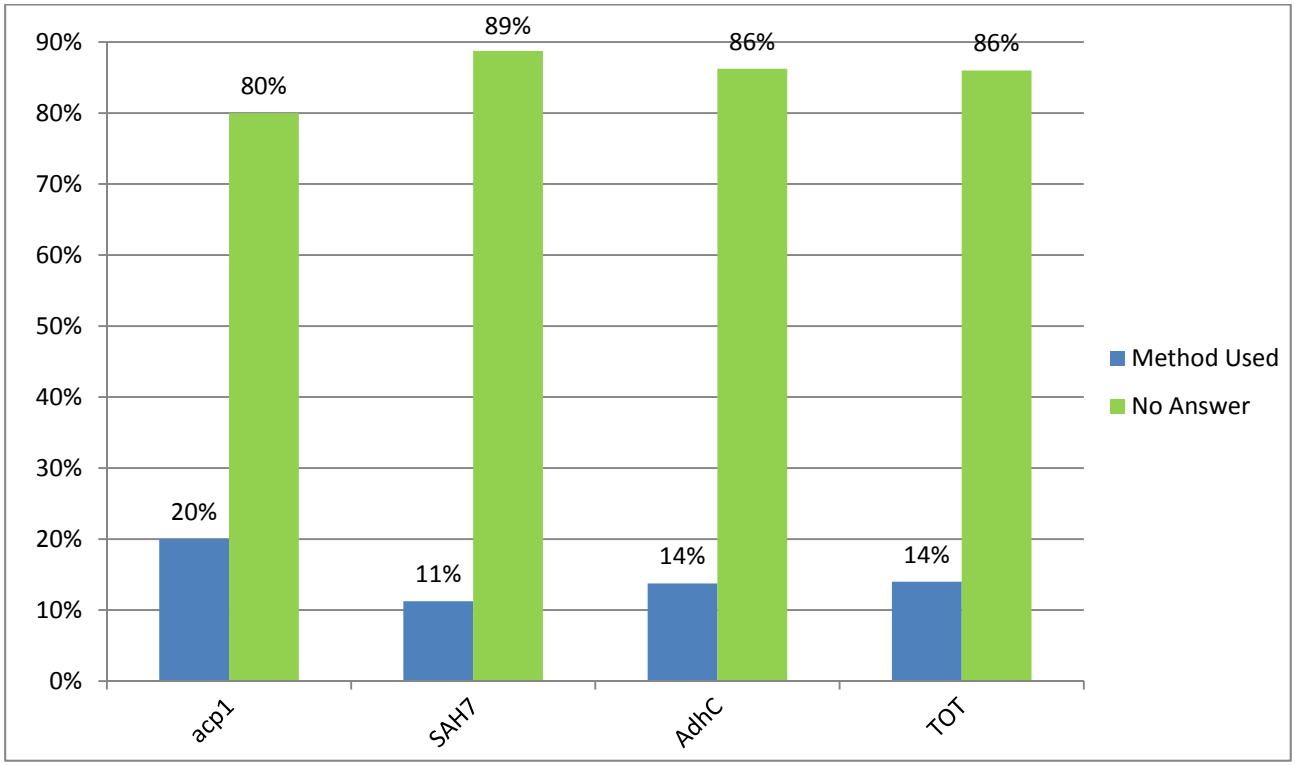
Legend: adh1 = Alcohol dehydrogenase1 gene; hmgA = High-mobility-group A gene; zSSIIb = Maize starch synthase IIb gene; TOT = Percentages for all quantitative taxon-specific maize methods

Figure 34 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Soybean Reference Genes



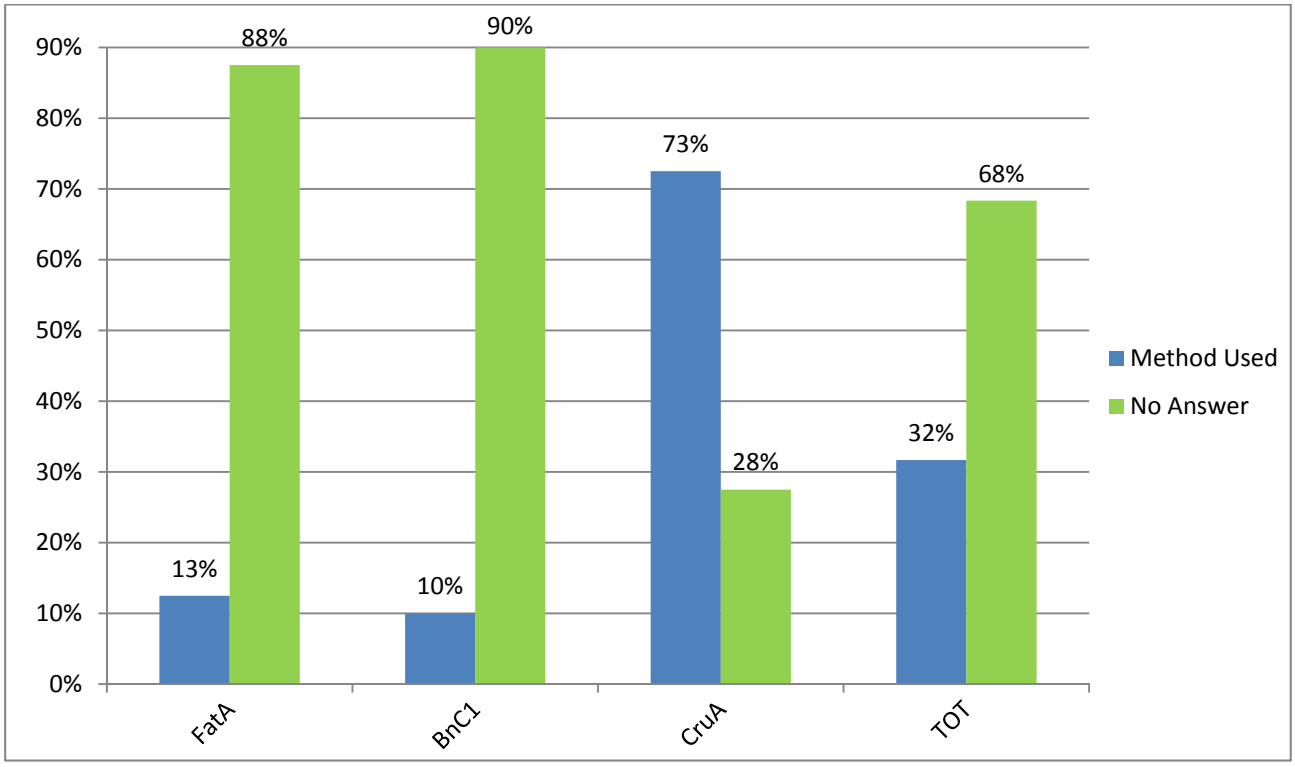
Legend: Le1 = Lectin gene; TOT = Percentages for all quantitative taxon-specific soybean methods

Figure 35 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Cotton Reference Genes



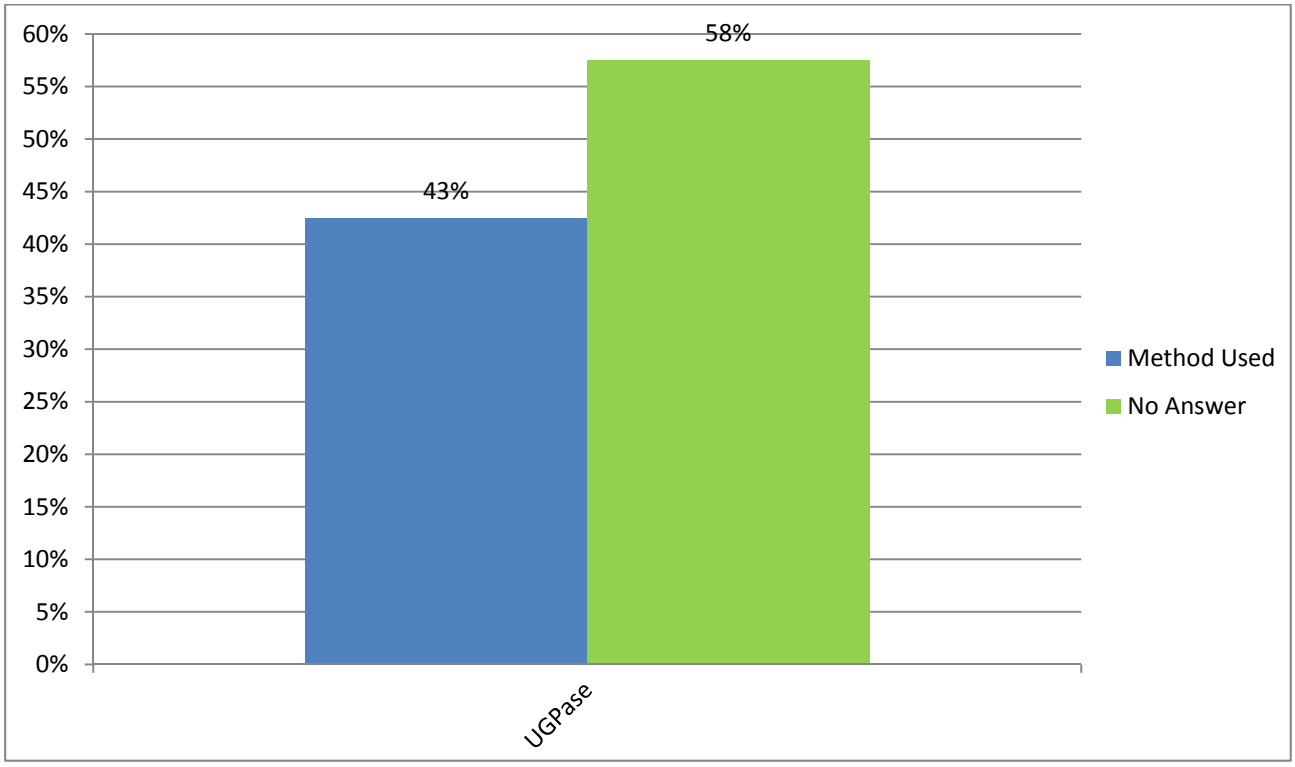
Legend: acp1 = Acyl carrier protein 1 gene; SAH7 = IVS of the putative *Sinapis Arabidopsis Homolog 7* protein gene; AdhC = Alcohol dehydrogenase C gene; TOT = Percentages for all quantitative taxon-specific cotton methods

Figure 36 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Oilseed Rape Reference Genes



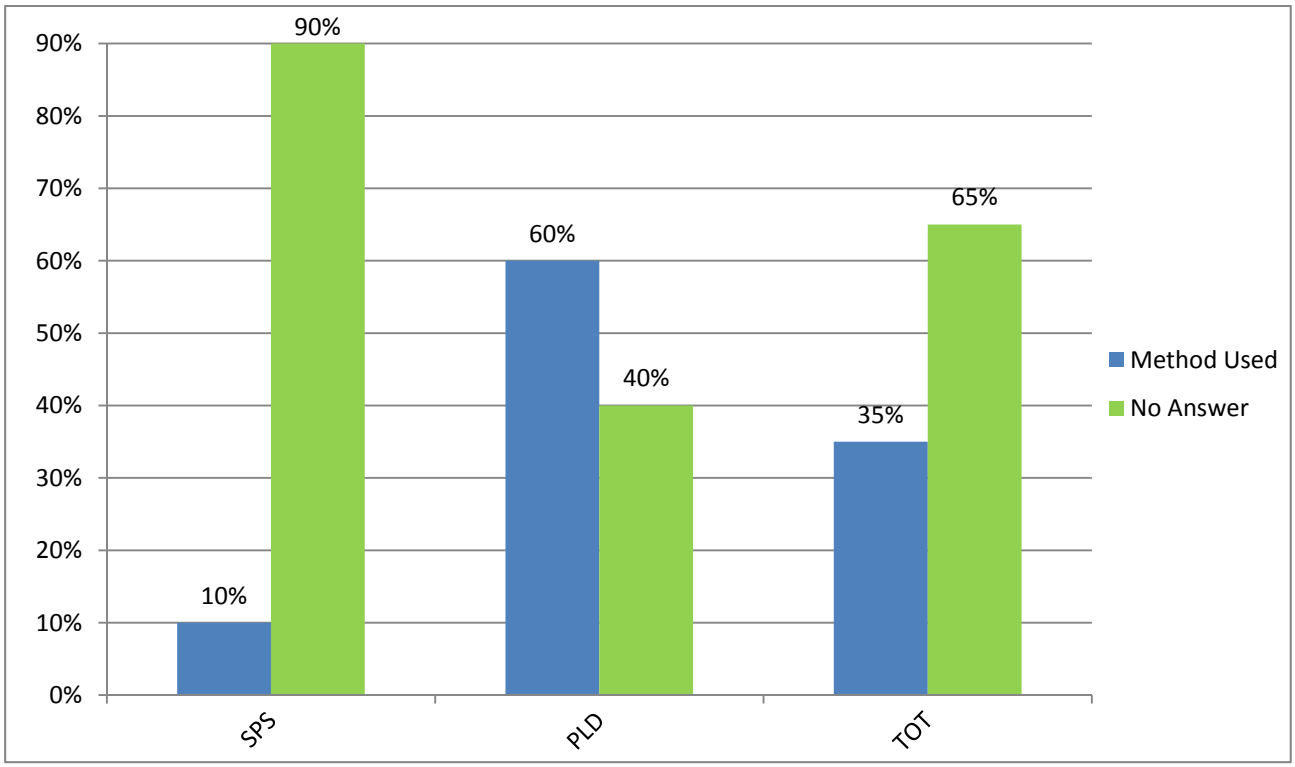
Legend: FatA = Acyl-ACP thioesterase gene; BnC1 = Cruciferin storage protein gene; CruA = Cruciferin A gene; TOT = Percentages for all quantitative taxon-specific oilseed rape methods

Figure 37 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Potato Reference Genes



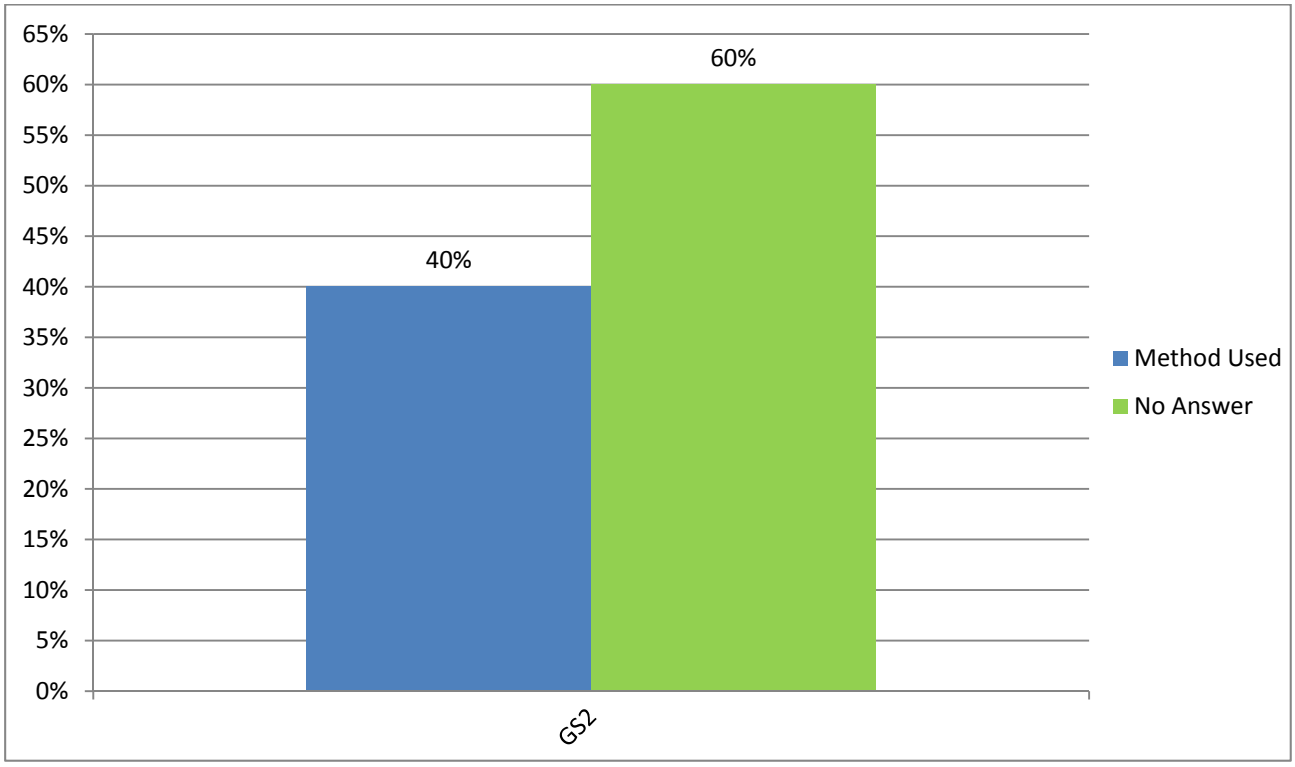
Legend: UGPase = UDP-glucose pyrophosphorylase gene

Figure 38 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Rice Reference Genes



Legend: SPS = Sucrose-phosphate synthase gene; PLD = Phospholipase D gene TOT = Percentages for all quantitative taxon-specific rice methods

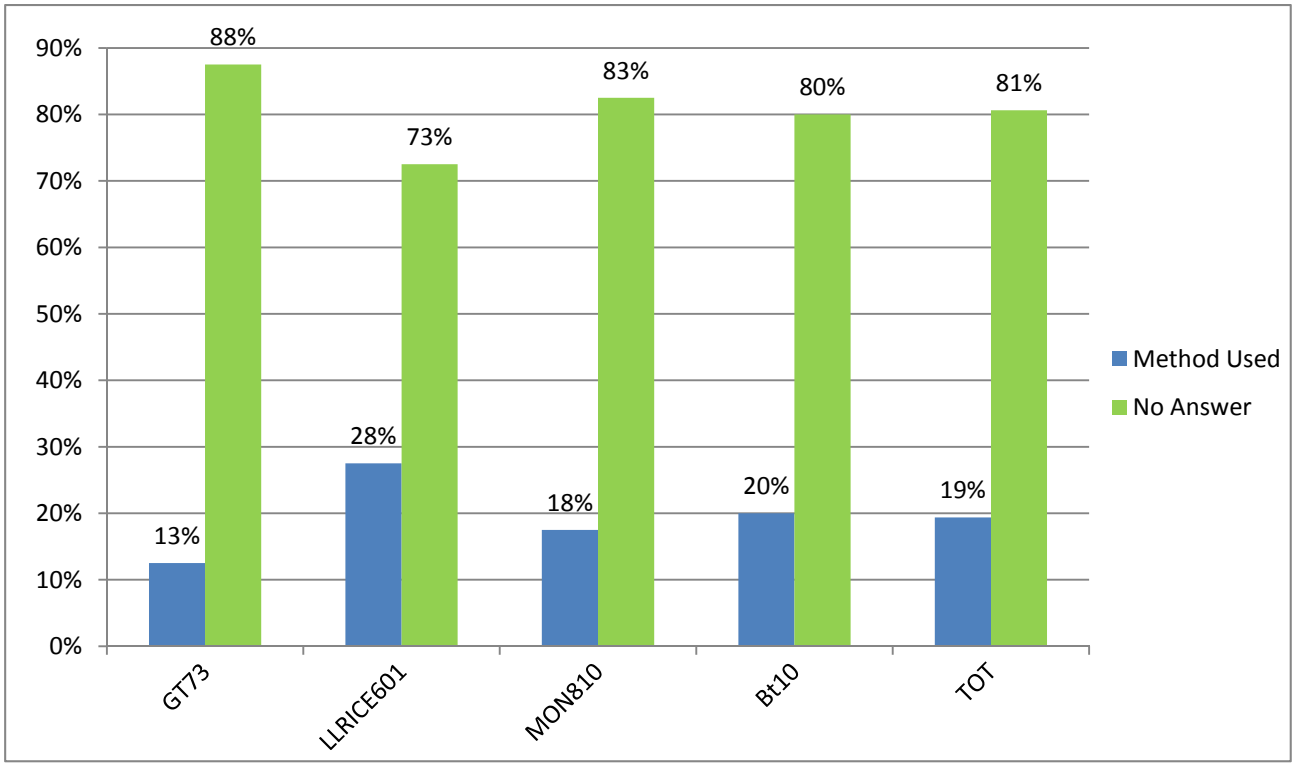
Figure 39 - Percentage of Respondents using Quantitative Taxon-specific Methods According to Sugar Beet Reference Genes



Legend: GS2 = Glutamine synthetase gene

e) Qualitative Event-specific Methods

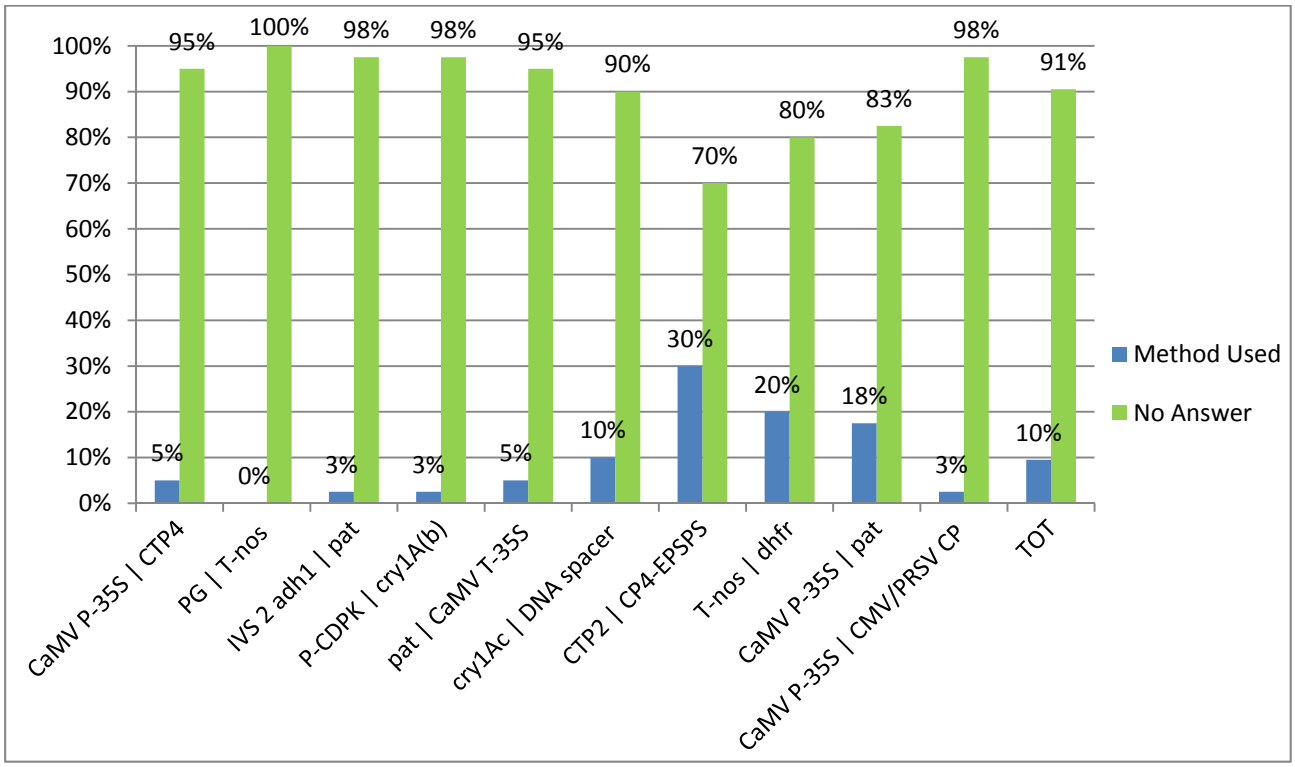
Figure 40 - Percentage of Respondents using Qualitative Event-specific Methods According to GMOs



Legend: TOT = Percentages for all qualitative event-specific methods

f) Qualitative Construct-specific Methods

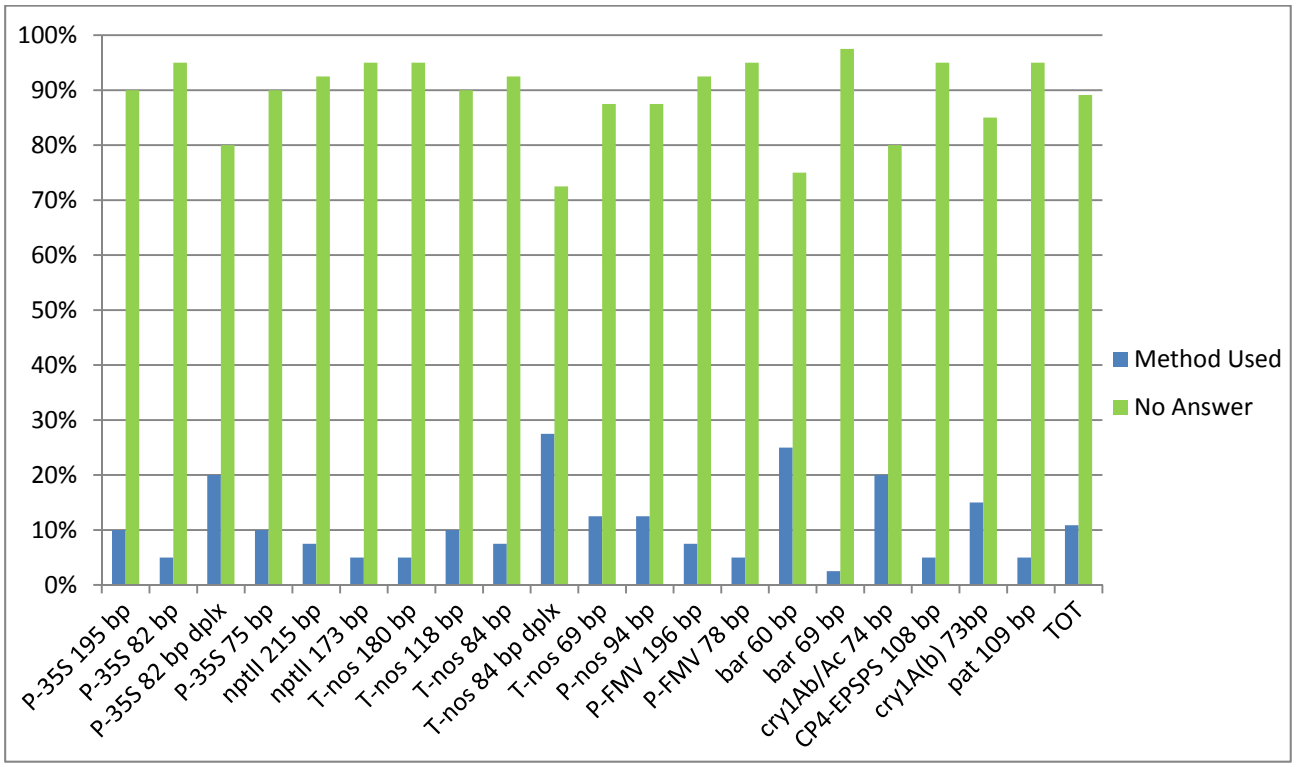
Figure 41 - Percentage of Respondents using Qualitative Construct-specific Methods According to Genetic Targets



Legend: CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; CTP4 = Chloroplast transit peptide coding sequence from *Petunia hybrida epsps* gene; PG = Polygalacturonase gene from *Solanum lycopersicum*; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; IVS 2 adh1 = Intervening sequence 2 from *Zea mays* alcohol dehydrogenase 1 gene; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* P-CDPK = Promoter of *Zea mays* calcium-dependent protein kinase gene; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; CTP2 = Chloroplast transit peptide 2 sequence from *Arabidopsis thaliana epsps* gene; CP4-EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; dhfr = Dihydrofolate reductase gene; CMV/PRSV CP = Chimeric *Cucumber mosaic virus* coat protein/*Papaya ring spot virus* coat protein; TOT = Percentages for all qualitative construct-specific methods

g) Qualitative Element-specific Methods

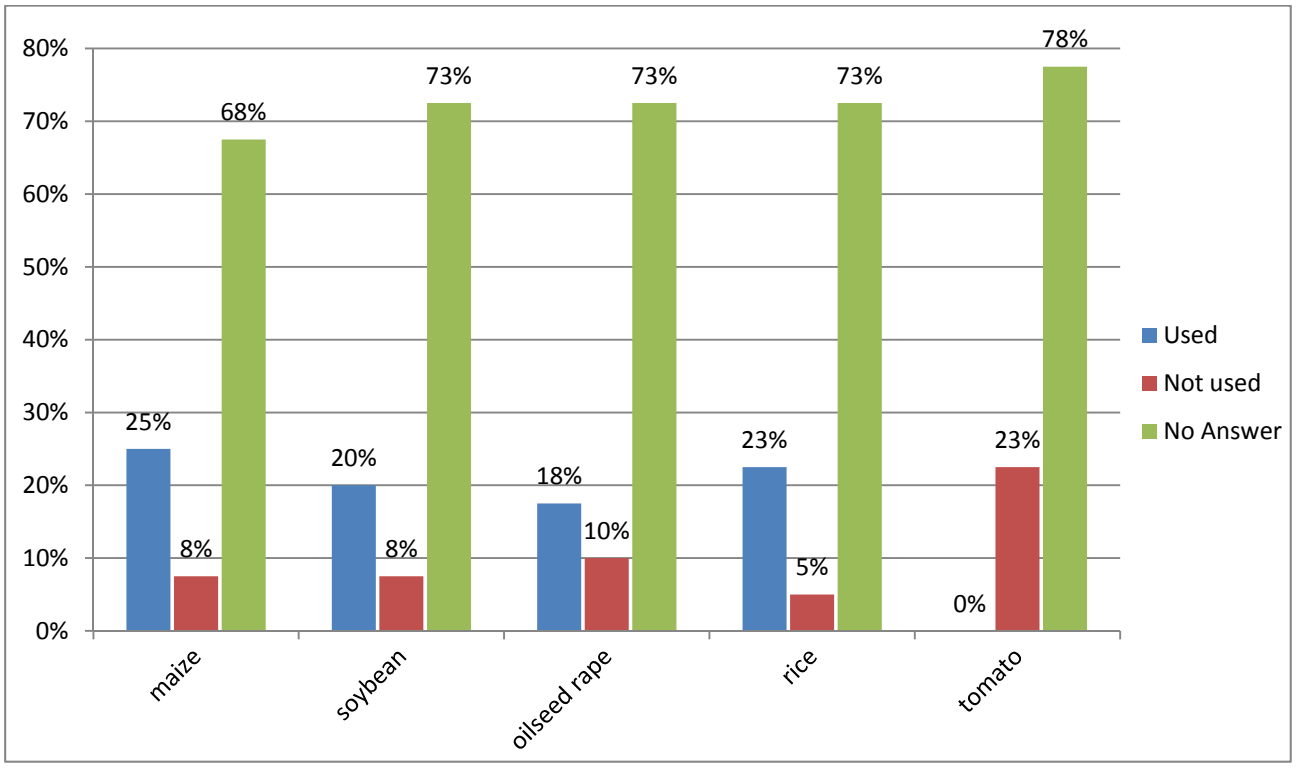
Figure 42 - Percentage of Respondents using Qualitative Element-specific Methods According to Genetic Targets



Legend: P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; nptII = Neomycin phosphotransferase II gene; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; P-nos = Nopaline synthase promoter from *Agrobacterium tumefaciens*; P-FMV = *Figwort Mosaic Virus* 35S promoter; bar = Phosphinothricin N-acetyl transferase gene from *Streptomyces hygroscopicus;* cryIAb/Ac = Synthetic construct derived from *Bacillus thuringiensis;* CP4-EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* TOT = Percentages for all qualitative element-specific methods

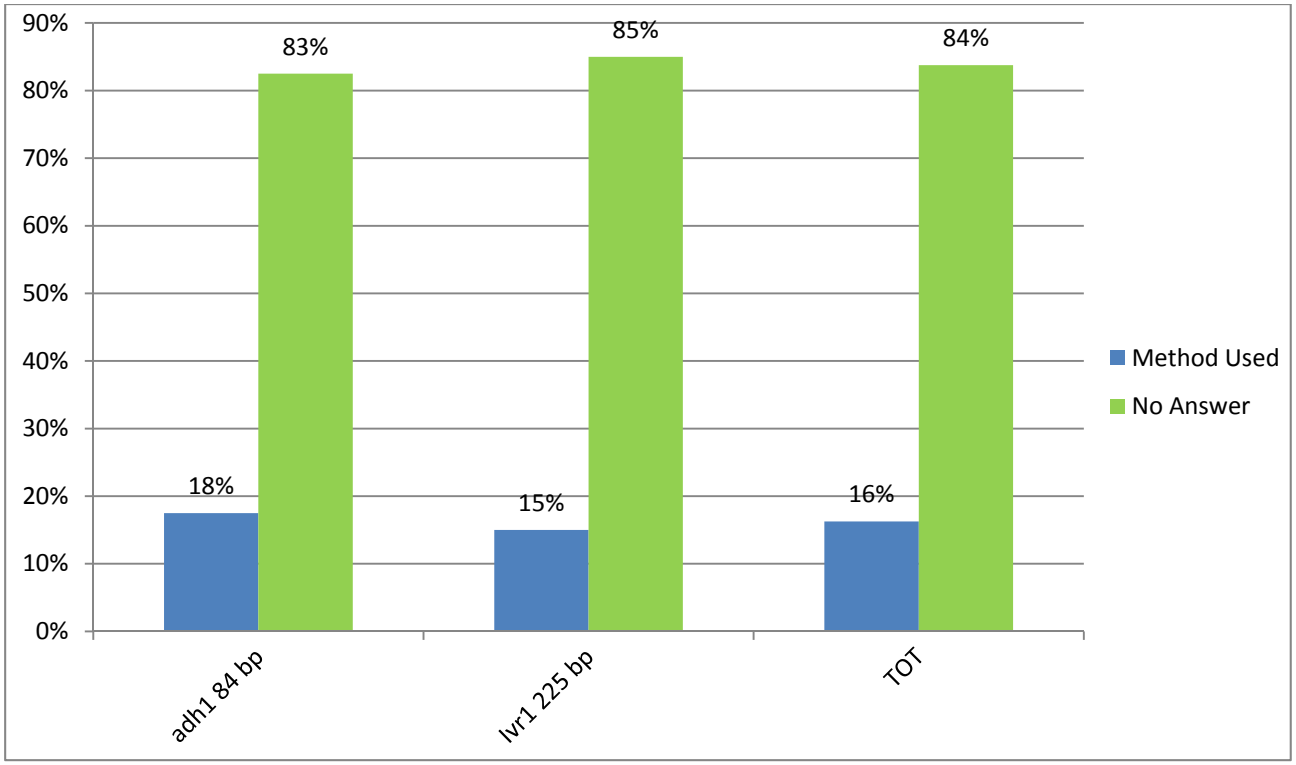
h) Qualitative Taxon-specific Methods

Figure 43 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Species



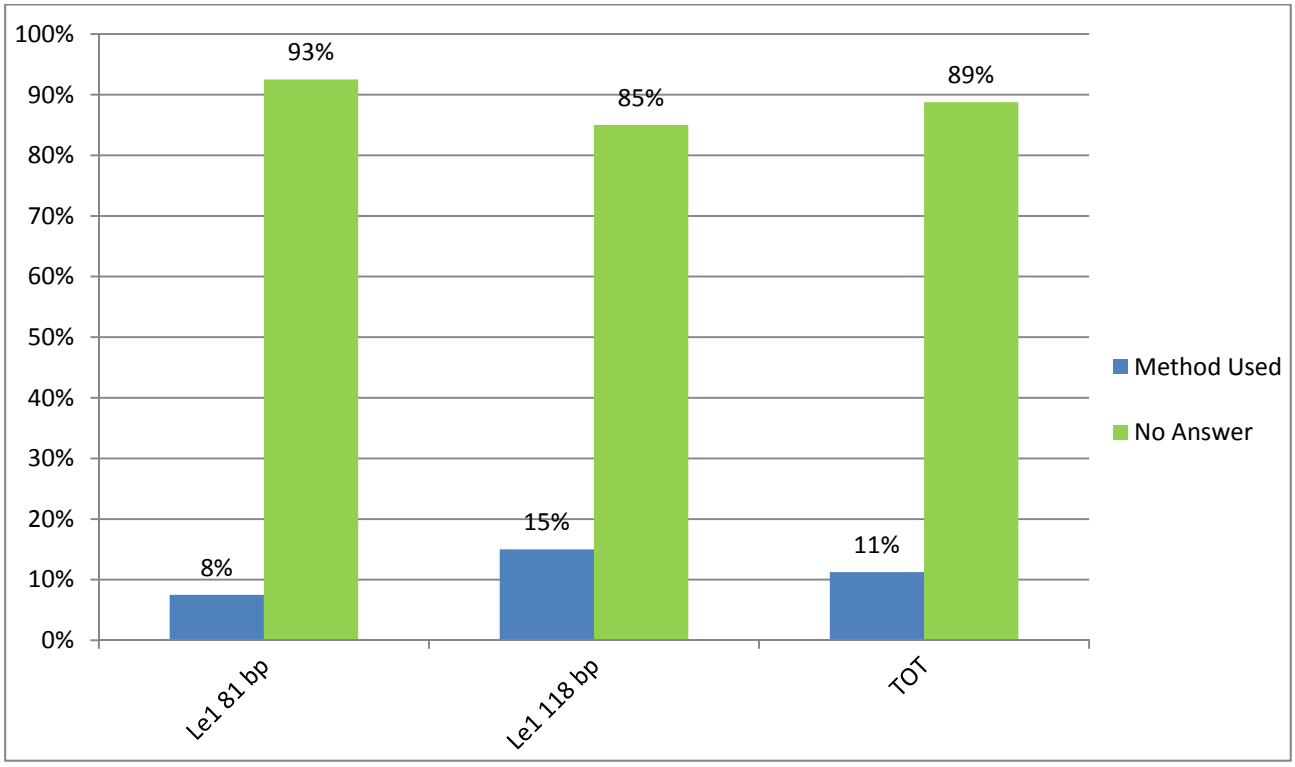
Legend: TOT = Percentages for all qualitative taxon-specific methods

Figure 44 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Maize Reference Genes



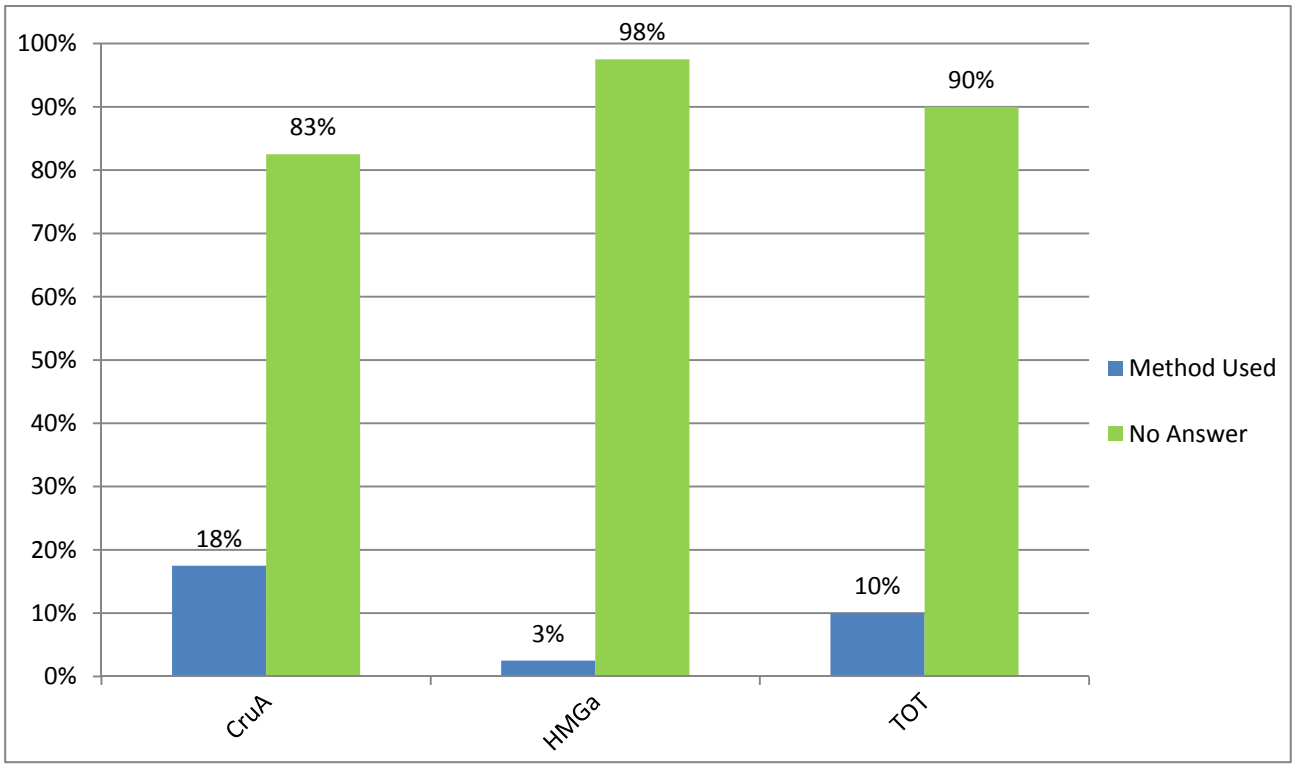
Legend: adh1 = Alcohol dehydrogenase1 gene; lvr1 = Invertase gene; TOT = Percentages for all qualitative taxon-specific maize methods

Figure 45 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Soybean Reference Genes



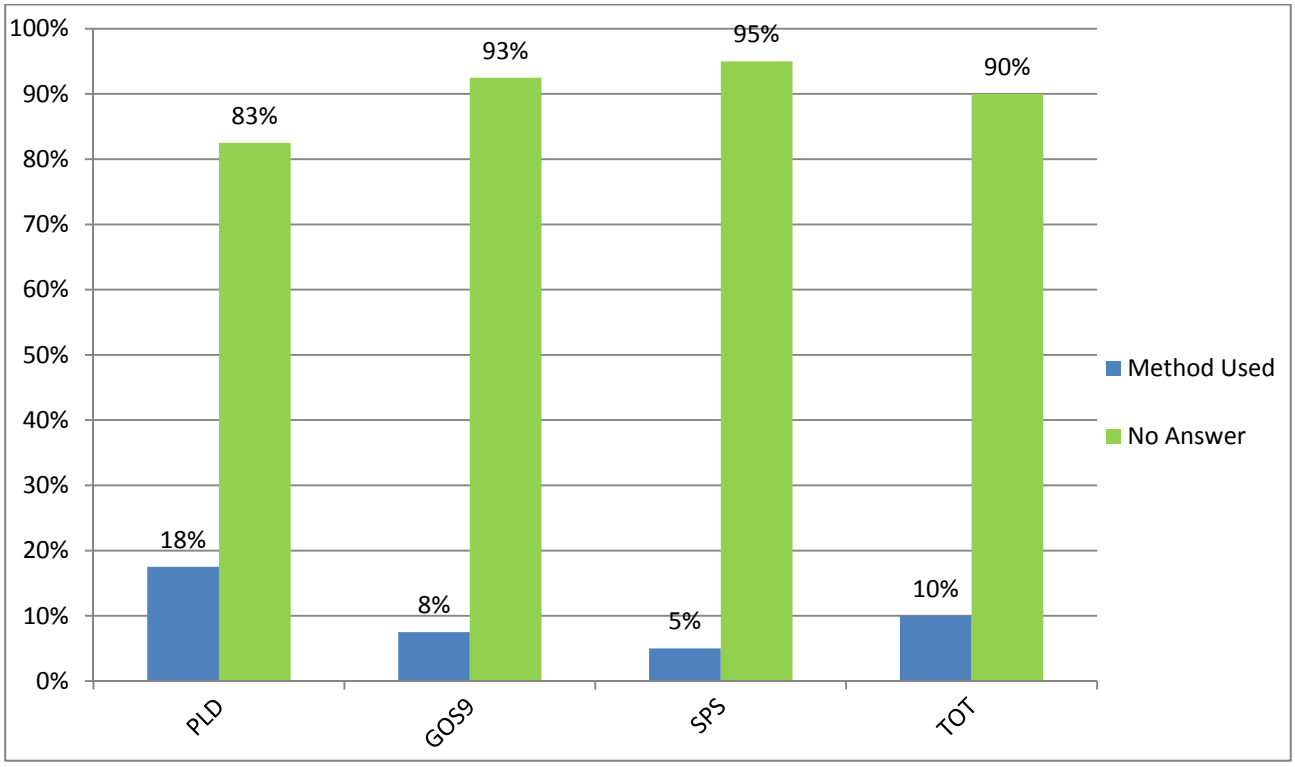
Legend: Le1 = Lectin gene; TOT = Percentages for all qualitative taxon-specific soybean methods

Figure 46 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Oilseed Rape Reference Genes



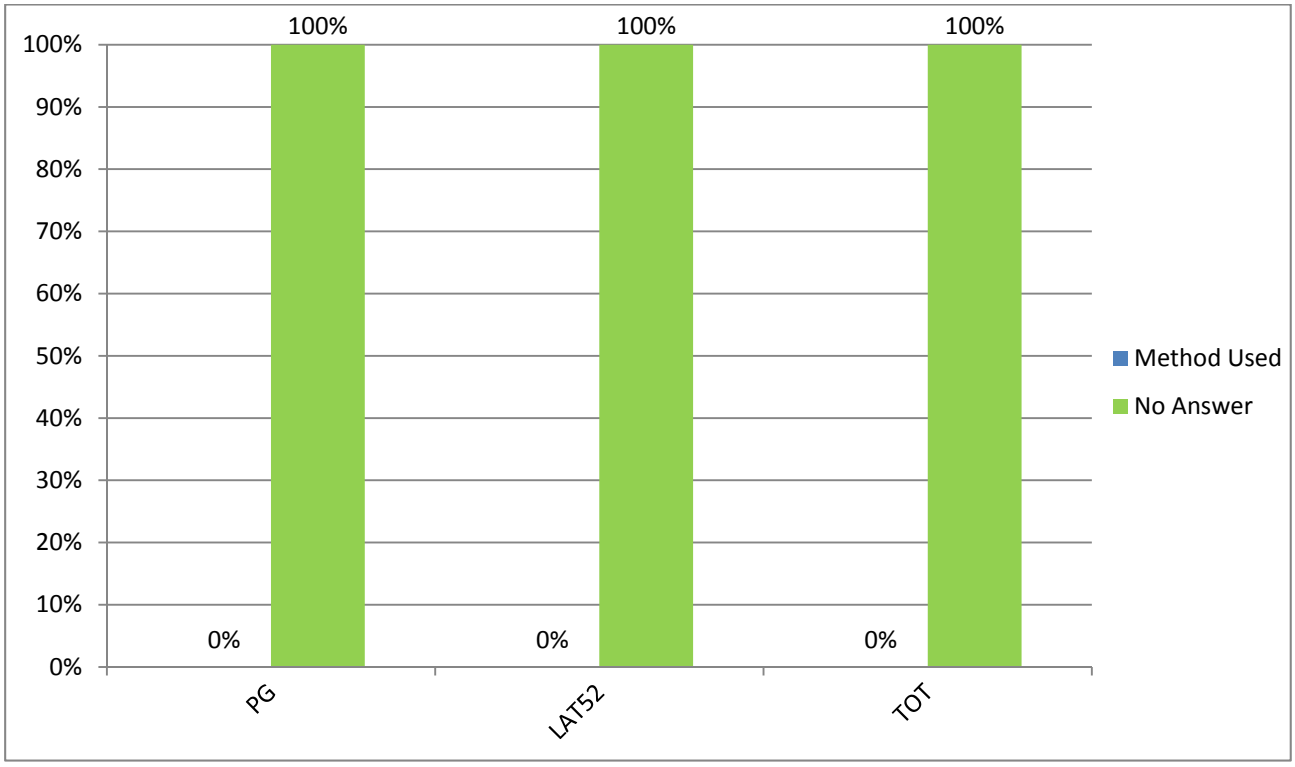
Legend: CruA = Cruciferin A gene; HMGa = High mobility group protein I/Y gene; TOT = Percentages for all qualitative taxon-specific oilseed rape methods

Figure 47 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Rice Reference Genes



Legend: PLD = Phospholipase D gene; GOS9 = Rice root-specific GOS9 gene; SPS = Sucrose-phosphate synthase gene; TOT = Percentages for all qualitative taxon-specific rice methods

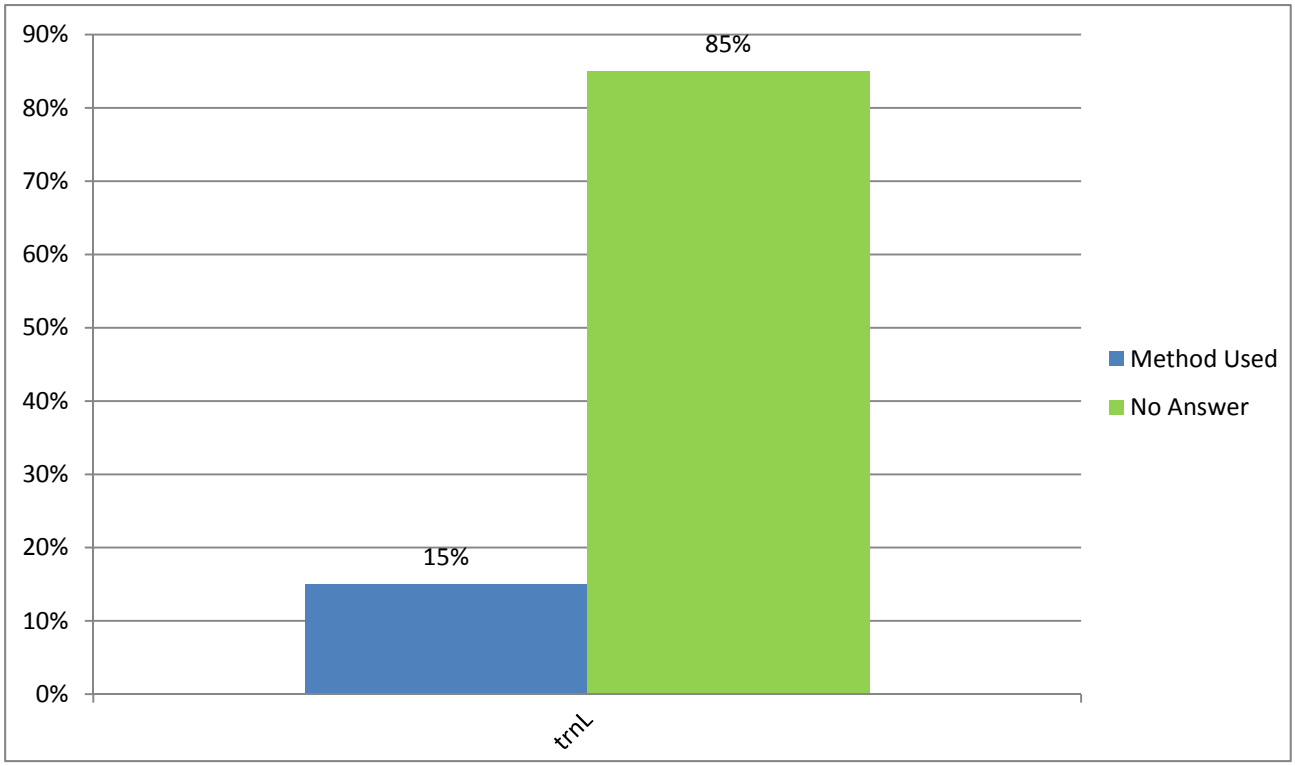
Figure 48 - Percentage of Respondents using Qualitative Taxon-specific Methods According to Tomato Reference Genes



Legend: PG = Polygalacturonase gene; LAT52 = LAT52 gene; TOT = Percentages for all qualitative taxon-specific tomato methods

i) Qualitative Plant-specific Methods

Figure 49 - Percentage of Respondents using Qualitative Plant-specific Methods



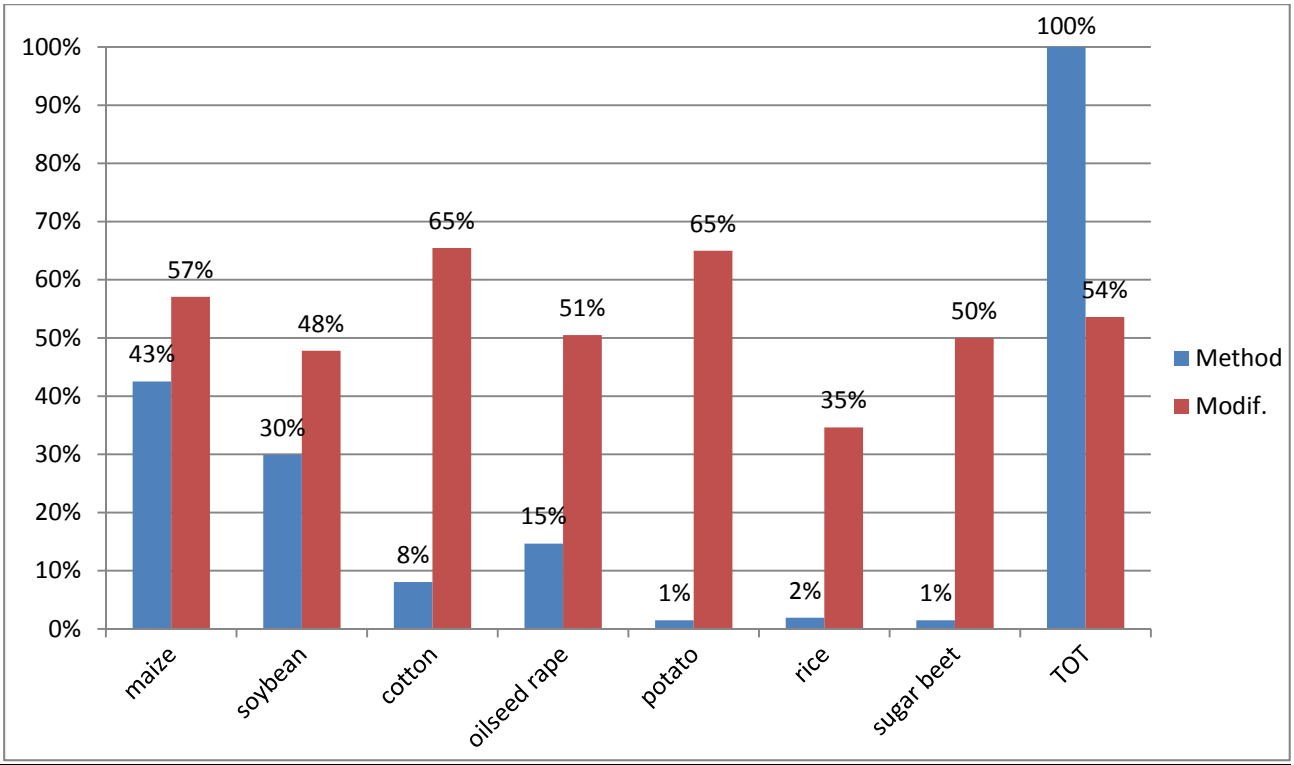
Legend: trnL = Chloroplast tRNA-Leu intron

11.2 Modifications of EU Reference Methods

The charts in this section present the rate of modification (number of modified methods over the total number of methods used) according to GM species, GM events, target elements or crops. Data on method modification are represented in percentages in the charts while the absolute values of respondents using the related methods are displayed in the tables below. The blue bars in the charts provide a visualisation of the relative use of the methods detecting the targets listed on the horizontal axis and the red bars their respective own rate of modification. These values are statistically significant only if many respondents have declared using the methods.

a) Quantitative Event-specific Methods

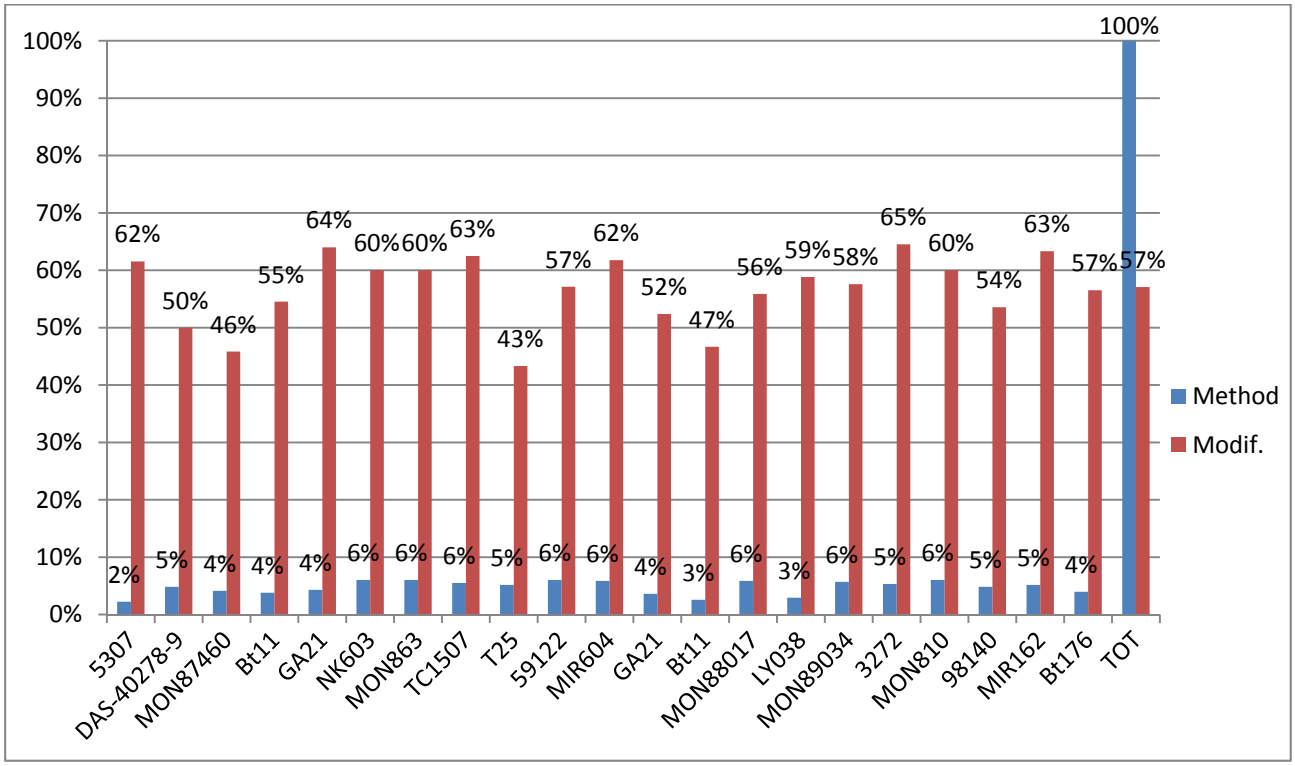
Figure 50 - Percentage of Methods used for Quantitative Event-specific Detection of GMOs According to Species and their own Rate of Modifications



QT EVE Modification	maize	soybean	cotton	oilseed rape	potato	rice	sugar beet	TOT
Methods Used	580	408	110	200	20	26	20	1364
Methods Modified	331	195	72	101	13	9	10	731
Rate of modification	57 %	48 %	65 %	51 %	65 %	35 %	50 %	54 %
% of Total Methods Used	43 %	30 %	8 %	15 %	1.5 %	1.9 %	1.5 %	100 %

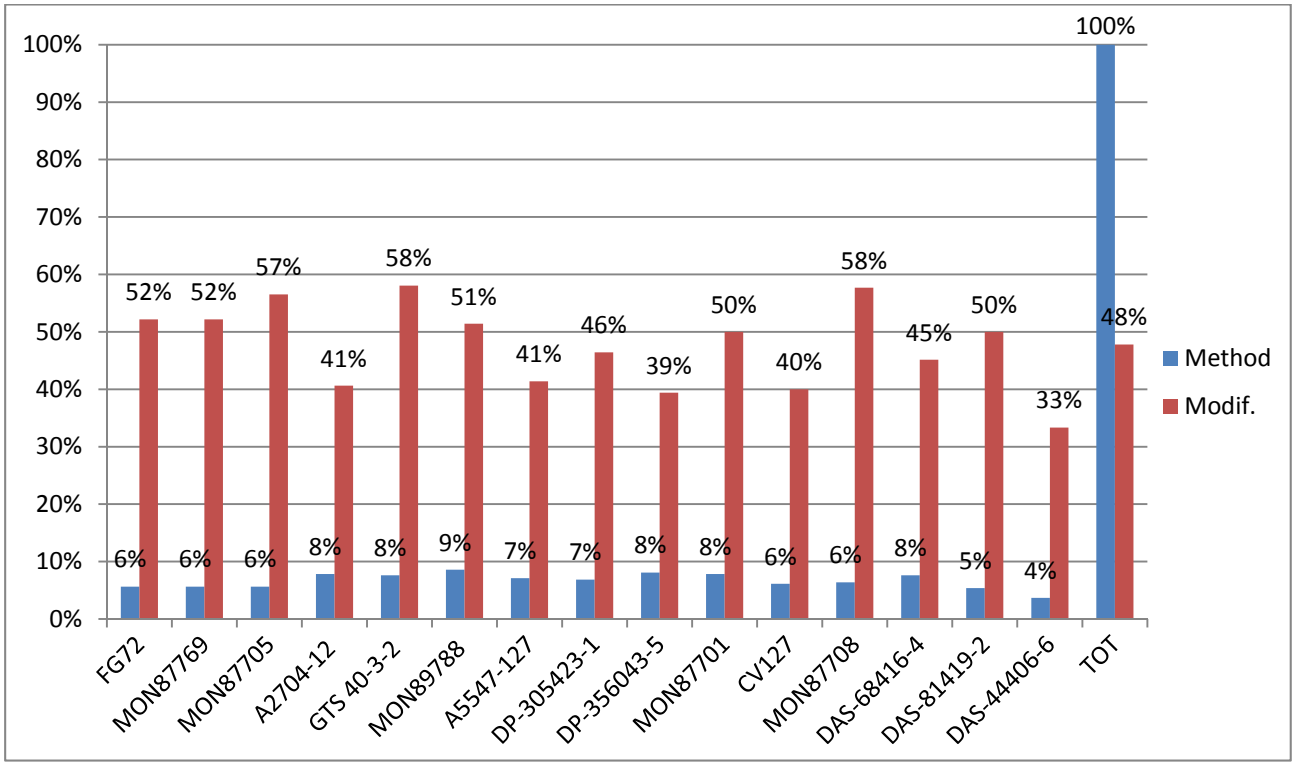
Legend: Method = Methods used; Modif = Methods modified (own ratio); TOT = Total methods used for quantitative event-specific detection of GMOs; QT = Quantitative; EVE = Event-specific methods;

Figure 51 - Percentage of Methods used for Quantitative Event-specific Detection of Maize GMOs and their own Rate of Modifications



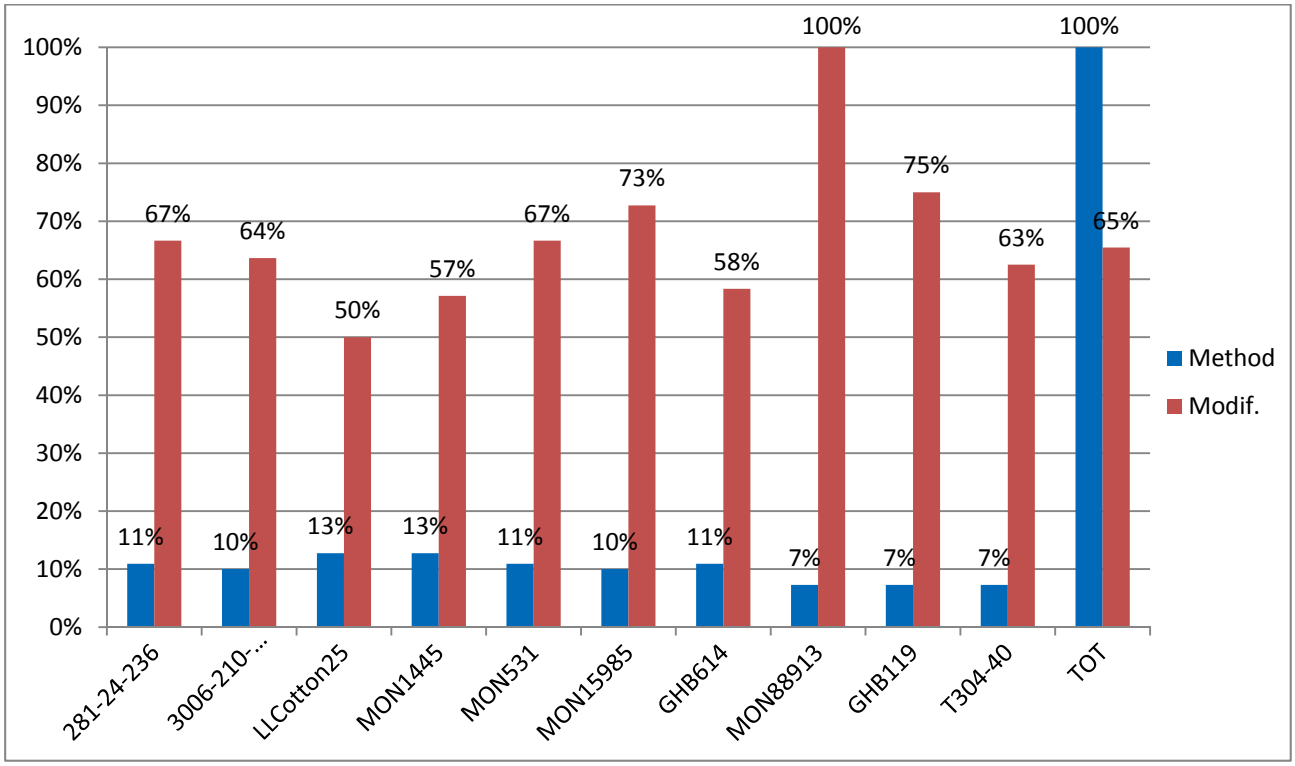
Legend: Method = Methods used; Modif = Methods modified; TOT = Total methods used for quantitative event-specific detection of maize GMOs

Figure 52 - Percentage of Methods used for Quantitative Event-specific Detection of Soybean GMOs and their own Rate of Modifications



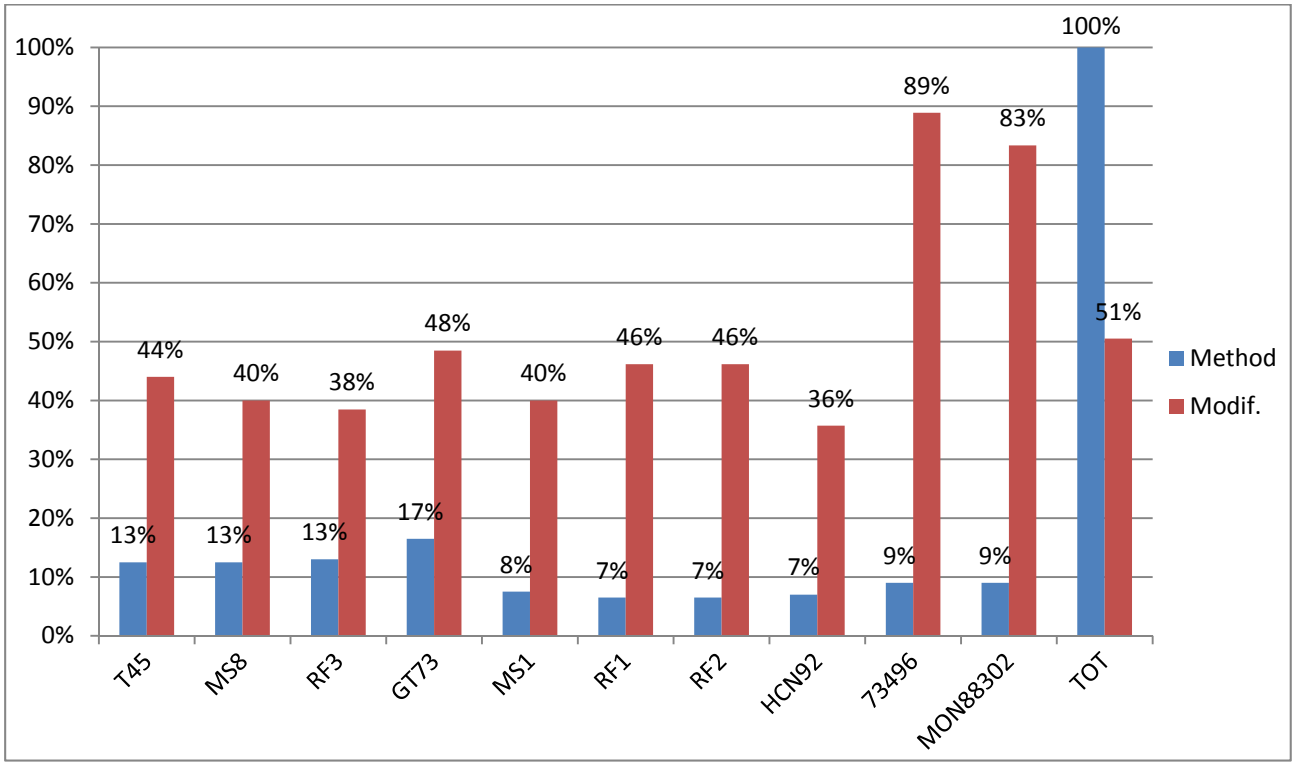
Legend: Method = Methods used; Modif = Methods modified; TOT = Total methods used for quantitative event-specific detection of soybean GMOs

Figure 53 - Percentage of Methods used for Quantitative Event-specific Detection of Cotton GMOs and their own Rate of Modifications



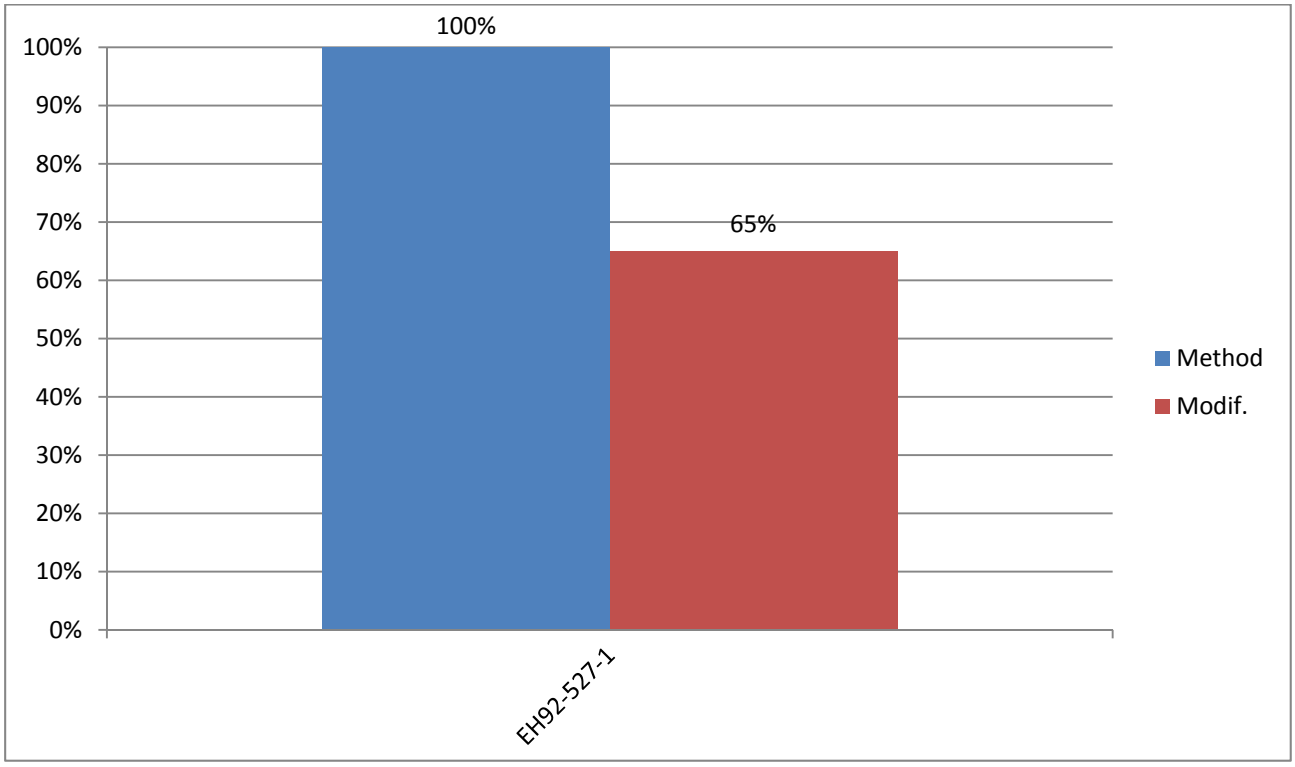
Legend: Method = Methods used; Modif = Methods modified; TOT = Total methods used for quantitative event-specific detection of cotton GMOs

Figure 54 - Percentage of Methods used for Quantitative Event-specific Detection of Oilseed Rape GMOs and their own Rate of Modifications



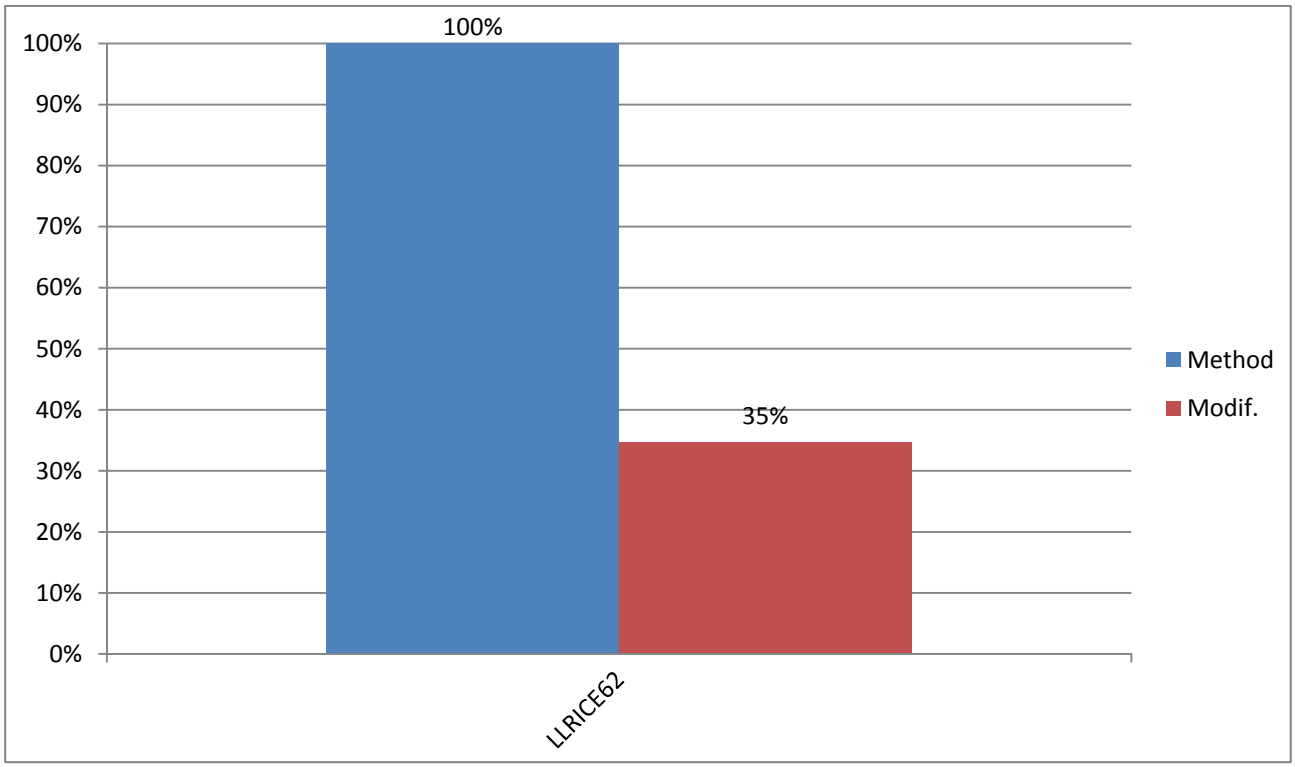
Legend: Method = Methods used; Modif = Methods modified; TOT = Total methods used for quantitative event-specific detection of oilseed rape GMOs

Figure 55 - Percentage of Methods used for Quantitative Event-specific Detection of Potato GMOs and their own Rate of Modification



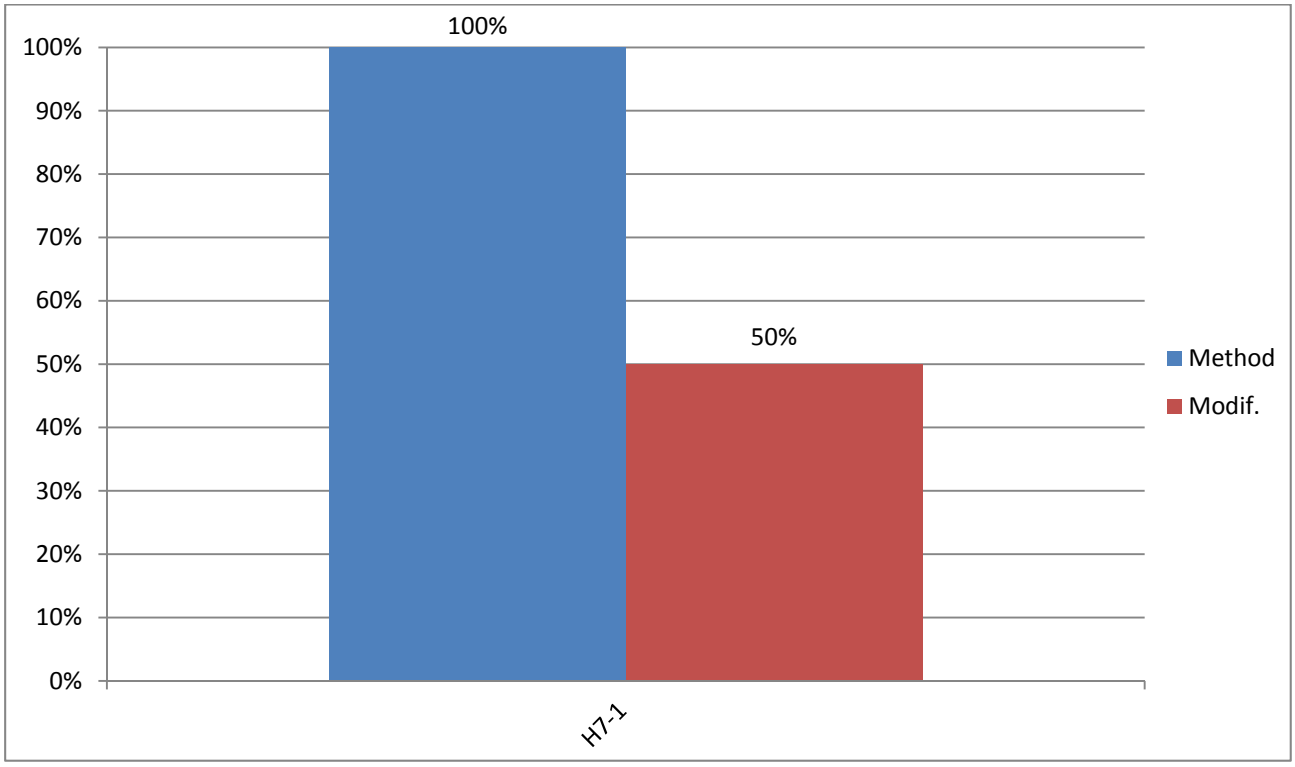
Legend: Method = Methods used; Modif = Methods modified

Figure 56 - Percentage of Methods used for Quantitative Event-specific Detection of Rice GMOs and their own Rate of Modification



Legend: Method = Methods used; Modif = Methods modified

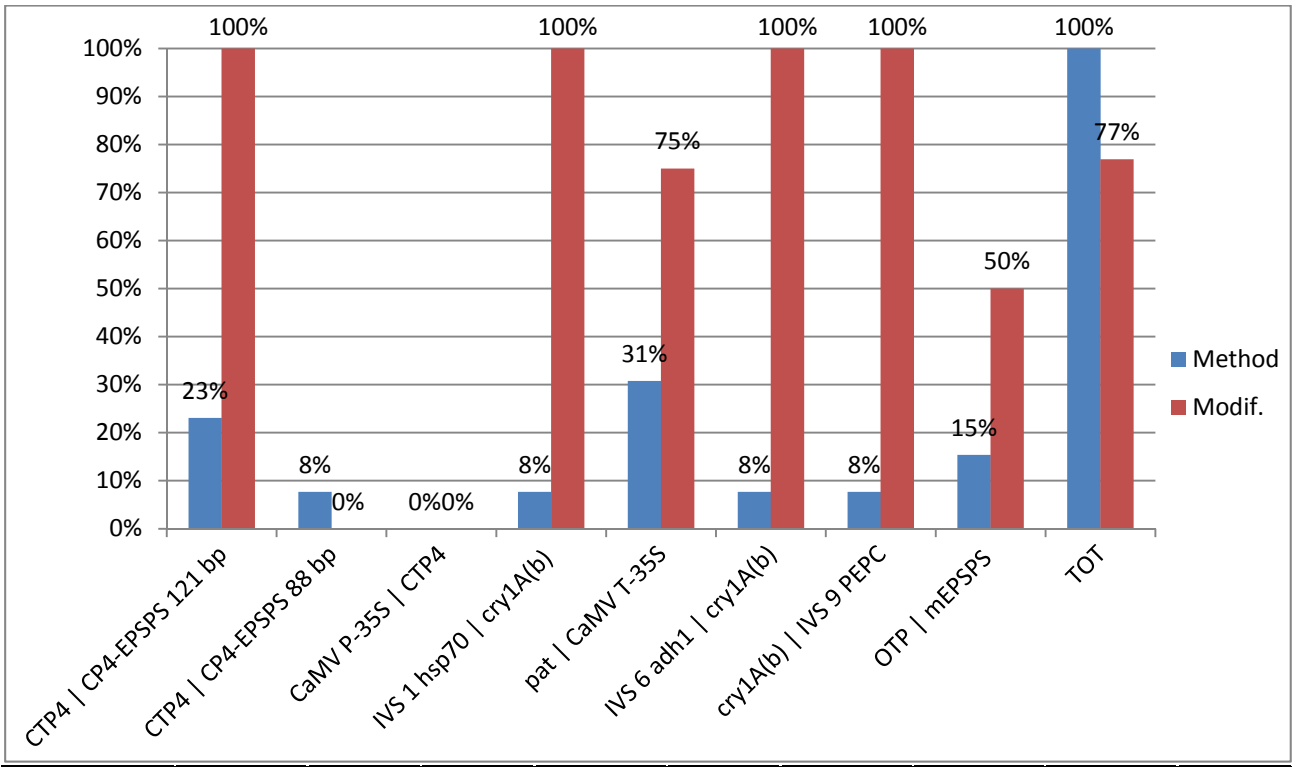
Figure 57 - Percentage of Methods used for Quantitative Event-specific Detection of Sugar Beet GMOs and their own Rate of Modification



Legend: Method = Methods used; Modif = Methods modified

b) Quantitative Construct-specific Methods

Figure 58 - Percentage of Methods used for Quantitative Construct-specific Detection of GMOs According to Genetic Targets and their own Rate of Modifications



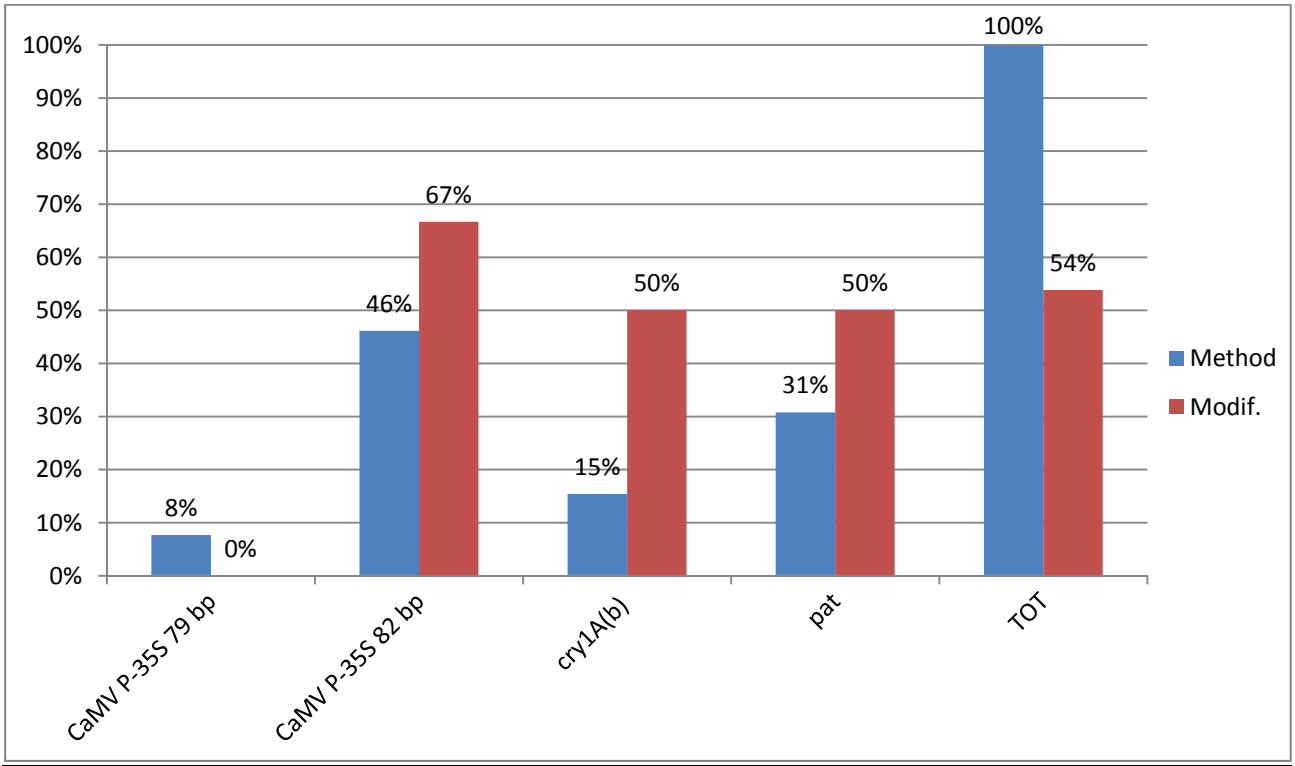
QT CON Modif.	CTP4 | CP4-EPSPS 121 bp	CTP4 | CP4-EPSPS 88 bp	CaMV P-35S | CTP4	IVS 1 hsp70 | cry1A(b)	pat | CaMV T-35S	IVS 6 adh1 | cry1A(b)	cry1A(b) | IVS 9 PEPC	OTP | mEPSPS	TOT
Methods Used	3	1	0	1	4	1	1	2	13

QT CON Modif.	CTP4 | CP4-EPSPS 121 bp	CTP4 | CP4-EPSPS 88 bp	CaMV P-35S | CTP4	IVS 1 hsp70 | cry1A(b)	pat | CaMV T-35S	IVS 6 adh1 | cry1A(b)	cry1A(b) | IVS 9 PEPC	OTP | mEPSPS	TOT
Methods Modified	3	0	0	1	3	1	1	1	10
Rate of Modification	100 %	0 %	0 %	100 %	75 %	100 %	100 %	50 %	77 %
% of Total Methods Used	23 %	7.7 %	0 %	7.7 %	31 %	7.7 %	7.7 %	15 %	100 %

Legend: Method = Methods used; Modif = Methods modified (own ratio); CTP4 = Chloroplast transit peptide coding sequence from *Petunia hybrida*; CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; IVS 1 hsp70 = Intervening sequence 1 from *Zea mays hsp70* gene; cry1A(b) = *cry1Ab* delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes*; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; IVS 6 adh1 = Intervening sequence 6 from *Zea mays* alcohol dehydrogenase 1 gene; IVS 9 PEPC = Intervening sequence 9 from the phospho-enol-pyruvate carboxylase gene; OTP = Optimized transit peptide sequence from *Zea mays*; mEPSPS = Point mutated epsps gene from *Zea mays*; TOT = Total methods used for quantitative construct-specific detection of GMOs; QT = Quantitative; CON = Construct-specific methods;

c) Quantitative Element-specific Methods

Figure 59 - Percentage of Methods used for Quantitative Element-specific Detection of GMOs According to Genetic Targets and their own Rate of Modifications

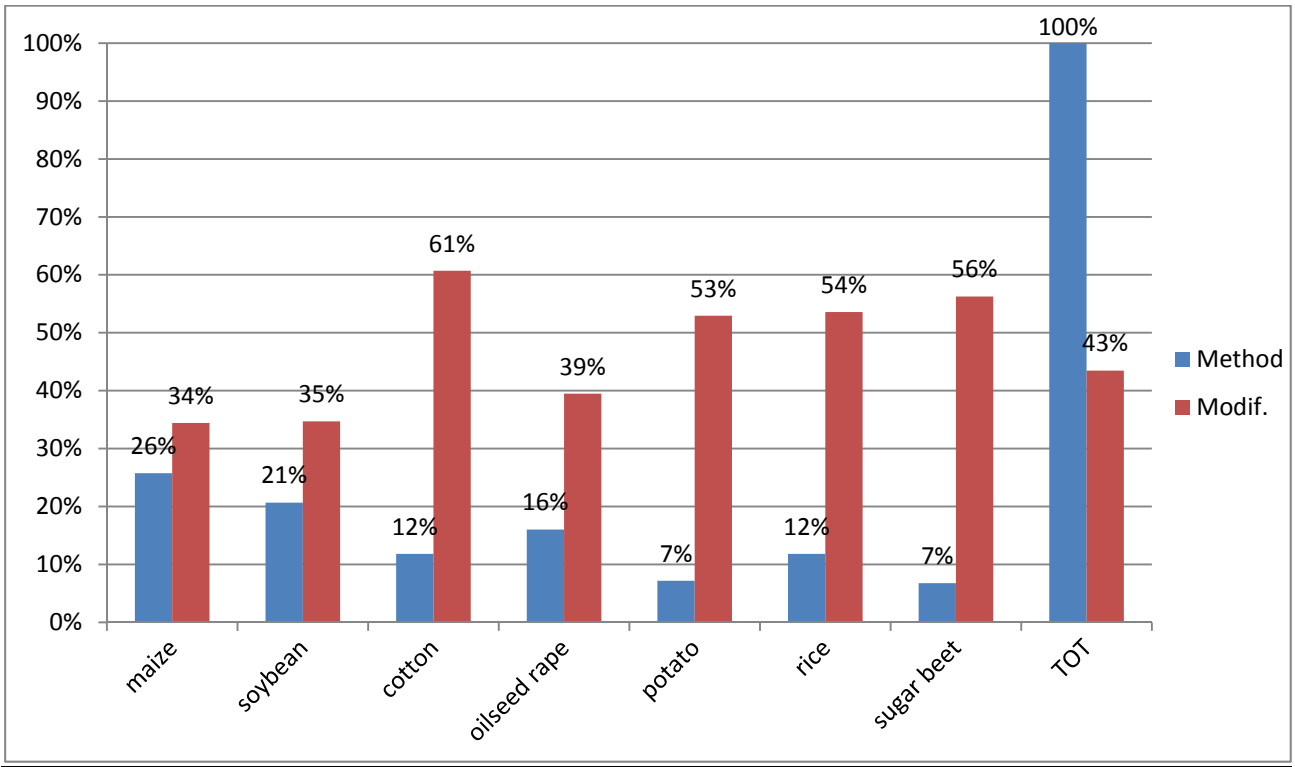


QT ELE Modification	CaMV P-35S 79 bp	CaMV P-35S 82 bp	cry1A(b)	pat	TOT
Methods Used	1	6	2	4	13
Methods Modified	0	4	1	2	7
Rate of Modification	0 %	67 %	50 %	50 %	54 %
% of Total Methods Used	7.7 %	46 %	15 %	31 %	100 %

Legend: Method = Methods used; Modif = Methods modified (own ratio); CaMV P-35S = *Cauliflower Mosaic Virus* 35S Promoter; cry1A (b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes;* TOT = Total methods used for quantitative element-specific detection of GMOs; QT = Quantitative; ELE = Element-specific methods;

d) Quantitative Taxon-specific Methods

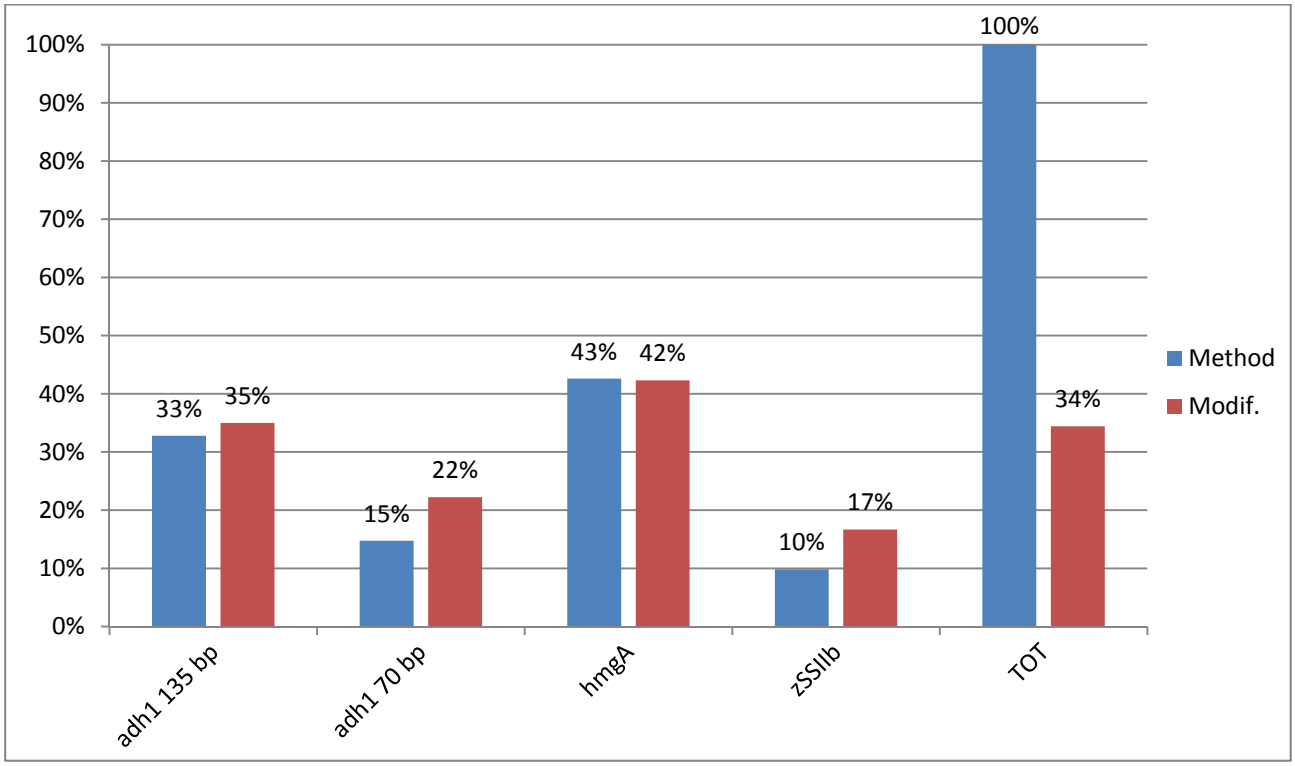
Figure 60 - Percentage of Methods used for Quantitative Taxon-specific Detection According to Species and their own Rate of Modifications



QT TAX Modification	maize	soybean	cotton	oilseed rape	potato	rice	sugar beet	TOT
Methods Used	61	49	28	38	17	28	16	237
Methods Modified	21	17	17	15	9	15	9	103
Rate of Modification	34 %	35 %	61 %	39 %	53 %	54 %	56 %	43 %
% of Total Methods Used	26 %	21 %	12 %	16 %	7.2 %	12 %	6.8 %	100%

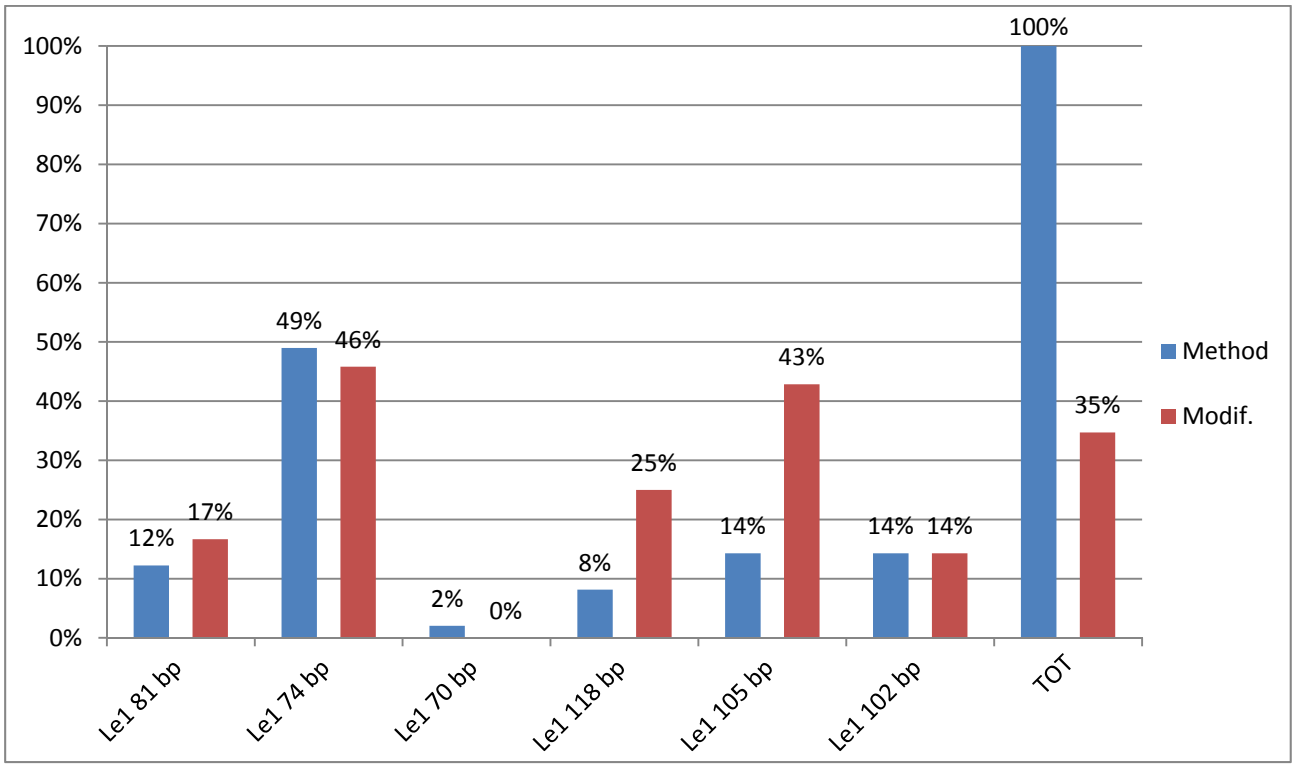
Legend: Method = Methods used; Modif = Methods modified (own ratio); TOT = Total methods used for quantitative taxon-specific detection of species; QT = Quantitative; TAX = Taxon-specific methods;

Figure 61 - Percentage of Methods used for Quantitative Detection of Maize Reference Genes and their own Rate of Modifications



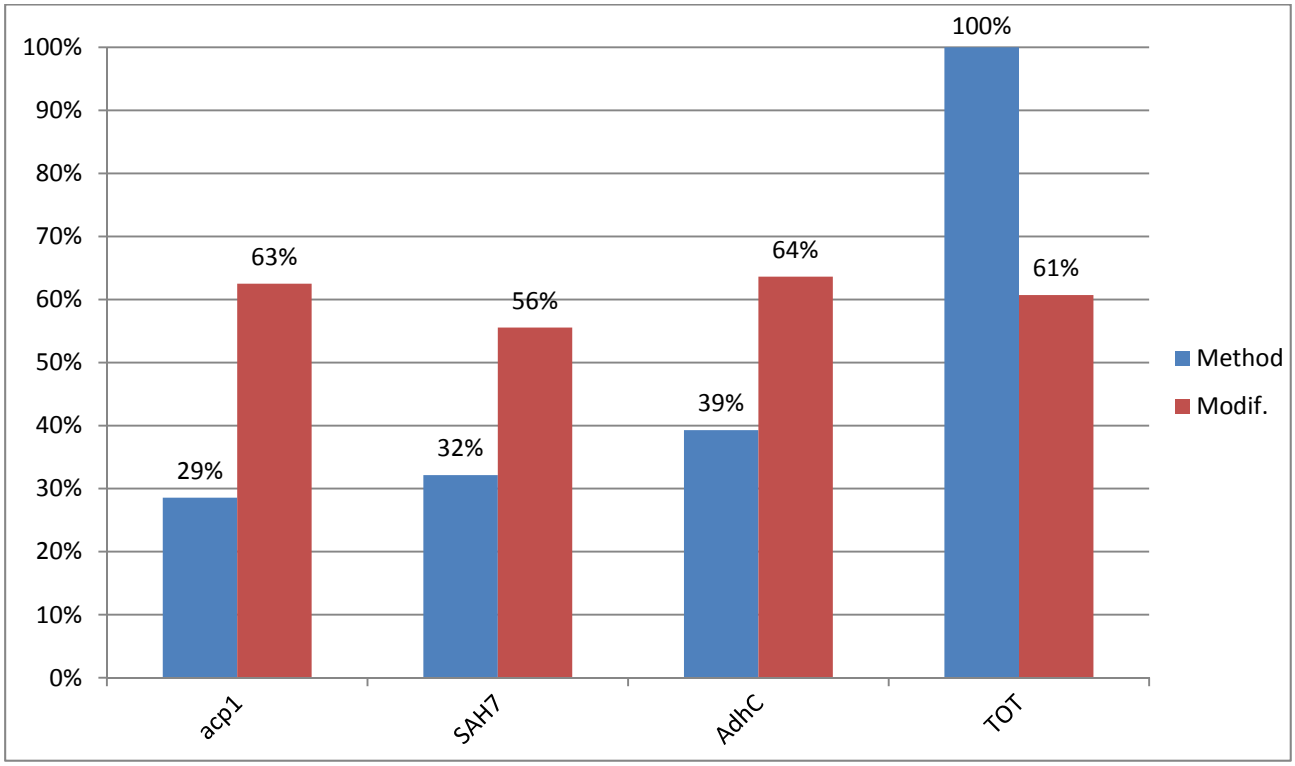
Legend: Method = Methods used; Modif = Methods modified; adh1 = Alcohol dehydrogenase1 gene; hmgA = High-mobility-group A gene; zSSIIb = Maize starch synthase IIb gene; TOT = Total methods used for quantitative taxon-specific detection of maize

Figure 62 - Percentage of Methods used for Quantitative Detection of Soybean Reference Genes and their own Rate of Modifications



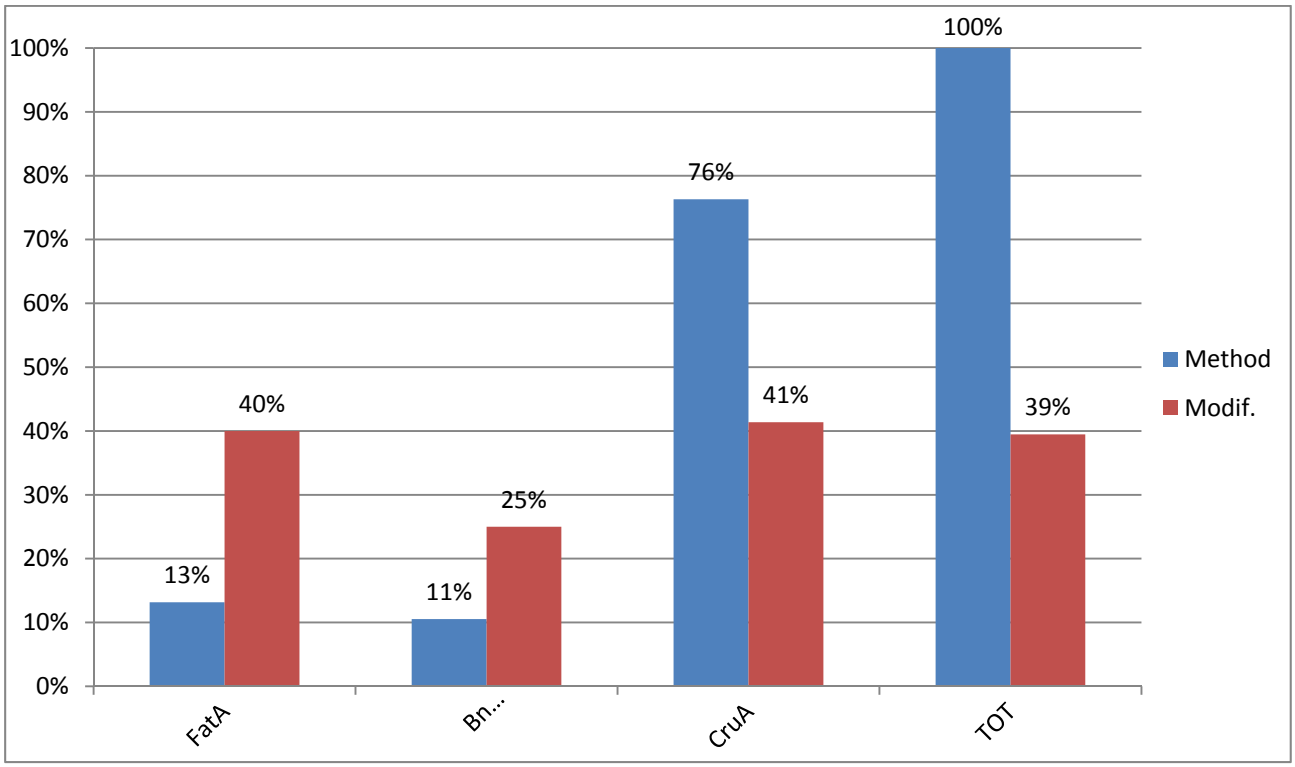
Legend: Method = Methods used; Modif = Methods modified; Le1 = Lectin gene; TOT = Total methods used for quantitative taxon-specific detection of soybean

Figure 63 - Percentage of Methods used for Quantitative Detection of Cotton Reference Genes and their own Rate of Modifications



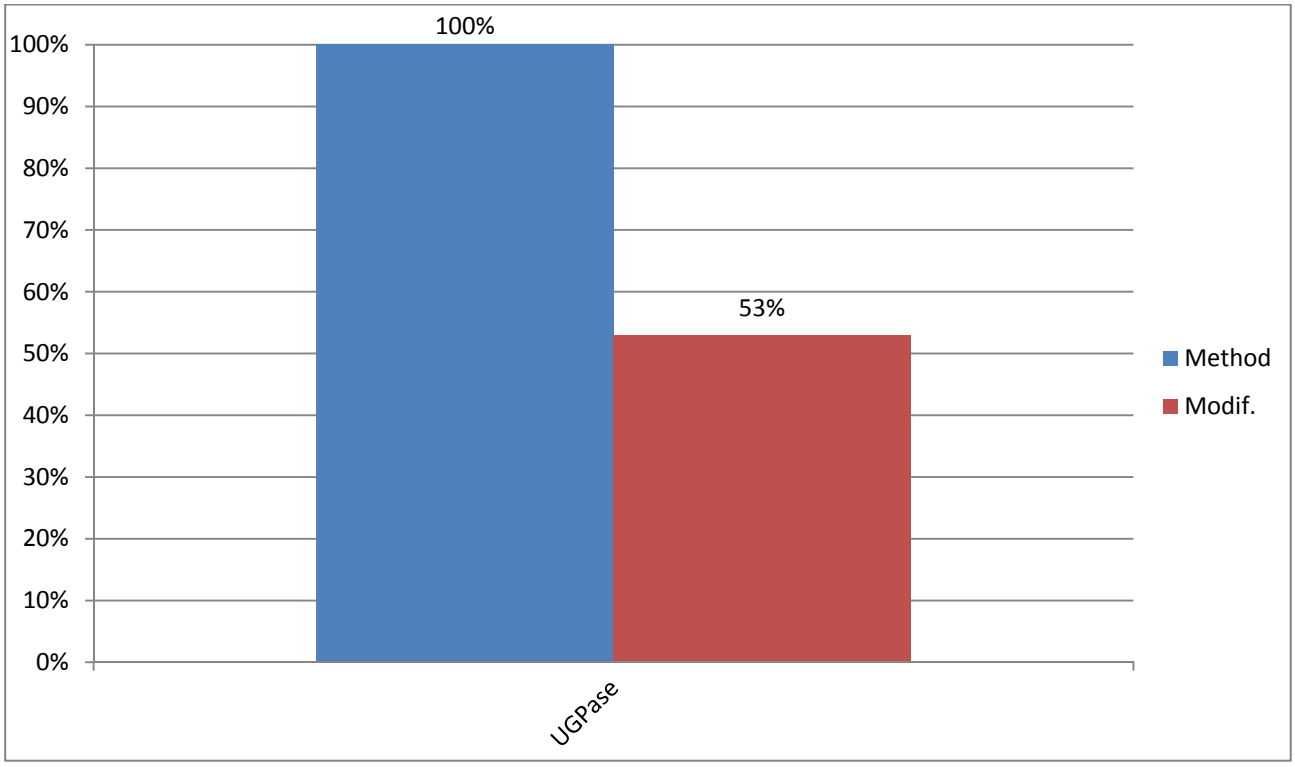
Legend: Method = Methods used; Modif = Methods modified; acp1 = Acyl carrier protein 1 gene; SAH7 = IVS of the putative *Sinapis Arabidopsis Homolog 7* protein gene; AdhC = Alcohol dehydrogenase C gene; TOT = Total methods used for quantitative taxon-specific detection of cotton

Figure 64 - Percentage of Methods used for Quantitative Detection of Oilseed Rape Reference Genes and their own Rate of Modifications



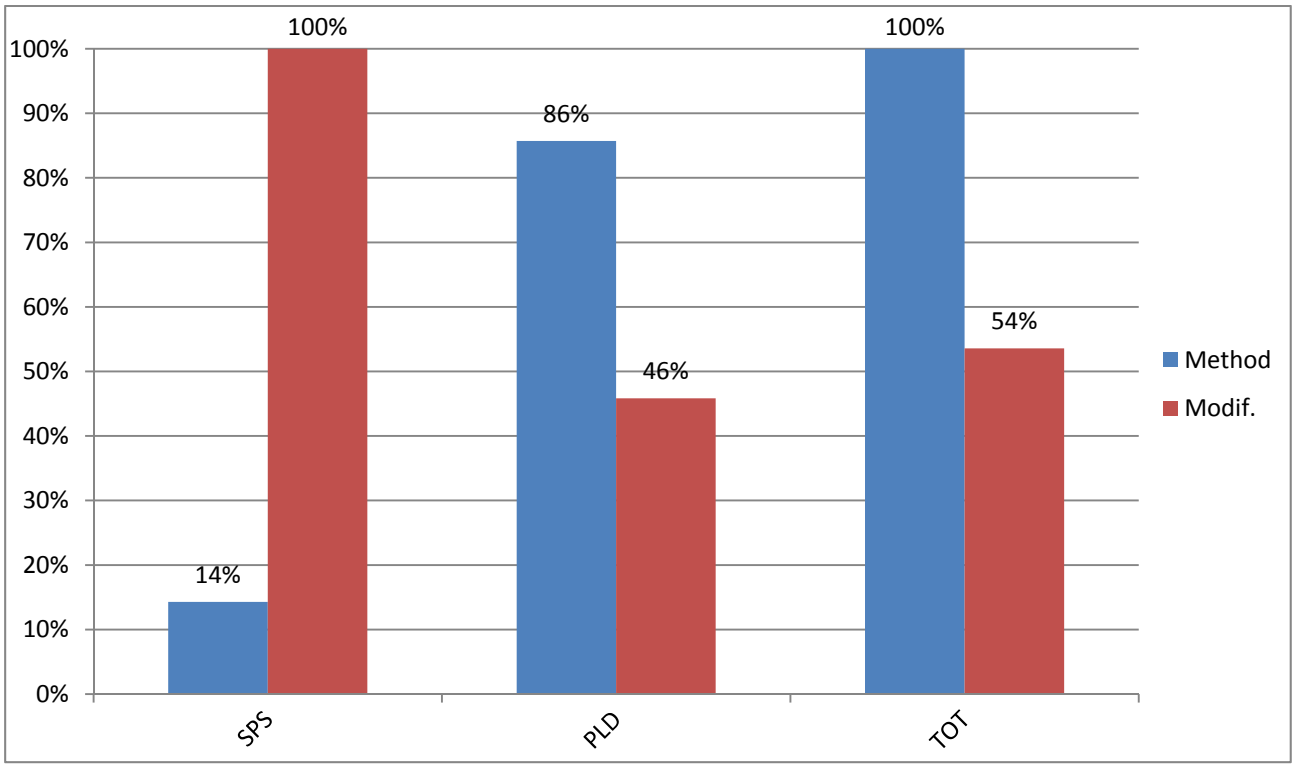
Legend: Method = Methods used; Modif = Methods modified; FatA = Acyl-ACP thioesterase gene; BnC1 = Cruciferin storage protein gene; CruA = Cruciferin A gene; TOT = Total methods used for quantitative taxon-specific detection of oilseed rape

Figure 65 - Percentage of Methods used for Quantitative Detection of Potato Reference Genes and their own Rate of Modification



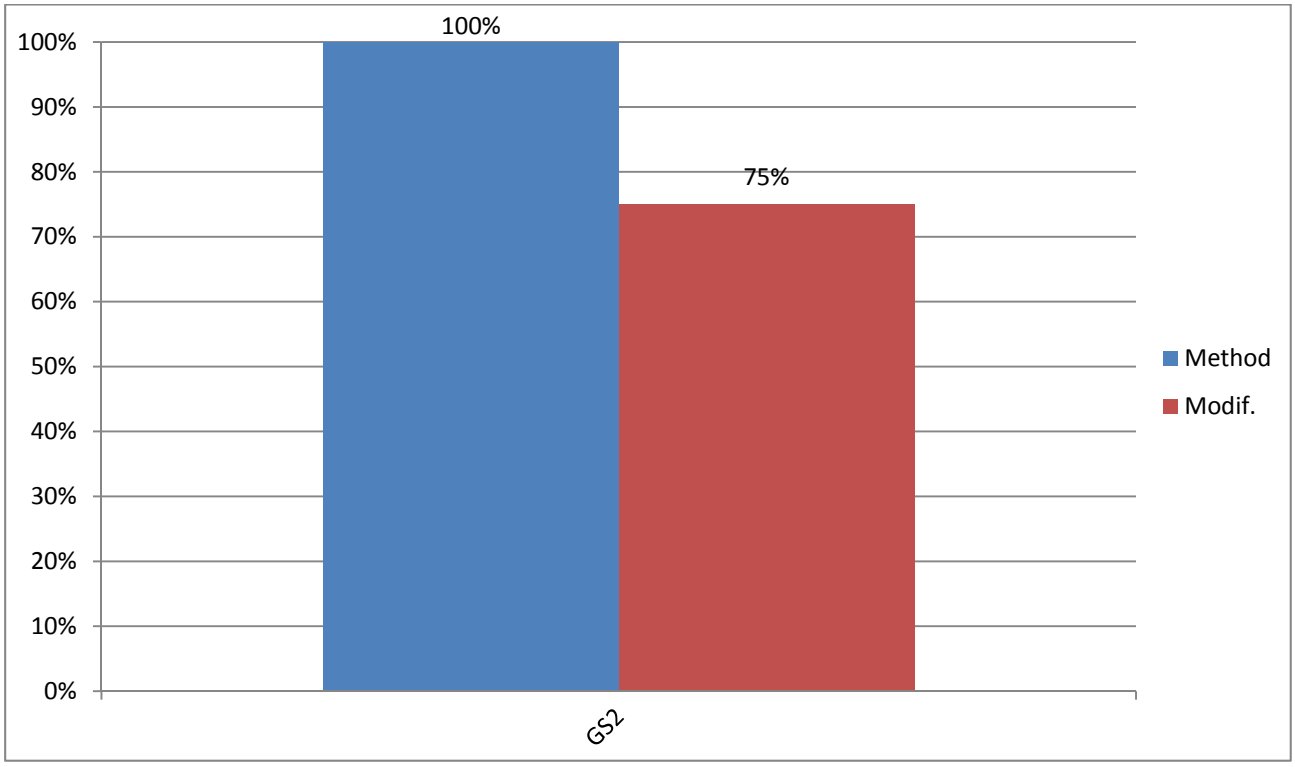
Legend: Method = Methods used; Modif = Methods modified; UGPase = UDP-glucose pyrophosphorylase gene

Figure 66 - Percentage of Methods used for Quantitative Detection of Rice Reference Genes and their own Rate of Modifications



Legend: Method = Methods used; Modif = Methods modified; SPS = Sucrose-phosphate synthase gene; PLD = Phospholipase D gene; TOT = Total methods used for quantitative taxon-specific detection of rice

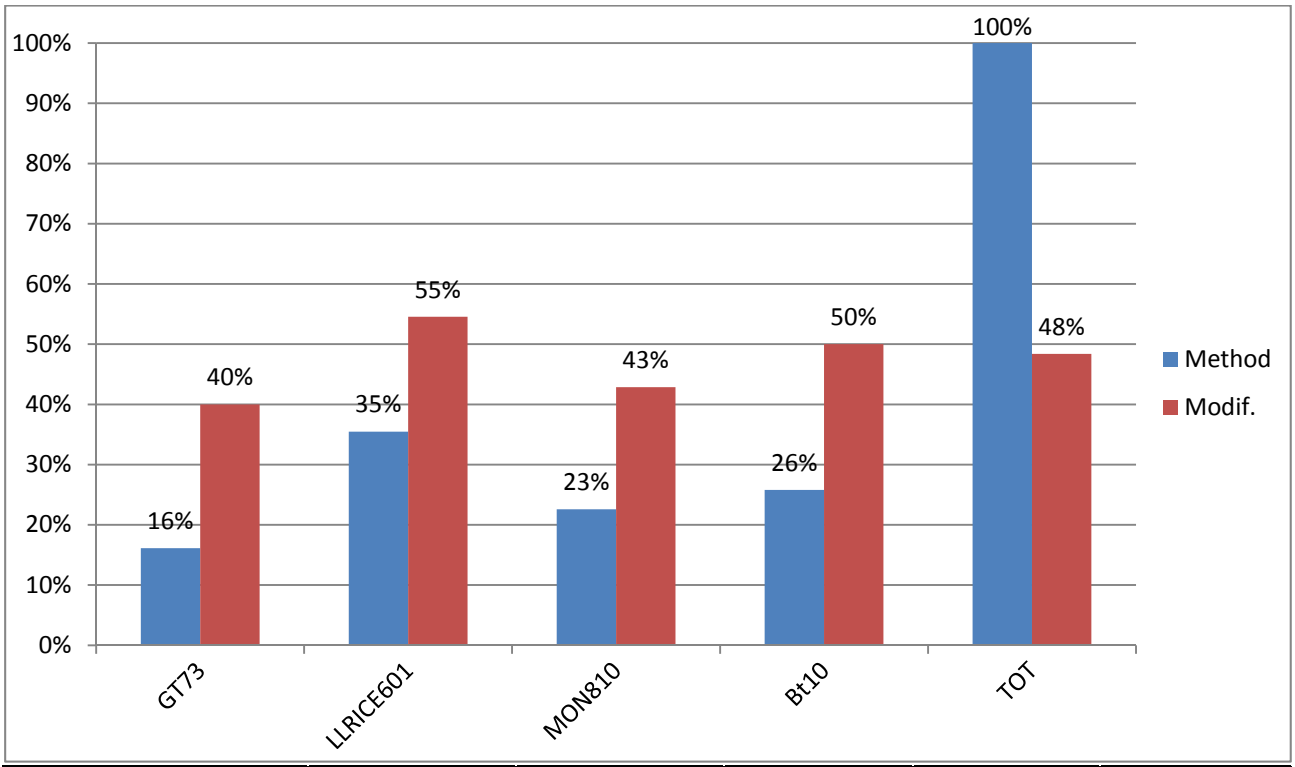
Figure 67 - Percentage of Methods used for Quantitative Detection of Sugar Beet Reference Genes and their own Rate of Modification



Legend: Method = Methods used; Modif = Methods modified; GS2 = Glutamine synthetase gene

e) Qualitative Event-specific Methods

Figure 68 - Percentage of Methods used for Qualitative Event-specific Detection According to GMOs and their own Rate of Modifications

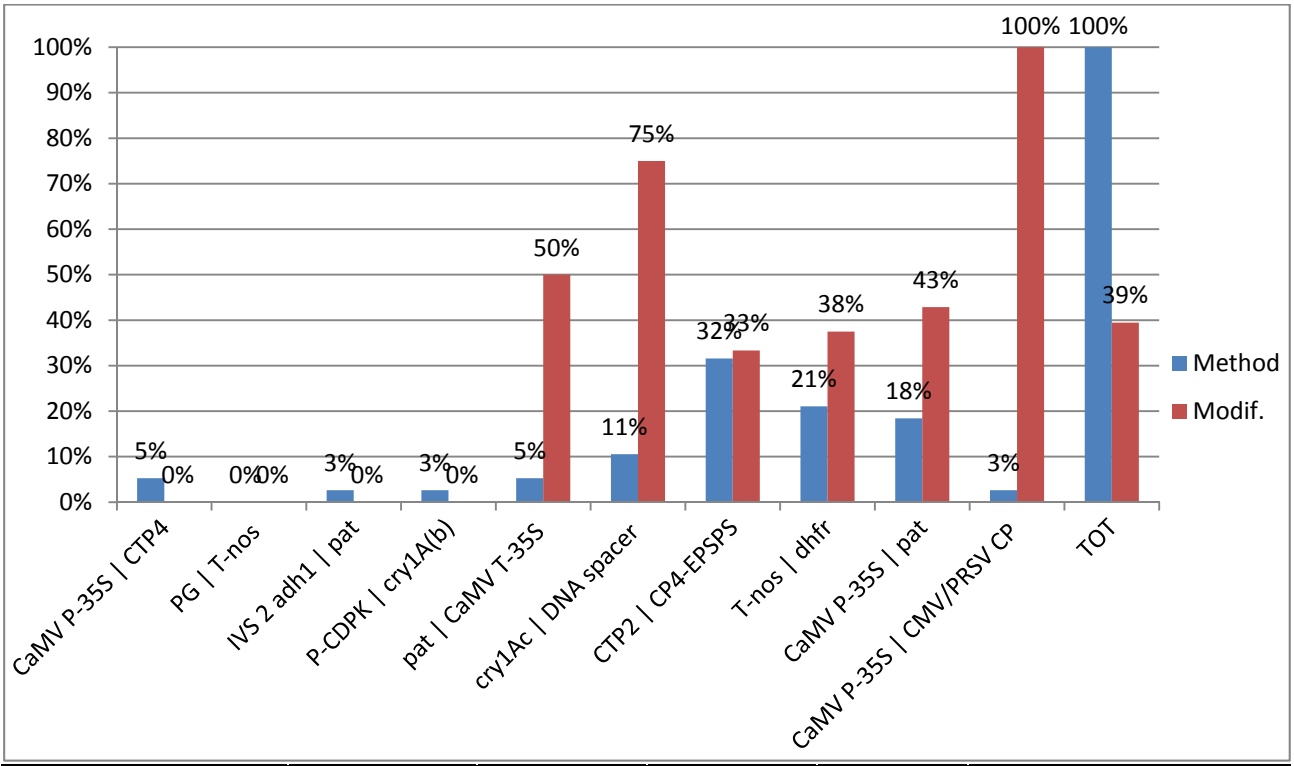


QL EVE Modification	GT73	LLRICE601	MON810	Bt10	TOT
Methods Used	5	11	7	8	31
Methods Modified	2	6	3	4	15
Rate of Modification	40 %	55 %	43 %	50 %	48 %
% of Total Methods Used	16 %	35 %	23 %	26 %	100 %

Legend: Method = Methods used; Modif = Methods modified (own ratio); TOT = Total methods used for qualitative event-specific detection of GMOs; QL = Qualitative; EVE = Event-specific methods;

f) Qualitative Construct-specific Methods

Figure 69 Percentage of Methods used for Qualitative Construct-specific Detection of GMOs According to Genetic Targets and their own Rate of Modification

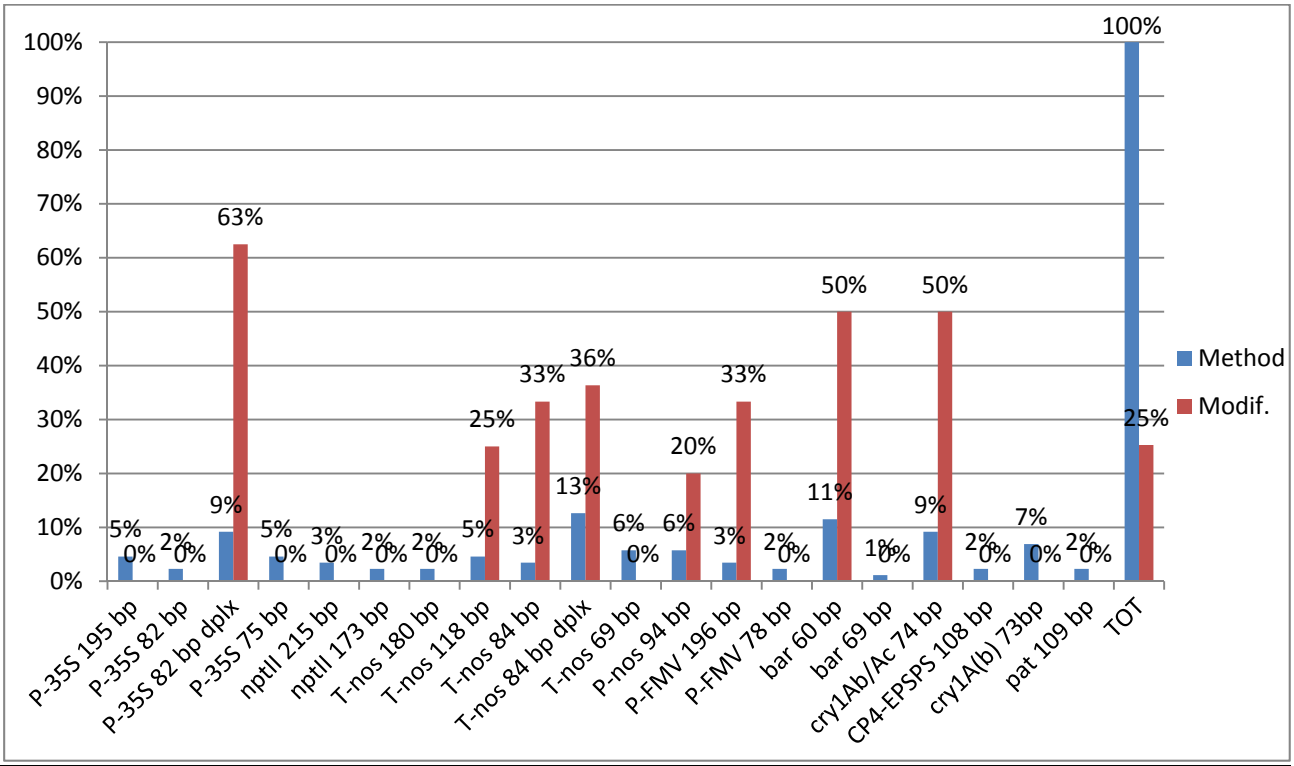


QL CON Modification	CaMV P-35S | CTP4	PG | T-nos	IVS 2 adh1 | pat	P-CDPK | cry1A(b)	pat | CaMV T-35S	
Methods Used	2	0	1	1	2	
Methods Modified	0	0	0	0	1	
Rate of Modification	0 %	0 %	0 %	0 %	50%	
% of Total Methods Used	5.3 %	0 %	2.6 %	2.6 %	5.3 %	
QL CON Modification	**cry1Ac | DNA spacer**	**CTP2 | CP4-EPSPS**	**T-nos | dhfr**	**CaMV P-35S | pat**	**CaMV P-35S | CMV/PRSV CP**	**TOT**
Methods Used	4	12	8	7	1	38
Methods Modified	3	4	3	3	1	15
Rate of Modification	75 %	33 %	38 %	43 %	100 %	39 %
% of Total Methods Used	11 %	32 %	21 %	18 %	2.6 %	100 %

Legend: Method = Methods used; Modif = Methods modified (own ratio); CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; CTP4 = Chloroplast transit peptide coding sequence from *Petunia hybrida epsps* gene; PG = Polygalacturonase gene from *Solanum lycopersicum*; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; IVS 2 adh1 = Intervening sequence 2 from *Zea mays* alcohol dehydrogenase 1 gene; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* P-CDPK = Promoter of *Zea mays* calcium-dependent protein kinase gene; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; CTP2 = Chloroplast transit peptide 2 sequence from *Arabidopsis thaliana epsps* gene; CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; dhfr = Dihydrofolate reductase gene; CMV/PRSV CP = Chimeric *Cucumber mosaic virus* coat protein/*Papaya ring spot virus* coat protein; TOT = Total methods used for qualitative construct-specific detection of GMOs; QL = Qualitative; CON = Construct-specific methods

g) Qualitative Element-specific Methods

Figure 70 - Percentage of Methods used for Qualitative Element-specific Detection of GMOs According to Genetic Targets and their own Rate of Modification



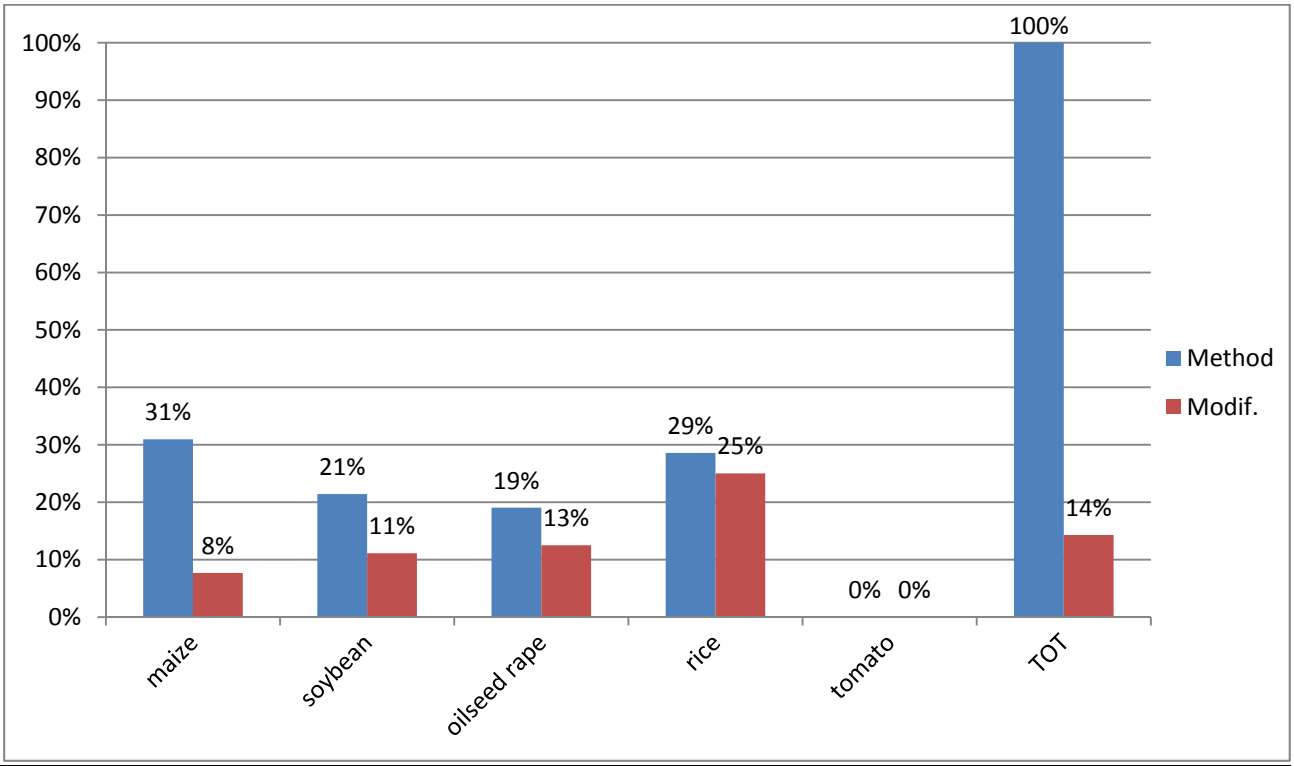
QL ELE Modification	CaMV P-35S 195 bp	CaMV P-35S 82 bp	CaMV P-35S 82 bp duplex	CaMV P-35S 75 bp	nptII 215 bp	nptII 173 bp	T-nos 180 bp
Methods Used	4	2	8	4	3	2	2
Methods Modified	0	0	5	0	0	0	0
Rate of Modification	0 %	0 %	63 %	0 %	0 %	0 %	0 %
% of Total Methods Used	4.6 %	2.3 %	9.2 %	4.6 %	3.4 %	2.3 %	2.3 %
QL ELE Modification	**T-nos 118 bp**	**T-nos 84 bp**	**T-nos 84 bp duplex**	**T-nos 69 bp**	**P-nos 94 bp**	**P-FMV 196 bp**	**P-FMV 78 bp**
Methods Used	4	3	11	5	5	3	2
Methods Modified	1	1	4	0	1	1	0
Rate of Modification	25 %	33 %	36 %	0 %	20 %	33 %	0 %
% of Total Methods Used	4.6 %	3.4 %	13 %	5.7 %	5.7 %	3.4 %	2.3 %
QL ELE Modification	**bar 60 bp**	**bar 69 bp**	**cry1Ab/Ac 74 bp**	**CP4-EPSPS 108 bp**	**cry1A(b) 73bp**	**pat 109 bp**	**TOT**
Methods used	10	1	8	2	6	2	87
Methods Modified	5	0	4	0	0	0	22
Rate of Modification	50 %	0 %	50 %	0 %	0 %	0 %	25 %
% of Total Methods Used	11 %	1.1 %	9.2 %	2.3 %	6.9 %	2.3 %	100 %

Legend: Method = Methods used; Modif = Methods modified (own ratio); P-35S = *Cauliflower Mosaic Virus* 35 S

Promoter; nptII = Neomycin phosphotransferase II gene; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; P-nos = Nopaline synthase promoter from *Agrobacterium tumefaciens*; P-FMV = *Figwort Mosaic Virus* 35S promoter; bar = Phosphinothricin N-acetyl transferase gene from *Streptomyces hygroscopicus;* cryIAb/Ac = Synthetic construct derived from *Bacillus thuringiensis;* CP4-EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* TOT = Total methods used for qualitative element-specific detection of GMOs; QL = Qualitative; ELE = Element-specific methods

h) Qualitative Taxon-specific Methods

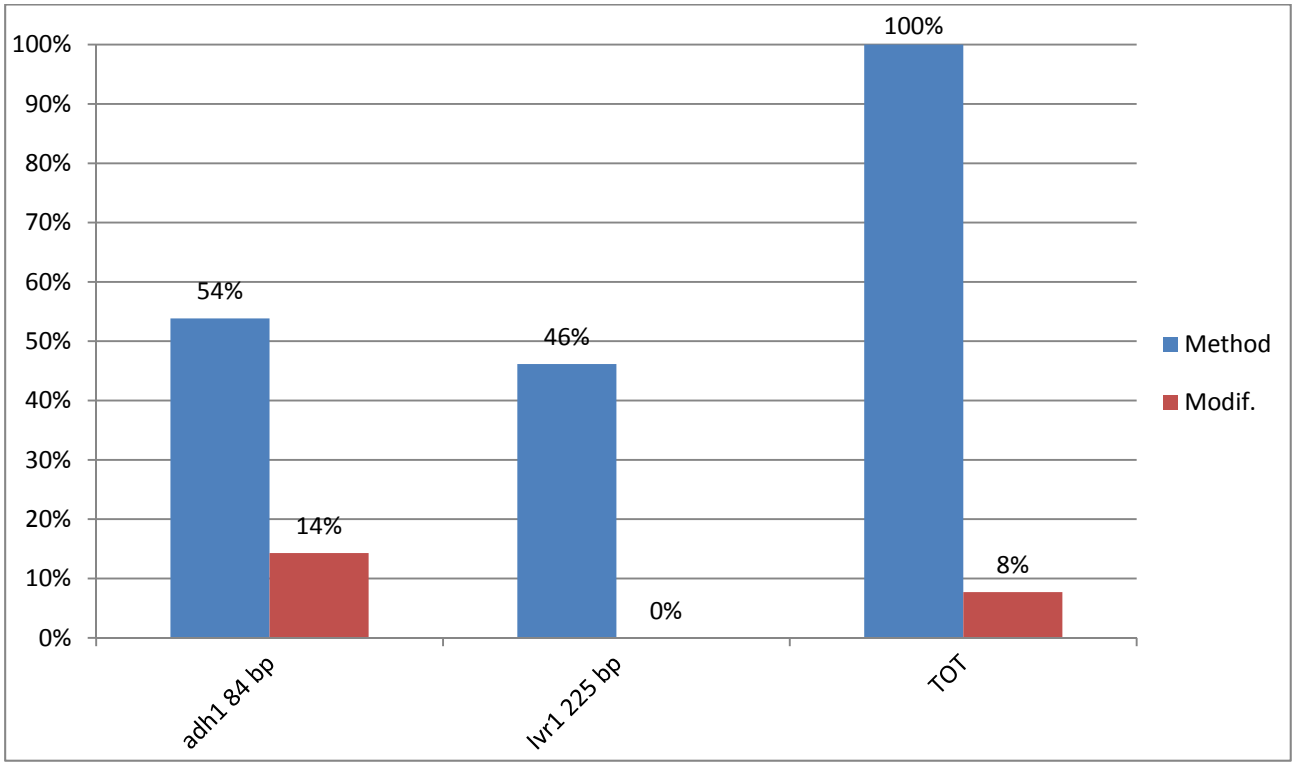
Figure 71 - Percentage of Methods used for Qualitative Taxon-specific Detection According to Species and their own Rate of Modifications



QL TAX Modification	maize	soybean	oilseed rape	rice	tomato	TOT
Methods Used	13	9	8	12	0	42
Methods Modified	1	1	1	3	0	6
Rate of Modification	7.7 %	11 %	13 %	25 %	0 %	14 %
% of Total Methods Used	31 %	21 %	19 %	29 %	0 %	100 %

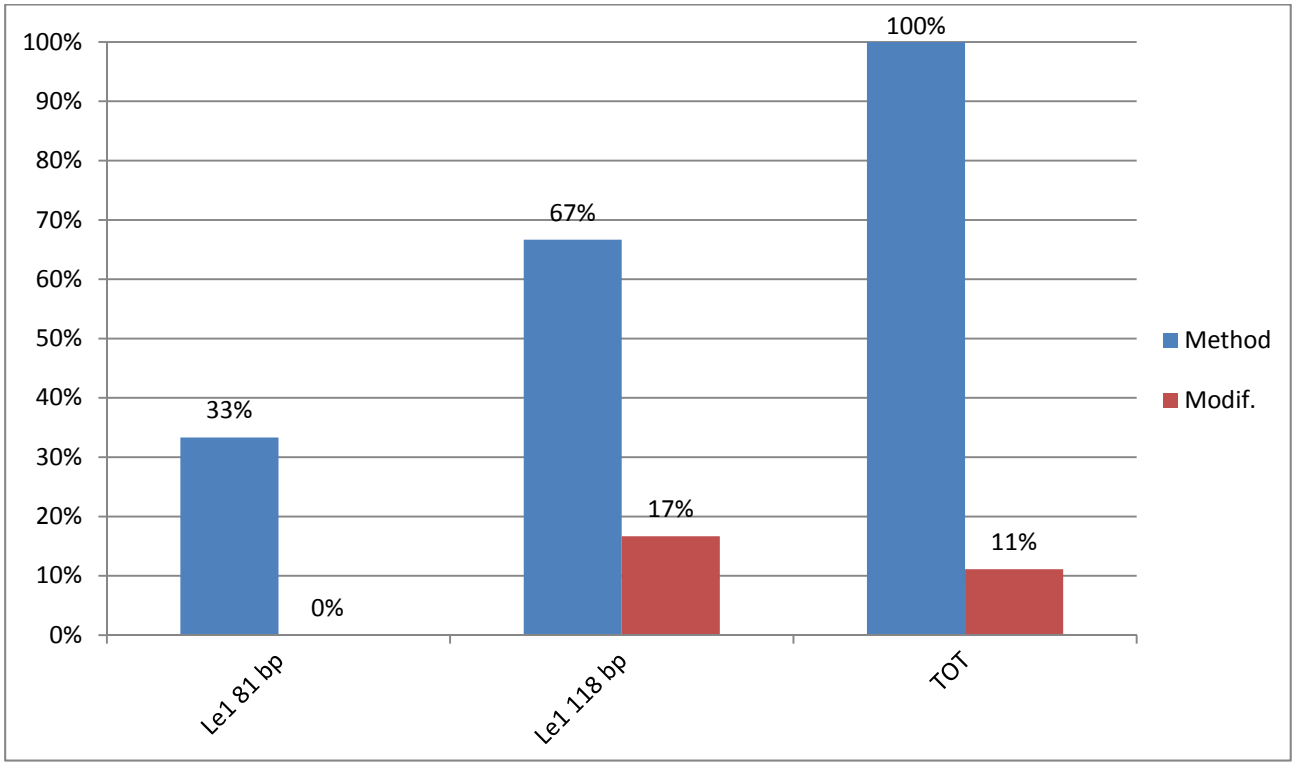
Legend: Method = Methods used; Modif = Methods modified (own ratio); TOT = Total methods used for qualitative taxon-specific detection of species; QL = Qualitative; TAX = Taxon-specific methods;

Figure 72 - Percentage of Methods used for Qualitative Detection of Maize Reference Genes and their own Rate of Modifications



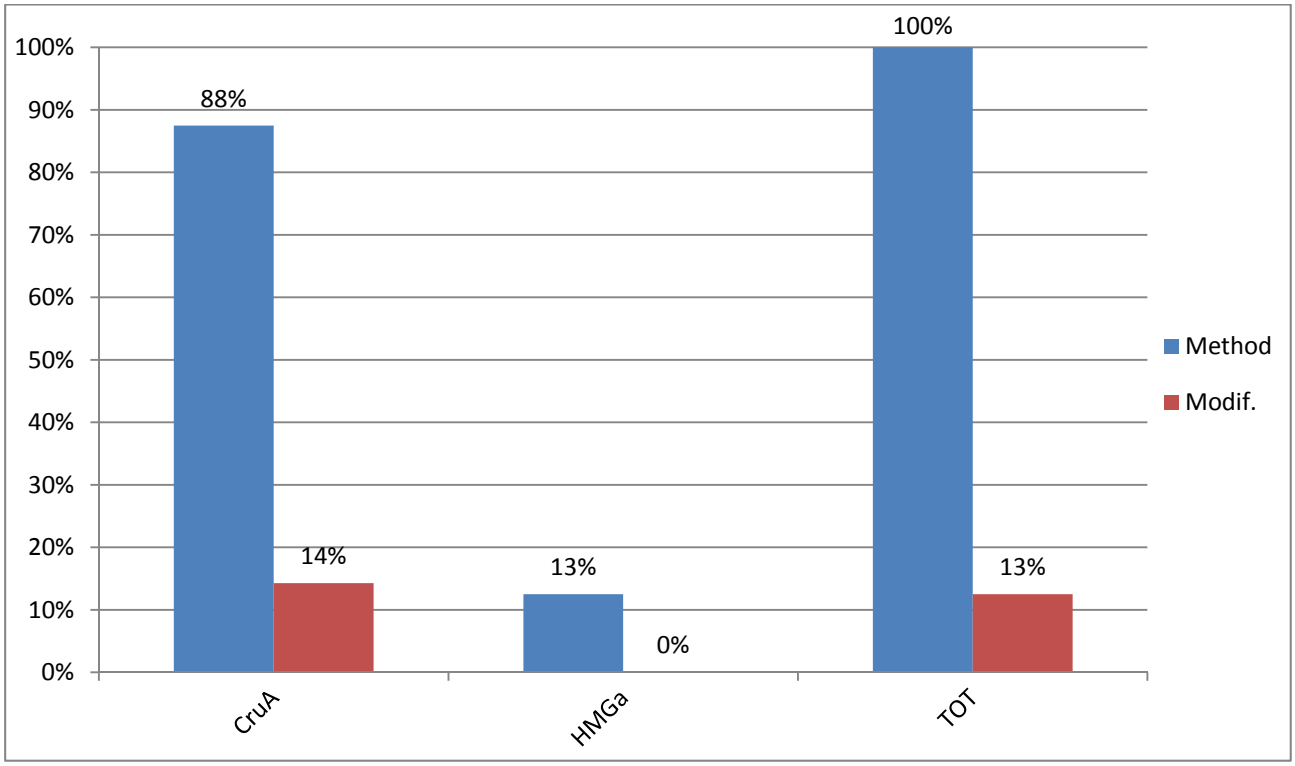
Legend: Method = Methods used; Modif = Methods modified; adh1 = Alcohol dehydrogenase1 gene; Ivr1 = Invertase gene; TOT = Total methods used for qualitative taxon-specific detection of maize

Figure 73 - Percentage of Methods used for Qualitative Detection of Soybean Reference Genes and their own Rate of Modifications



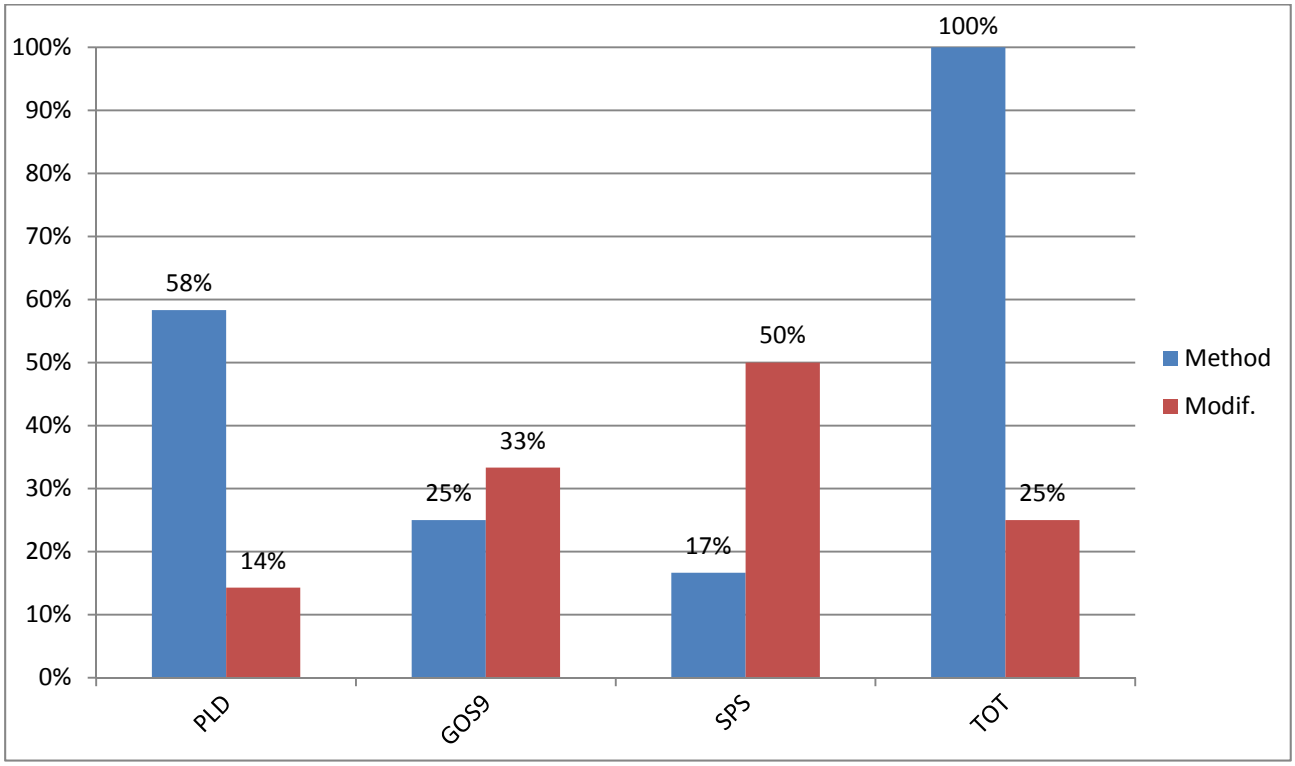
Legend: Method = Methods used; Modif = Methods modified; Le1 = Lectin gene; TOT = Total methods used for qualitative taxon-specific detection of soybean

Figure 74 - Percentage of Methods used for Qualitative Detection of Oilseed Rape Reference Genes and their own Rate of Modifications



Legend: Method = Methods used; Modif = Methods modified; CruA = Cruciferin A gene; HMGa = High mobility group protein I/Y gene; TOT = Total methods used for qualitative taxon-specific detection of oilseed rape

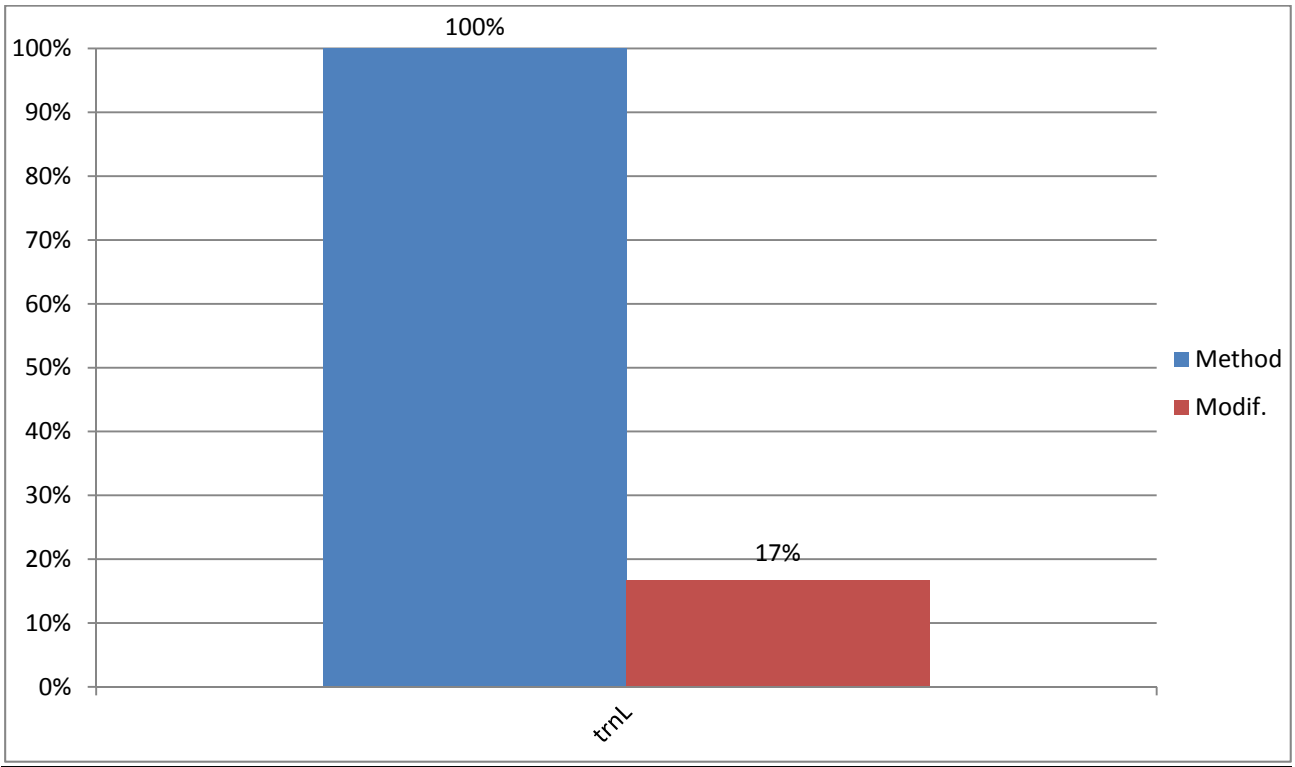
Figure 75 - Percentage of Methods used for Qualitative Detection of Rice Reference Genes and their own Rate of Modifications



Legend: Method = Methods used; Modif = Methods modified; PLD = Phospholipase D gene; GOS9 = Rice root-specific GOS9 gene; SPS = Sucrose-phosphate synthase gene; TOT = Total methods used for qualitative taxon-specific detection of rice

i) Qualitative Plant-specific Methods

Figure 76 - Percentage of Methods used for Qualitative Plant-specific Detection and their own Rate of Modifications



QL PLN Modification	trnL	TOT
Methods Used	6	6
Methods Modified	1	1
Rate of Modification	17 %	17 %
% of Total Methods Used	100 %	100 %

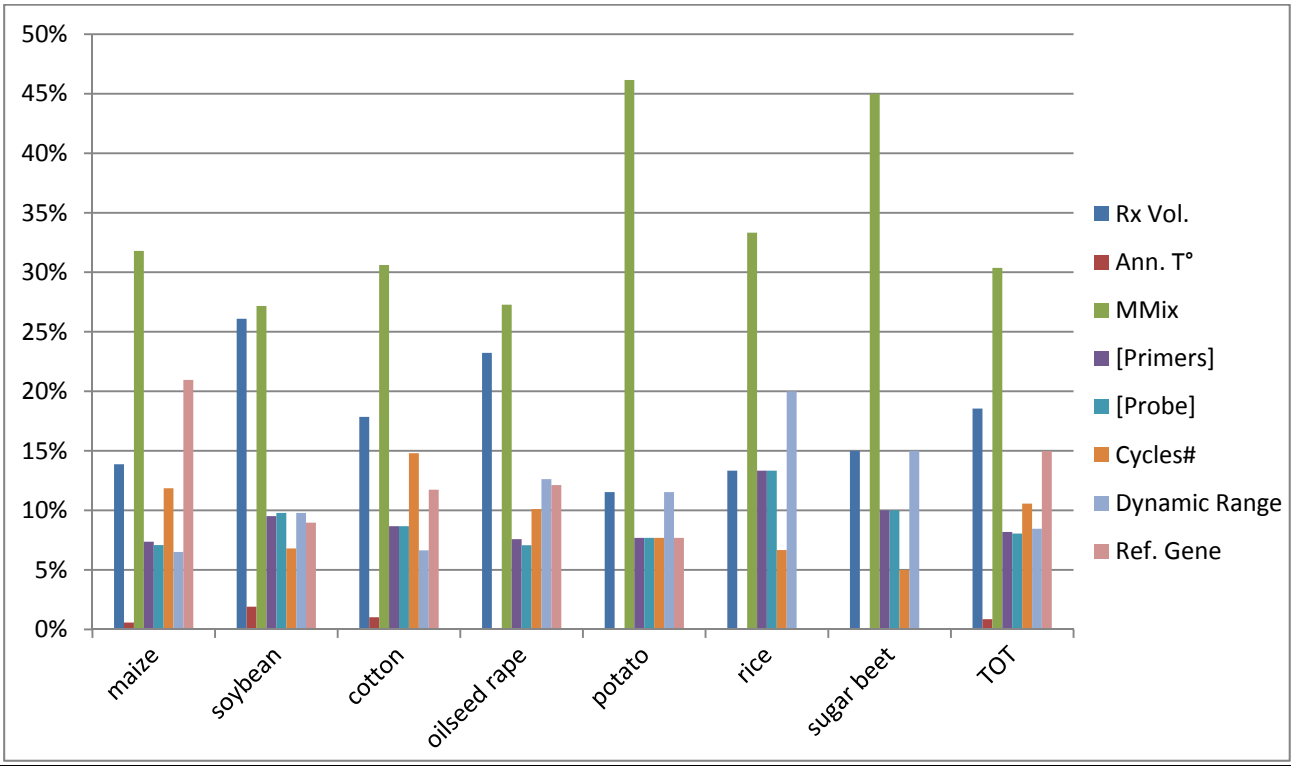
Legend: Method = Methods used; Modif = Method modified (own ratio); QL = Qualitative; PLN = Plant-specific methods

11.3 Type and Frequency of Modifications for EU Reference Methods

The charts in this section present the frequencies of the different type of modifications according to GM species, GM events, target elements or crops. The bars in the column charts represent the relative contribution of each type of modification to the total number of modifications implemented in each method's classification.

a) Quantitative Event-specific Methods

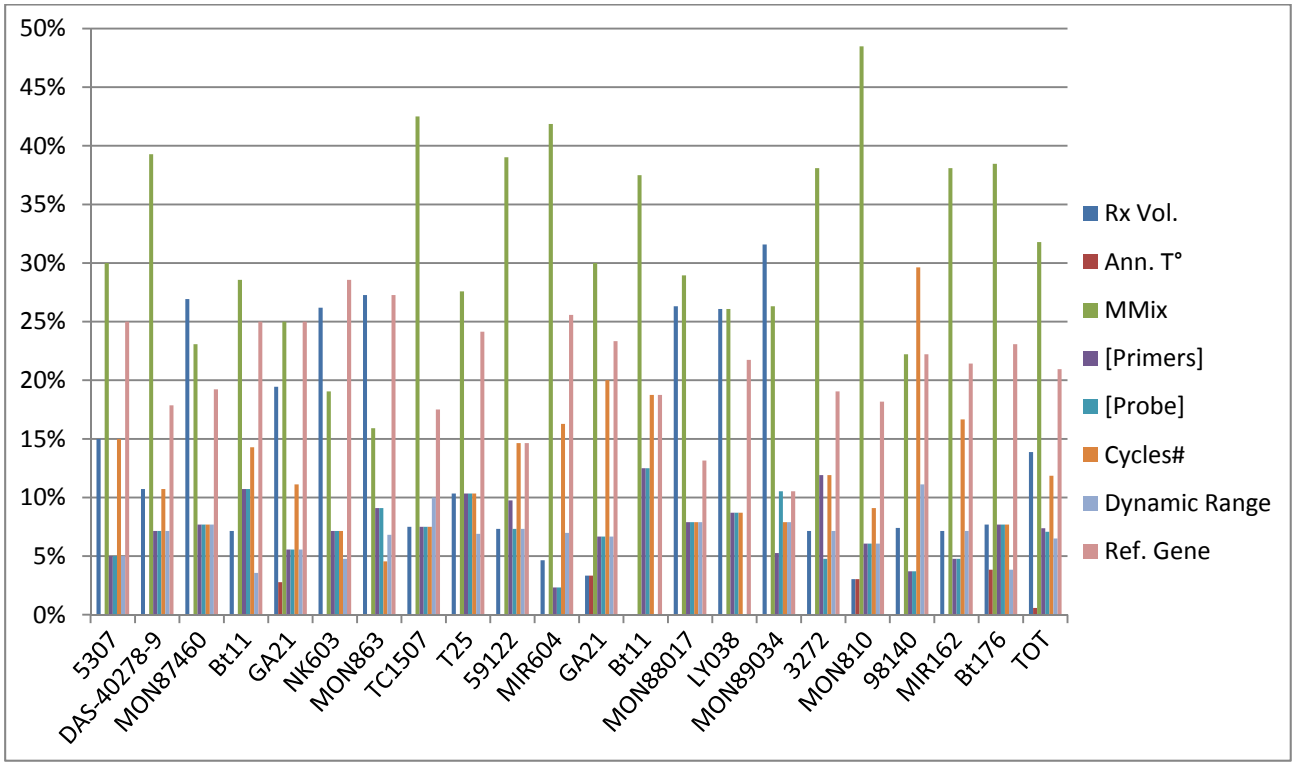
Figure 77 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to GM Species



QT EVE Modification	maize	soybean	cotton	oilseed rape	potato	rice	sugar beet	TOT
Rx Vol.	96	96	35	46	3	2	3	281
Ann. T°	4	7	2	0	0	0	0	13
MMix	220	100	60	54	12	5	9	460
[Primers]	51	35	17	15	2	2	2	124
[Probe]	49	36	17	14	2	2	2	122
Cycles#	82	25	29	20	2	1	1	160
Dynamic Range	45	36	13	25	3	3	3	128
Ref. Gene	145	33	23	24	2	0	0	227
Total Modifications	692	368	196	198	26	15	20	1515

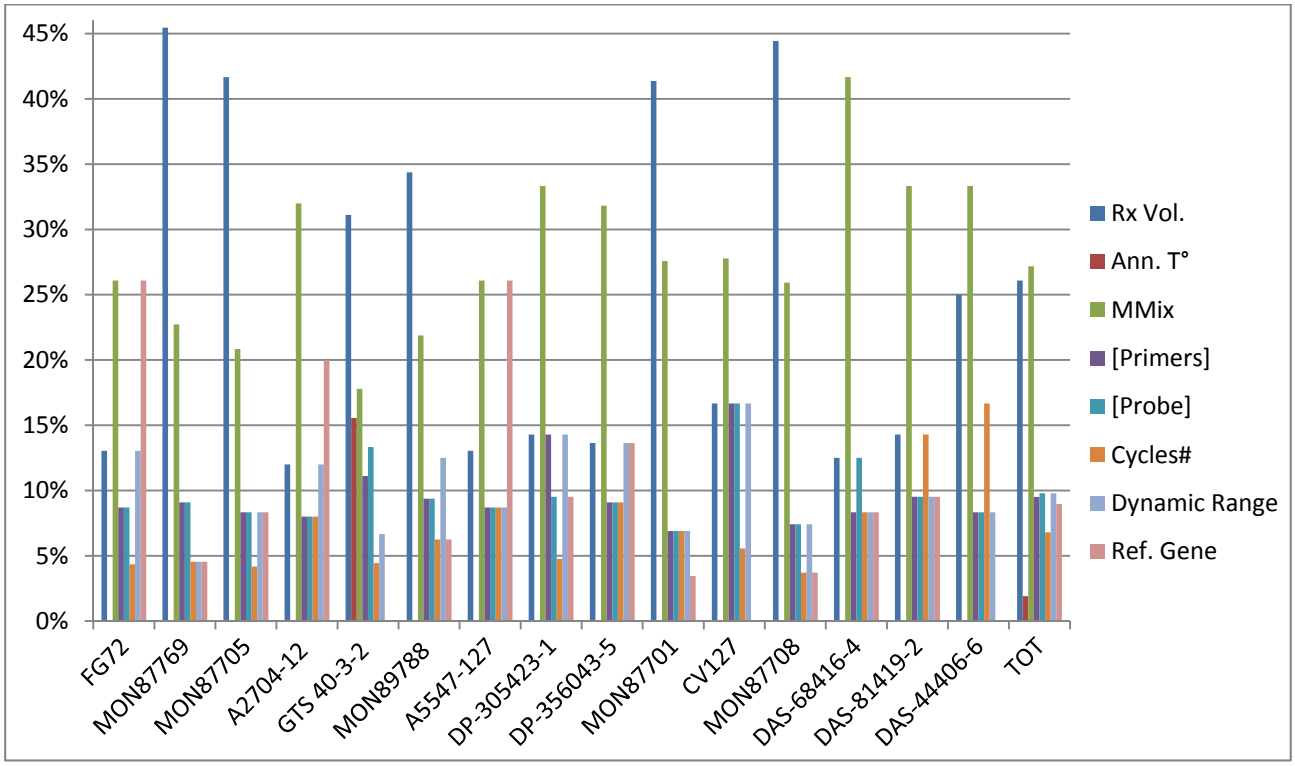
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific methods; QT = Quantitative; EVE = Event-specific methods;

Figure 78 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Maize GMOs



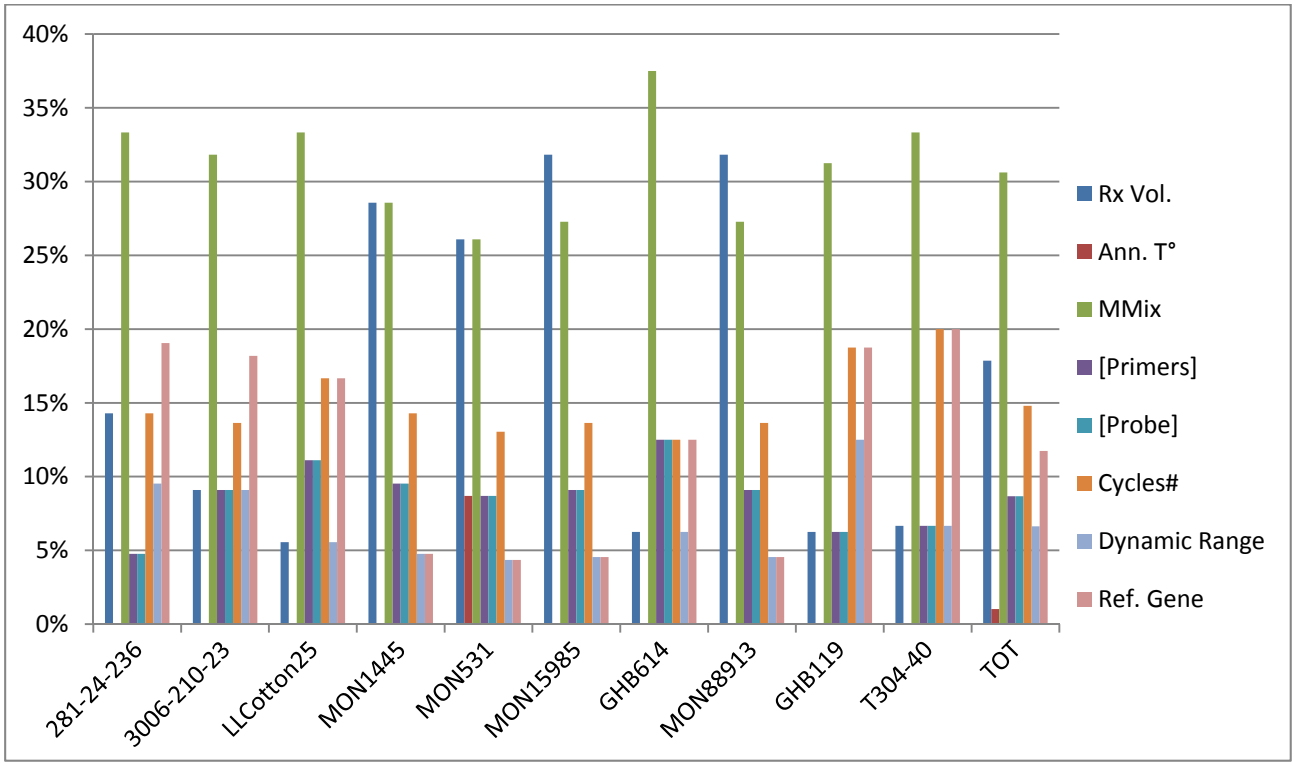
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific maize methods

Figure 79 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Soybean GMOs



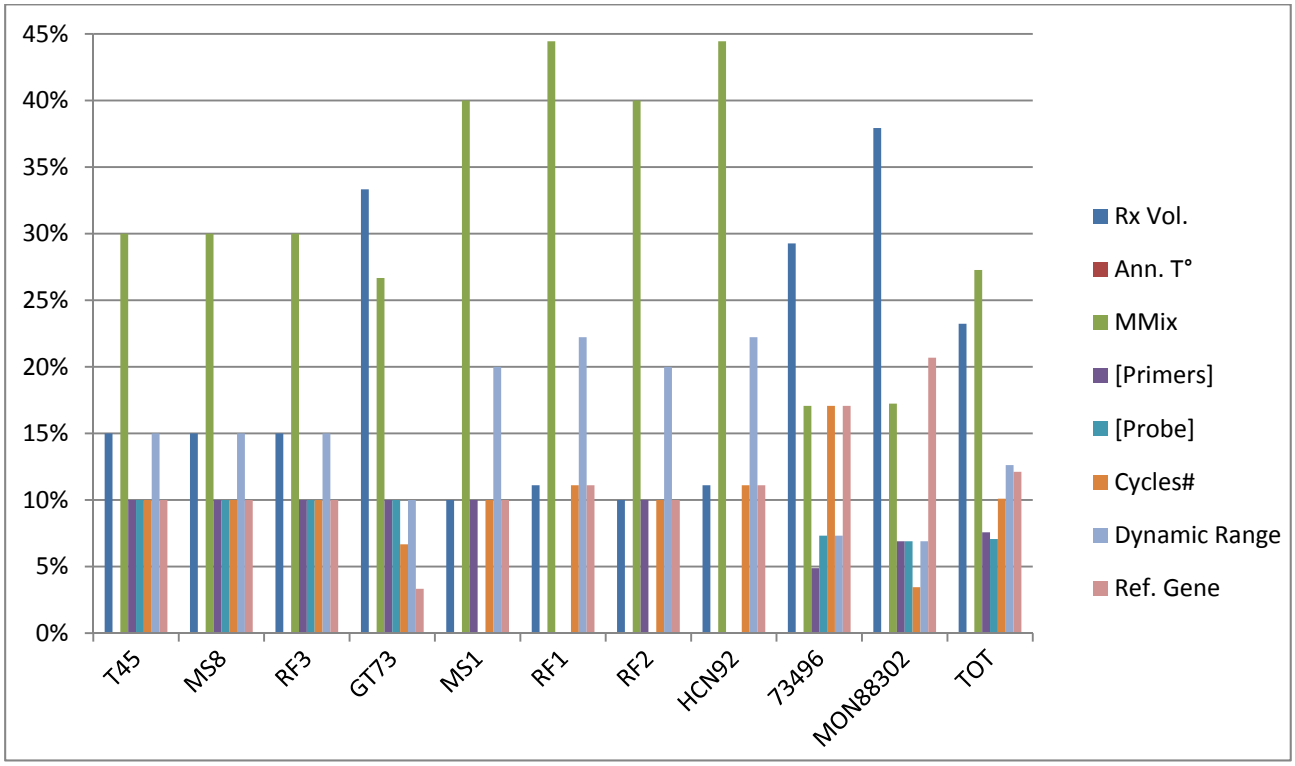
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific soybean methods

Figure 80 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Cotton GMOs



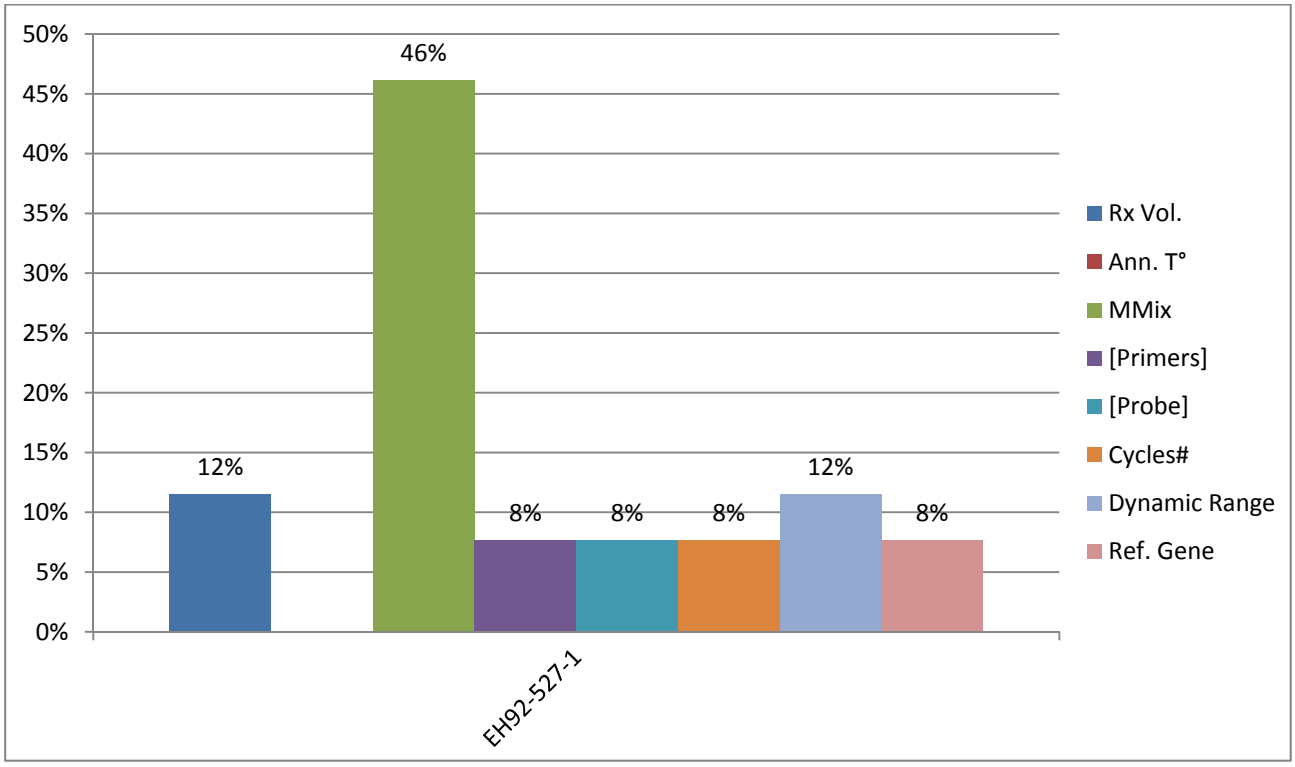
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific cotton methods

Figure 81 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Oilseed Rape GMOs



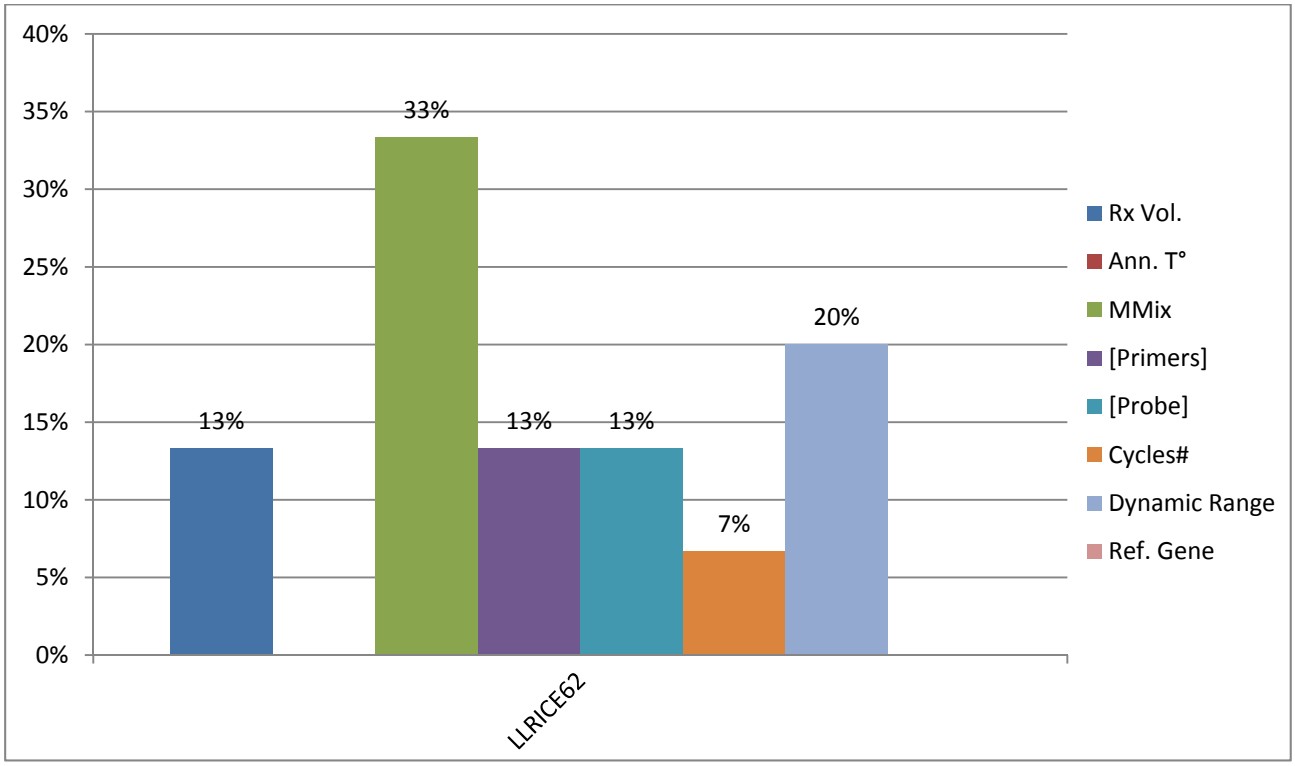
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative event-specific oilseed rape methods

Figure 82 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Potato GMOs



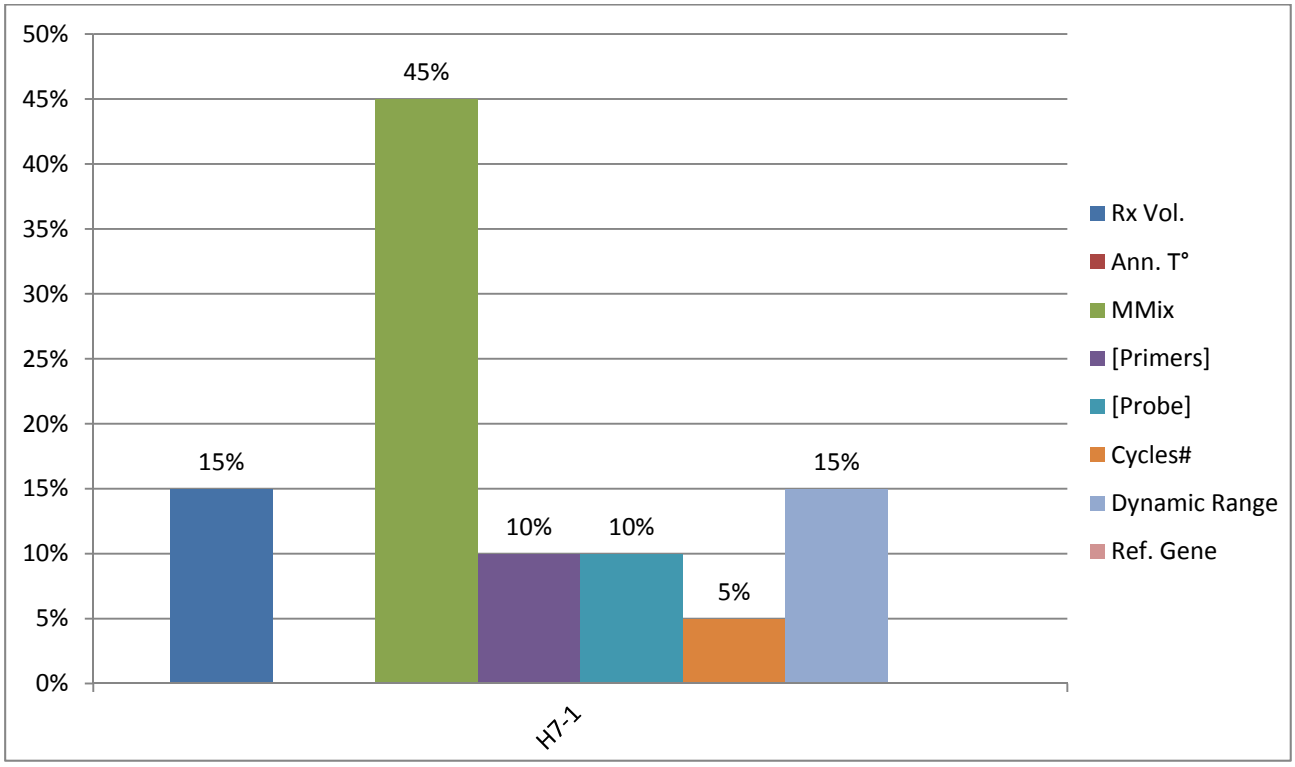
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene

Figure 83 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Rice GMOs



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene

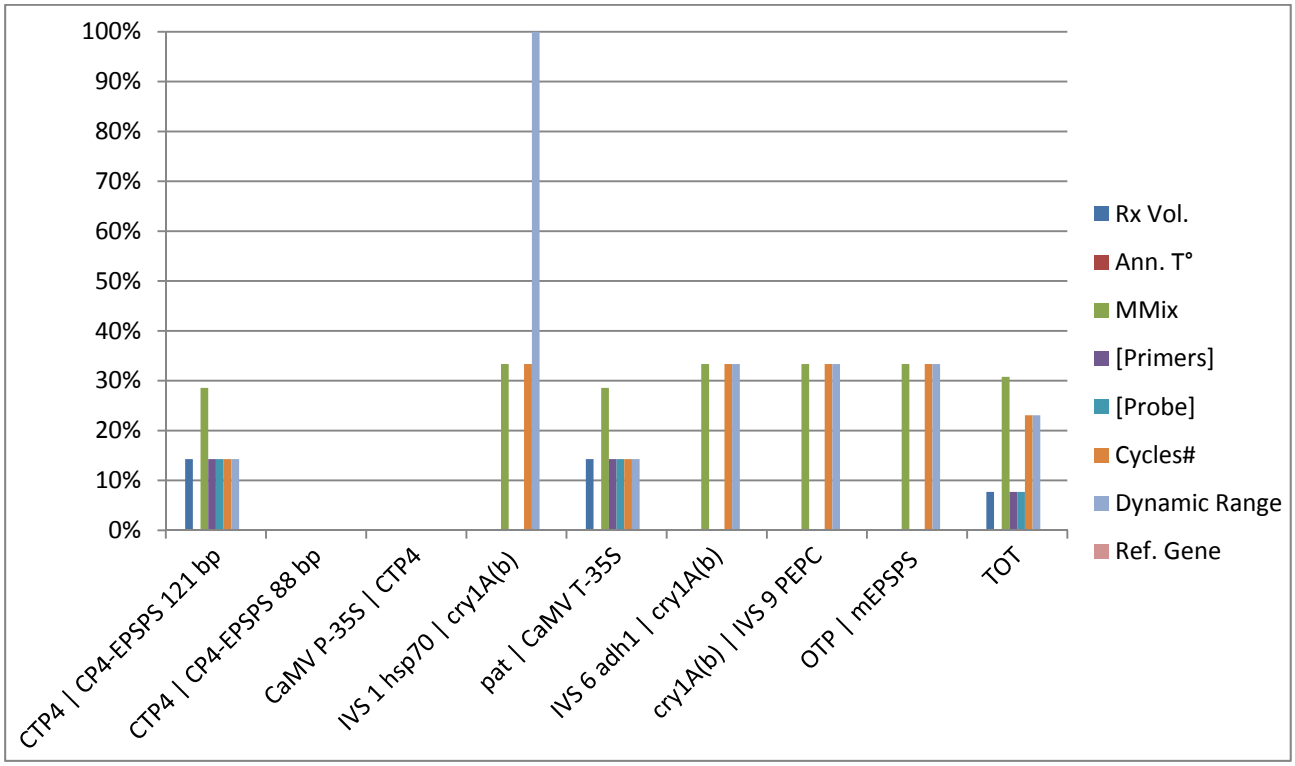
Figure 84 - Type and Frequency of Modifications for Quantitative Event-specific Methods According to Sugar Beet GMOs



Legend: Rx Vol. = PCR reaction volume; Ann. T° =Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene

b) Quantitative Construct-specific Methods

Figure 85 - Type and Frequency of Modifications for Quantitative Construct-specific Methods According to Genetic Targets

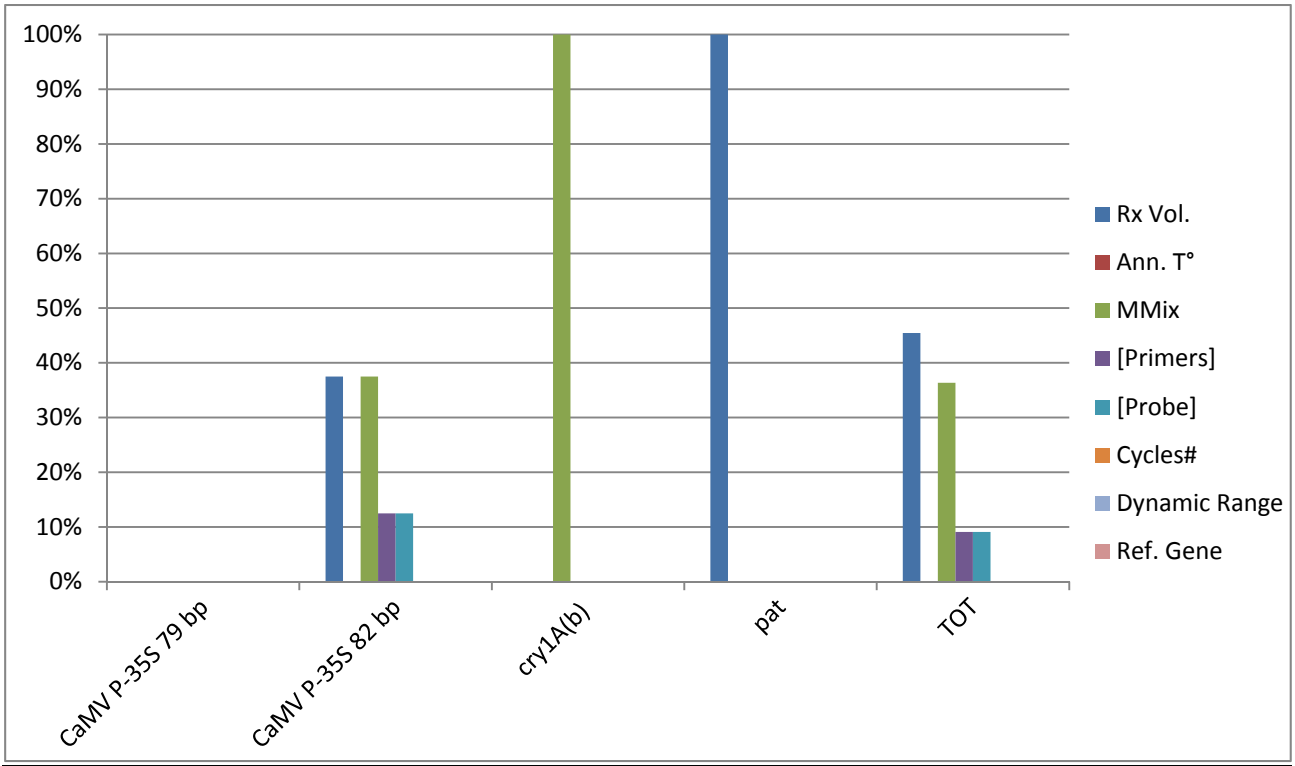


QT CON Modification	CTP4 | CP4-EPSPS 121 bp	CTP4 | CP4-EPSPS 88 bp	CaMV P-35S | CTP4	IVS 1 hsp70 | cry1A(b)	pat | CaMV T-35S	IVS 6 adh1 | cry1A(b)	cry1A(b) | IVS 9 PEPC	OTP | mEPSPS	TOT
Rx Vol.	1	0	0	0	1	0	0	0	2
Ann. T°	0	0	0	0	0	0	0	0	0
MMix	2	0	0	1	2	1	1	1	8
[Primers]	1	0	0	0	1	0	0	0	2
[Probe]	1	0	0	0	1	0	0	0	2
Cycles#	1	0	0	1	1	1	1	1	6
Dynamic Range	1	0	0	1	1	1	1	1	6
Ref. Gene	0	0	0	0	0	0	0	0	0
Total Modifications	7	0	0	3	7	3	3	3	26

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; CTP4 = Chloplast transit peptide coding sequence from *Petunia hybrida*; CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; IVS 1 hsp70 = Intervening sequence 1 from *Zea mays hsp70* gene; cry1A(b) = *cry1Ab* delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes*; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; IVS 6 adh1 = Intervening sequence 6 from *Zea mays* alcohol dehydrogenase 1 gene; IVS 9 PEPC = Intervening sequence 9 from the phospho-enol-pyruvate carboxylase gene; OTP = Optimized transit peptide sequence from *Zea mays*; mEPSPS = Point mutated epsps gene from *Zea mays*; TOT = Type and frequency of modifications for all quantitative construct-specific methods; QT = Quantitative; CON = Construct-specific methods

c) Quantitative Element-specific Methods

Figure 86 - Type and Frequency of Modifications for Quantitative Element-specific Methods According to Genetic Targets



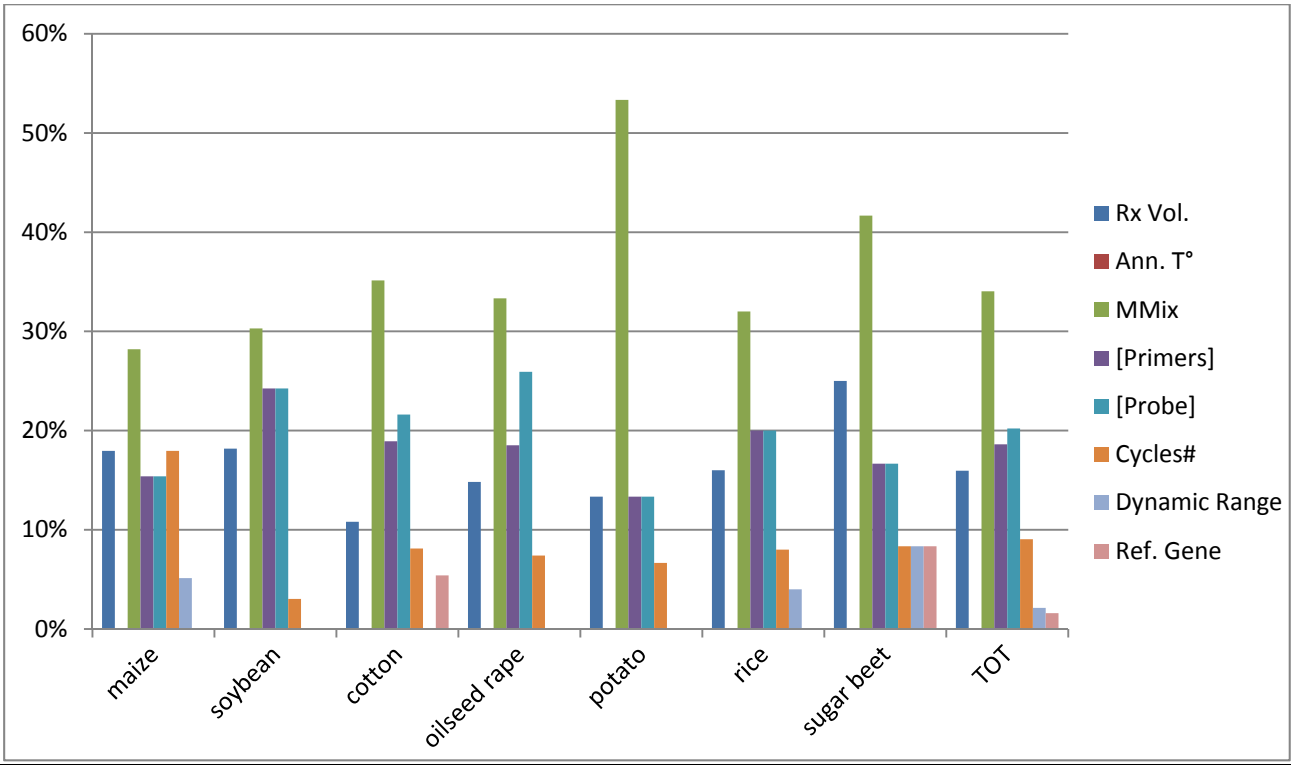
QT ELE Modification	CaMV P-35S 79 bp	CaMV P-35S 82 bp	cry1A(b)	pat	TOT
Rx Vol.	0	3	0	2	5
Ann. T°	0	0	0	0	0
MMix	0	3	1	0	4

QT ELE Modification	CaMV P-35S 79 bp	CaMV P-35S 82 bp	cry1A(b)	pat	TOT
[Primers]	0	1	0	0	1
[Probe]	0	1	0	0	1
Cycles#	0	0	0	0	0
Dynamic Range	0	0	0	0	0
Ref. Gene	0	0	0	0	0
Total Modifications	0	8	1	2	11

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; CaMV P-35S = *Cauliflower Mosaic Virus* 35S Promoter; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes;* TOT = Type and frequency of modifications for all quantitative element-specific methods; QT = Quantitative; ELE = Element-specific methods;

d) Quantitative Taxon-specific Methods

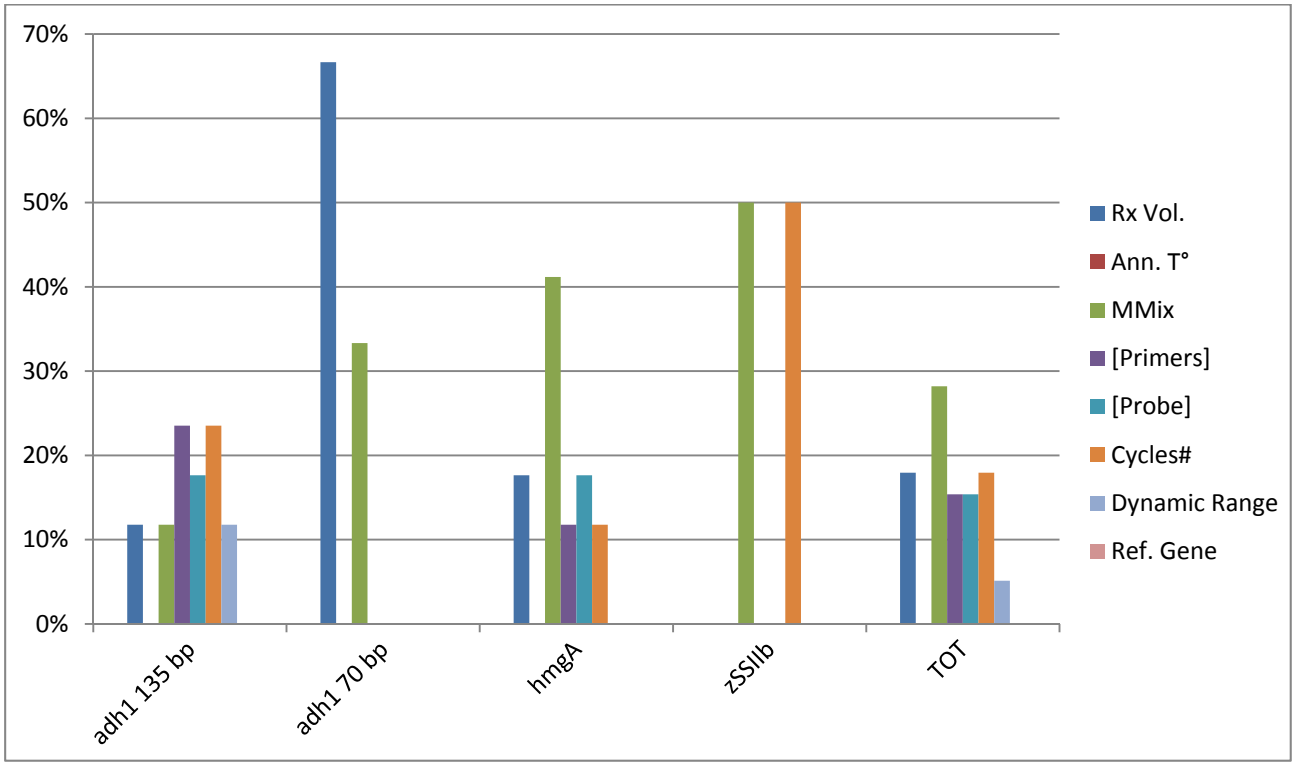
Figure 87 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Species



QT TAX Modification	maize	soybean	cotton	oilseed rape	potato	rice	sugar beet	TOT
Rx Vol.	7	6	4	4	2	4	3	30
Ann. T°	0	0	0	0	0	0	0	0
MMix	11	10	13	9	8	8	5	64
[Primers]	6	8	7	5	2	5	2	35
[Probe]	6	8	8	7	2	5	2	38
Cycles#	7	1	3	2	1	2	1	17
Dynamic Range	2	0	0	0	0	1	1	4
Ref. Gene	0	0	2	0	0	0	1	3
Total Modifications	39	33	37	27	15	25	12	188

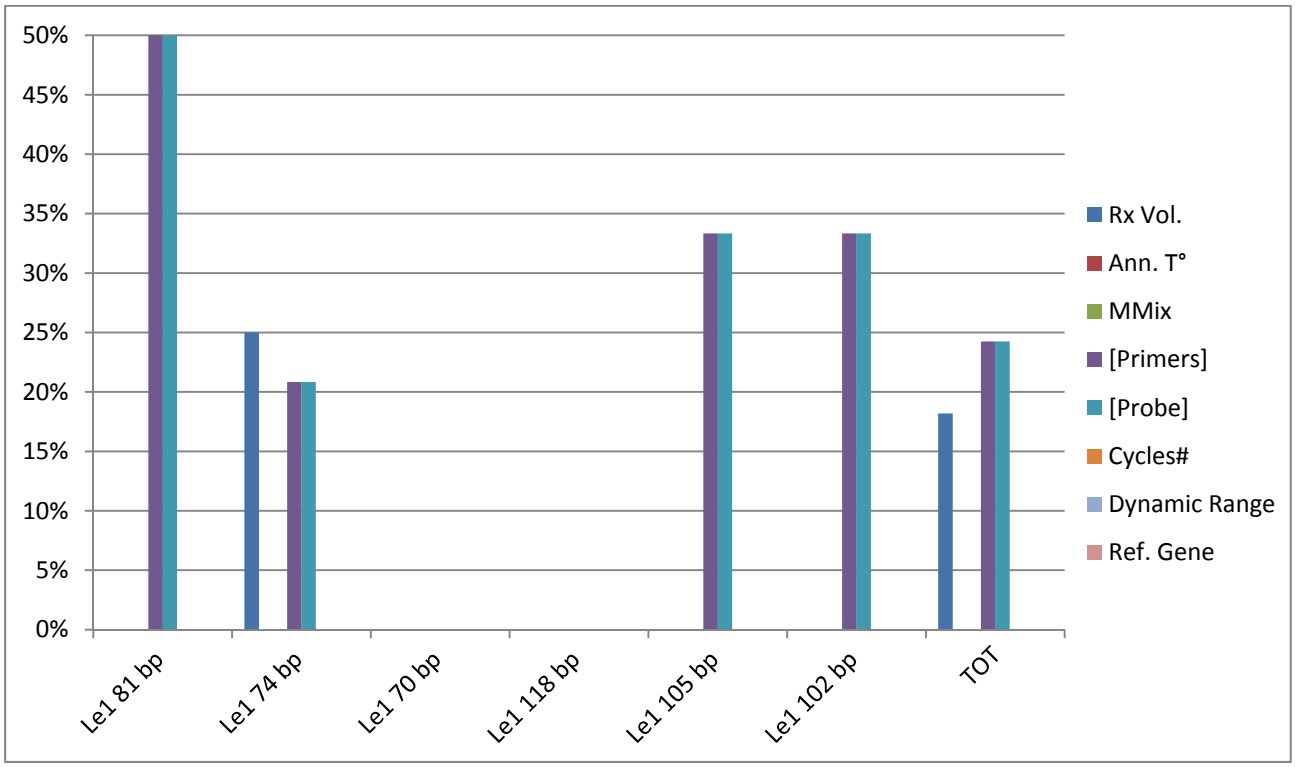
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all quantitative taxon-specific methods; QT = Quantitative; TAX = Taxon-specific methods;

Figure 88 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Maize Reference Genes



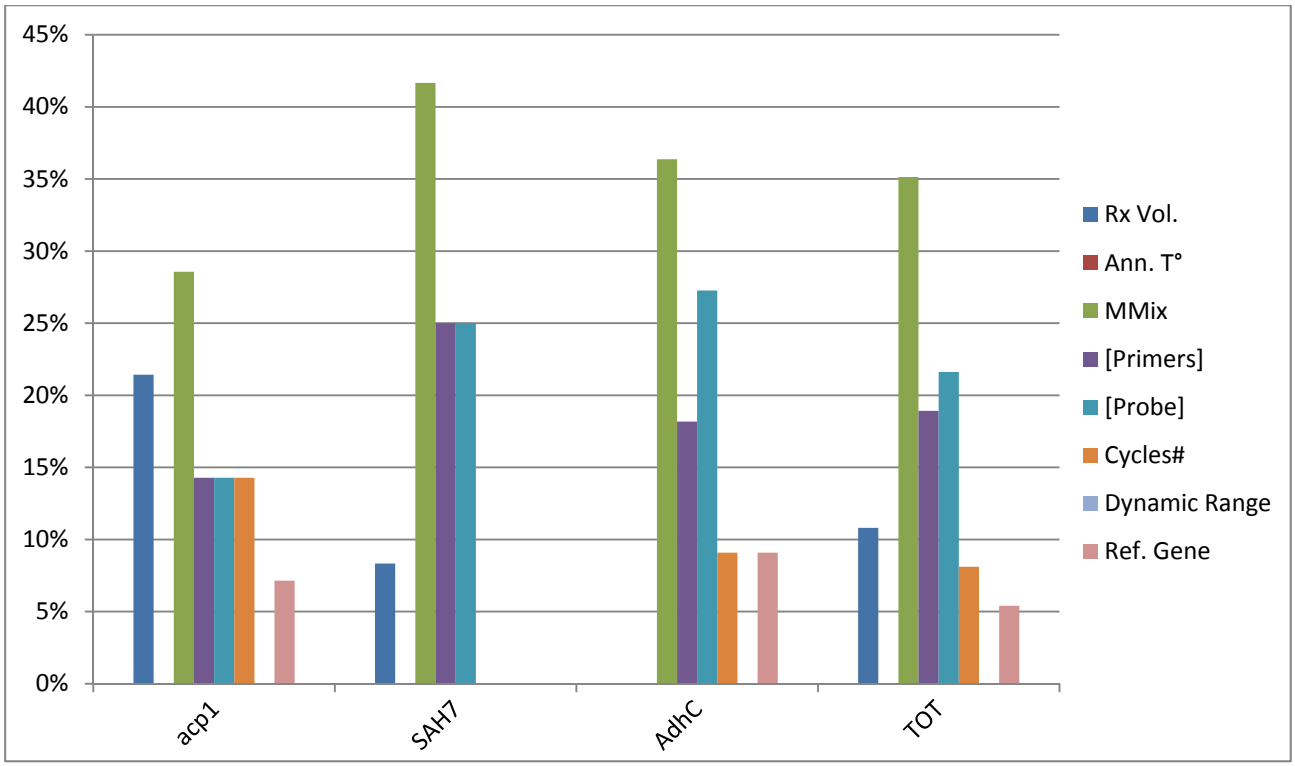
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; adh1 = Alcohol dehydrogenase1 gene; hmgA = High-mobility-group A gene; zSSIIb = Maize starch synthase IIb gene; TOT = Type and frequency of modifications for all quantitative taxon-specific maize methods

Figure 89 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Soybean Reference Genes



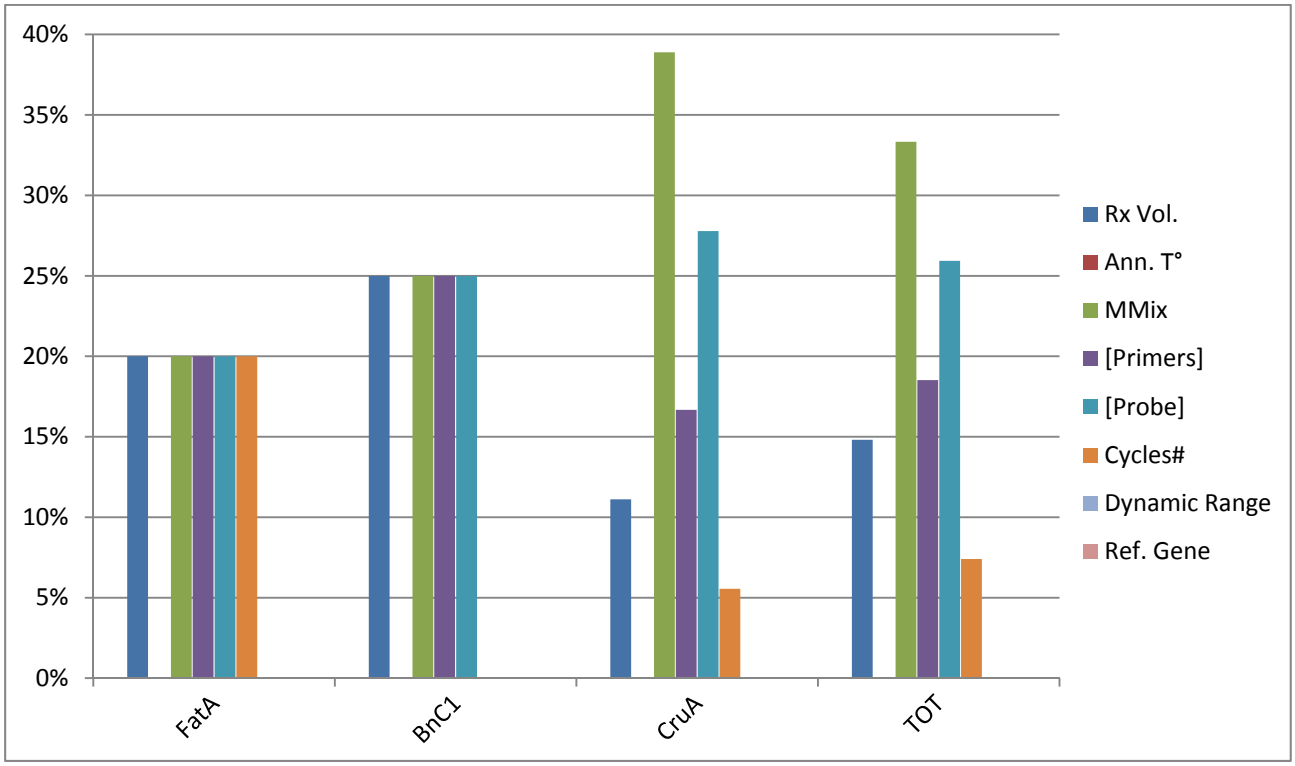
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; Le1 = Lectin gene; TOT = Type and frequency of modifications for all quantitative taxon-specific soybean methods

Figure 90 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Cotton Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; acp1 = Acyl carrier protein 1 gene; SAH7 = IVS of the putative *Sinapis Arabidopsis Homolog 7* protein gene; AdhC = Alcohol dehydrogenase C gene; TOT = Type and frequency of modifications for all quantitative taxon-specific cotton methods

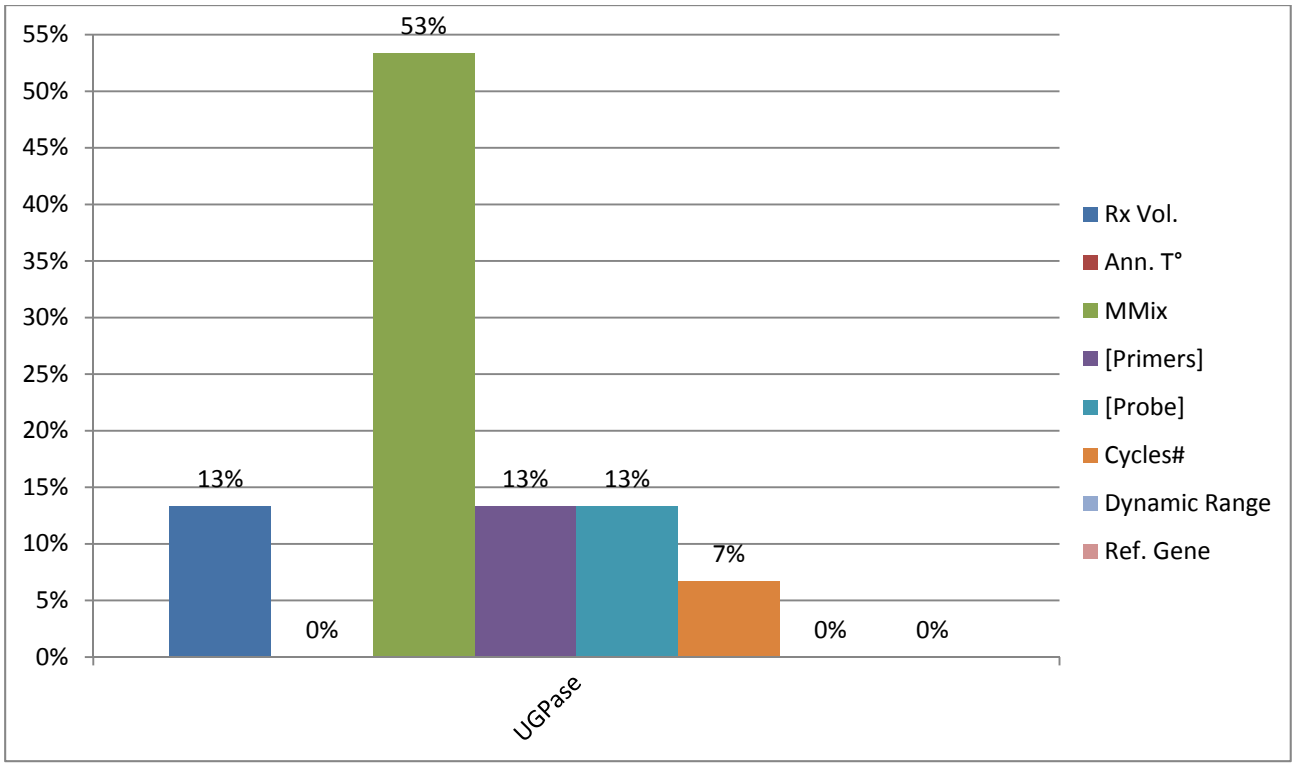
Figure 91 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Oilseed Rape Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference

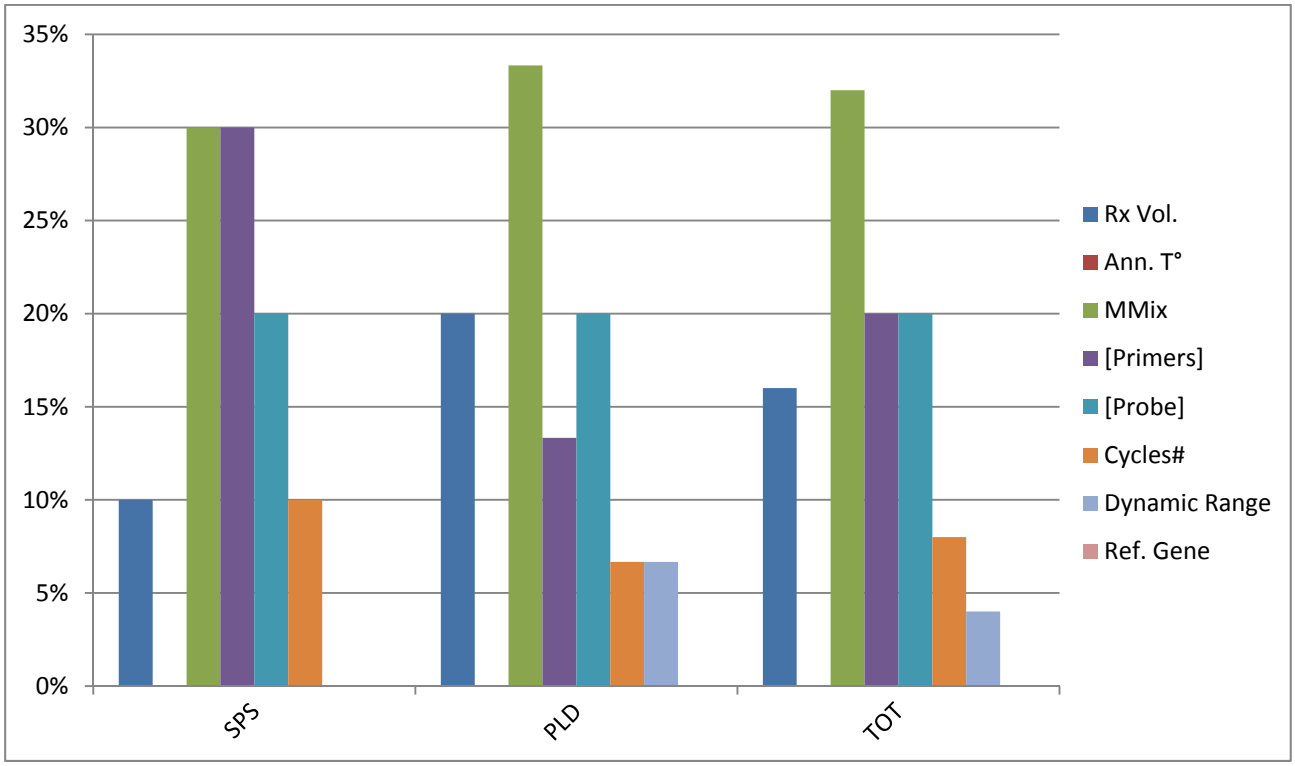
gene; FatA = Acyl-ACP thioesterase gene; BnC1 = Cruciferin storage protein gene; CruA = Cruciferin A gene; TOT = Type and frequency of modifications for all quantitative taxon-specific oilseed rape methods

Figure 92 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Potato Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; UGPase = UDP-glucose pyrophosphorylase gene

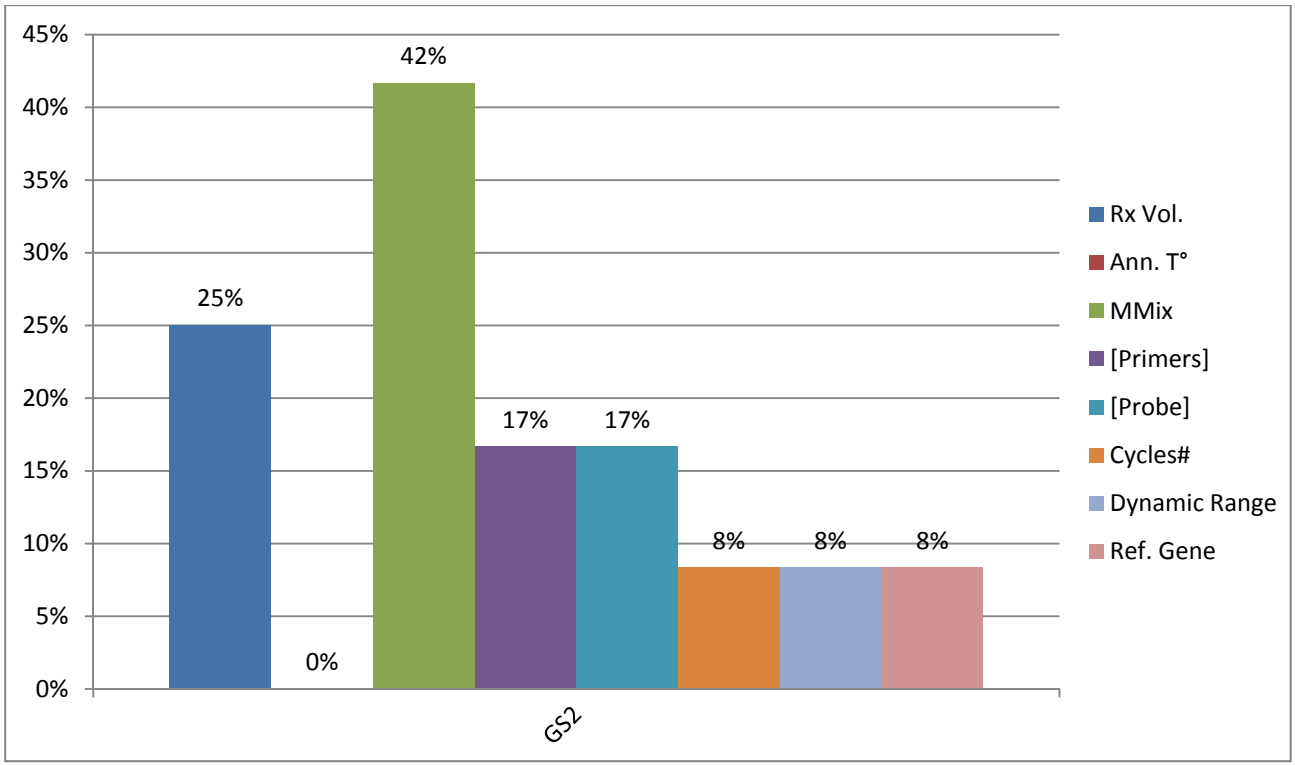
Figure 93 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Rice Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference

gene; SPS = Sucrose-phosphate synthase gene; PLD = Phospholipase D gene; TOT = Type and frequency of modifications for all quantitative taxon-specific rice methods

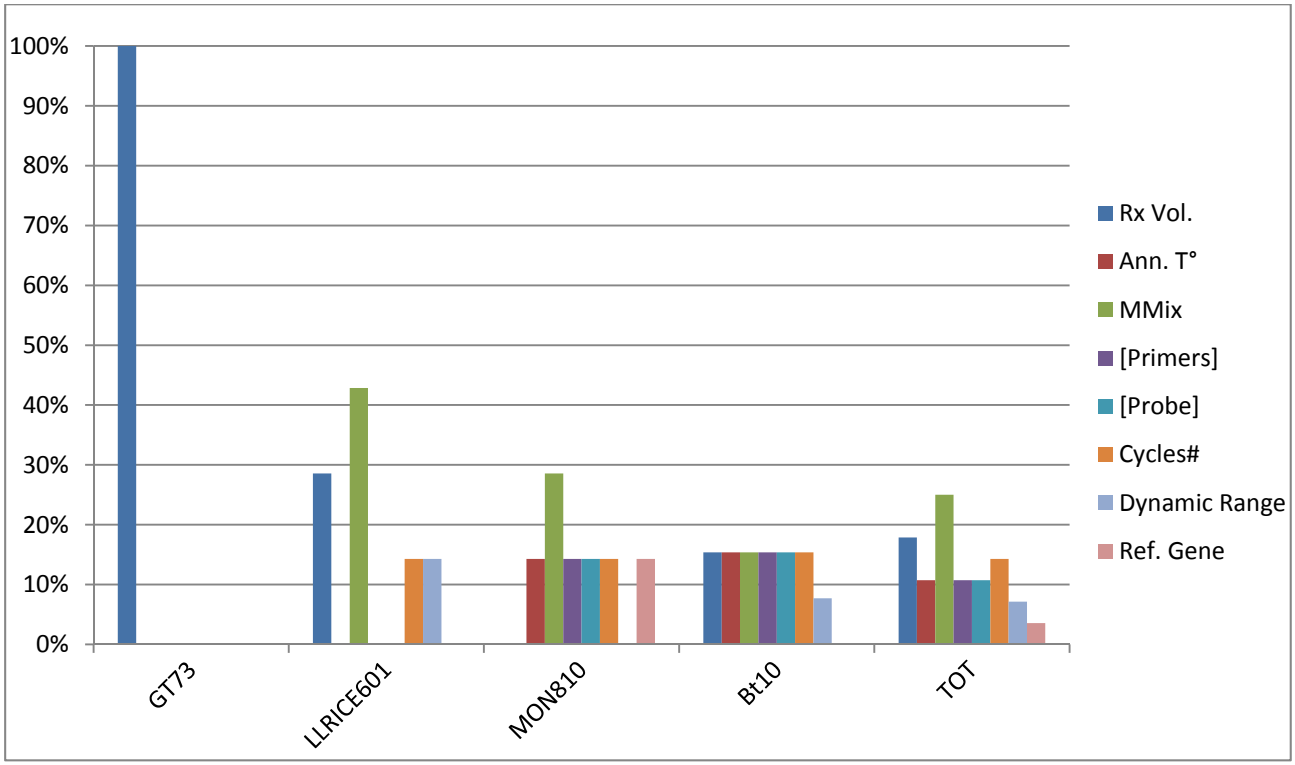
Figure 94 - Type and Frequency of Modifications for Quantitative Taxon-specific Methods According to Sugar Beet Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; GS2 = Glutamine synthetase gene

e) Qualitative Event-specific Methods

Figure 95 - Type and Frequency of Modifications for Qualitative Event-specific Methods According to GMOs

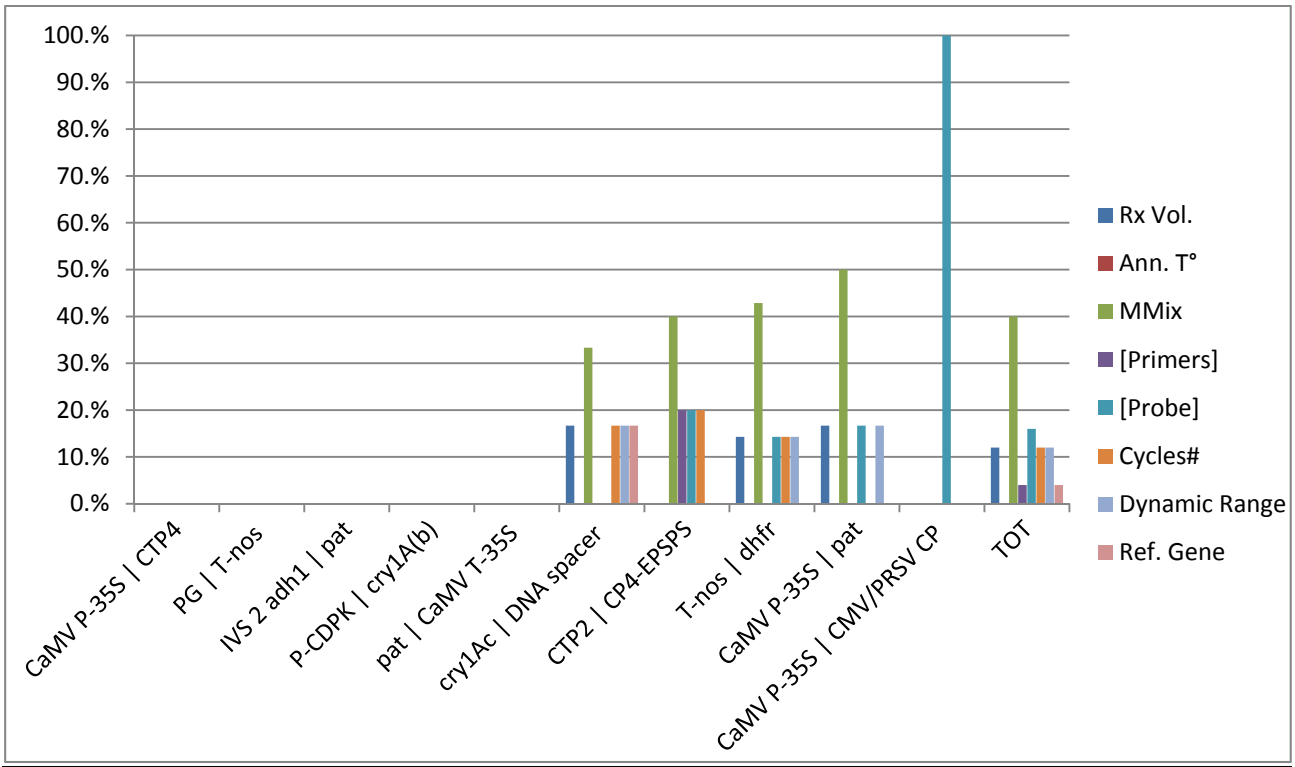


QL EVE Modification	GT73	LLRICE601	MON810	Bt10	TOT
Rx Vol.	1	2	0	2	5
Ann. T°	0	0	1	2	3
MMix	0	3	2	2	7
[Primers]	0	0	1	2	3
[Probe]	0	0	1	2	3
Cycles#	0	1	1	2	4
Dynamic Range	0	1	0	1	2
Ref. Gene	0	0	1	0	1
Total Modifications	1	7	7	13	28

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all qualitative event-specific methods; QL = Qualitative; EVE = Event-specific methods;

f) Qualitative Construct-specific Methods

Figure 96 - Type and Frequency of Modifications for Qualitative Construct-specific Methods According to Genetic Targets



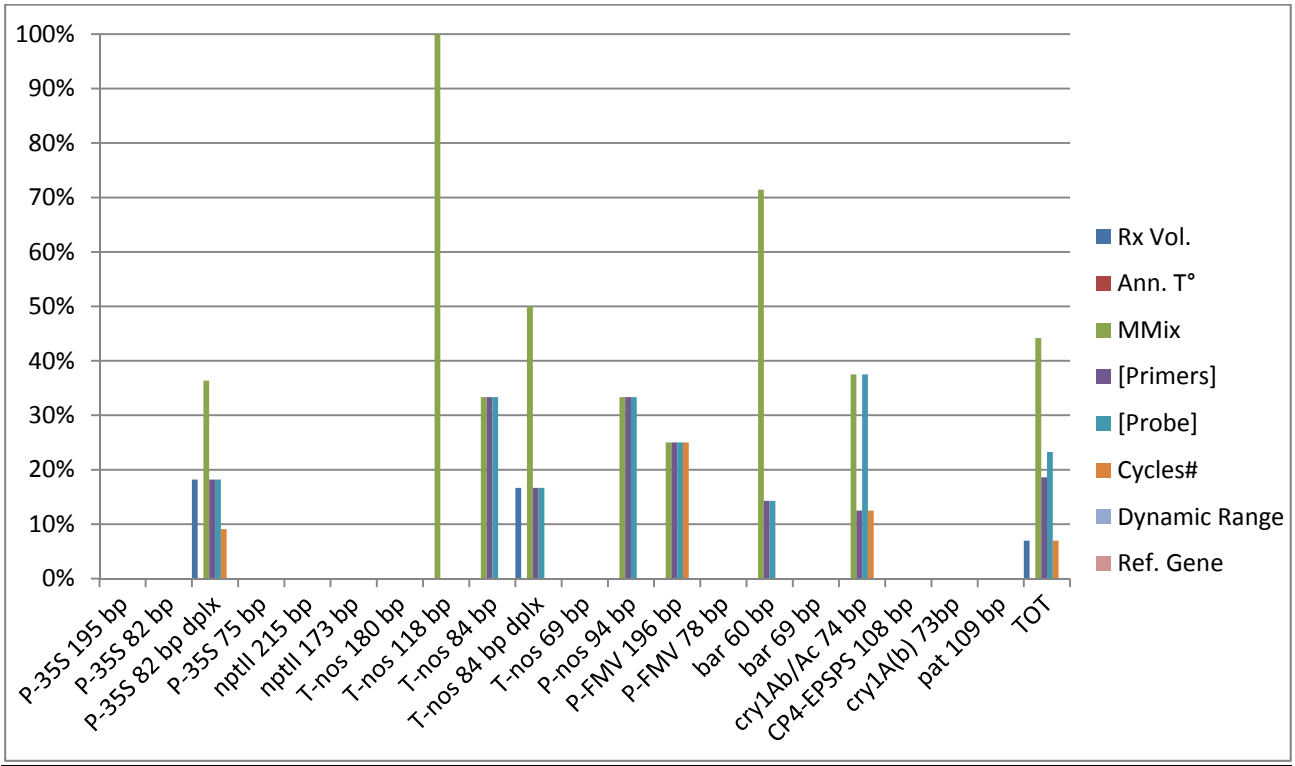
QL CON Modification	CaMV P-35S | CTP4	PG | T-nos	IVS 2 adh1 | pat	P-CDPK | cry1A(b)	pat | CaMV T-35S	
Rx Vol.	0	0	0	0	0	
Ann. T°	0	0	0	0	0	
MMix	0	0	0	0	0	
[Primers]	0	0	0	0	0	
[Probe]	0	0	0	0	0	
Cycles#	0	0	0	0	0	
Dynamic Range	0	0	0	0	0	
Ref. Gene	0	0	0	0	0	
Total Modifications	0	0	0	0	0	

QL CON Modification	cry1Ac | DNA spacer	CTP2 | CP4-EPSPS	T-nos | dhfr	CaMV P-35S | pat	CaMV P-35S | CMV/PRSV CP	TOT
Rx Vol.	1	0	1	1	0	3
Ann. T°	0	0	0	0	0	0
MMix	2	2	3	3	0	10
[Primers]	0	1	0	0	0	1
[Probe]	0	1	1	1	1	4
Cycles#	1	1	1	0	0	3
Dynamic Range	1	0	1	1	0	3
Ref. Gene	1	0	0	0	0	1
Total Modifications	6	5	7	6	1	25

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; CaMV P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; CTP4 = Chloplast transit peptide coding sequence from *Petunia hybrida epsps* gene; PG = Polygalacturonase gene from *Solanum lycopersicum*; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; IVS 2 adh1 = Intervening sequence 2 from *Zea mays* alcohol dehydrogenase 1 gene; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* P-CDPK = Promoter of *Zea mays* calcium-dependent protein kinase gene; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; CaMV T-35S = *Cauliflower Mosaic Virus* 35S Terminator; CTP2 = Chloroplast transit peptide 2 sequence from *Arabidopsis thaliana epsps* gene; CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; dhfr = Dihydrofolate reductase gene; CMV/PRSV CP = Chimeric *Cucumber mosaic virus* coat protein/*Papaya ring spot virus* coat protein; TOT = Type and frequency of modifications for all qualitative construct-specific methods; QL = Qualitative; CON = Construct-specific methods

g) Qualitative Element-specific Methods

Figure 97 - Type and Frequency of Modifications for Qualitative Element-specific Methods According to Genetic Targets



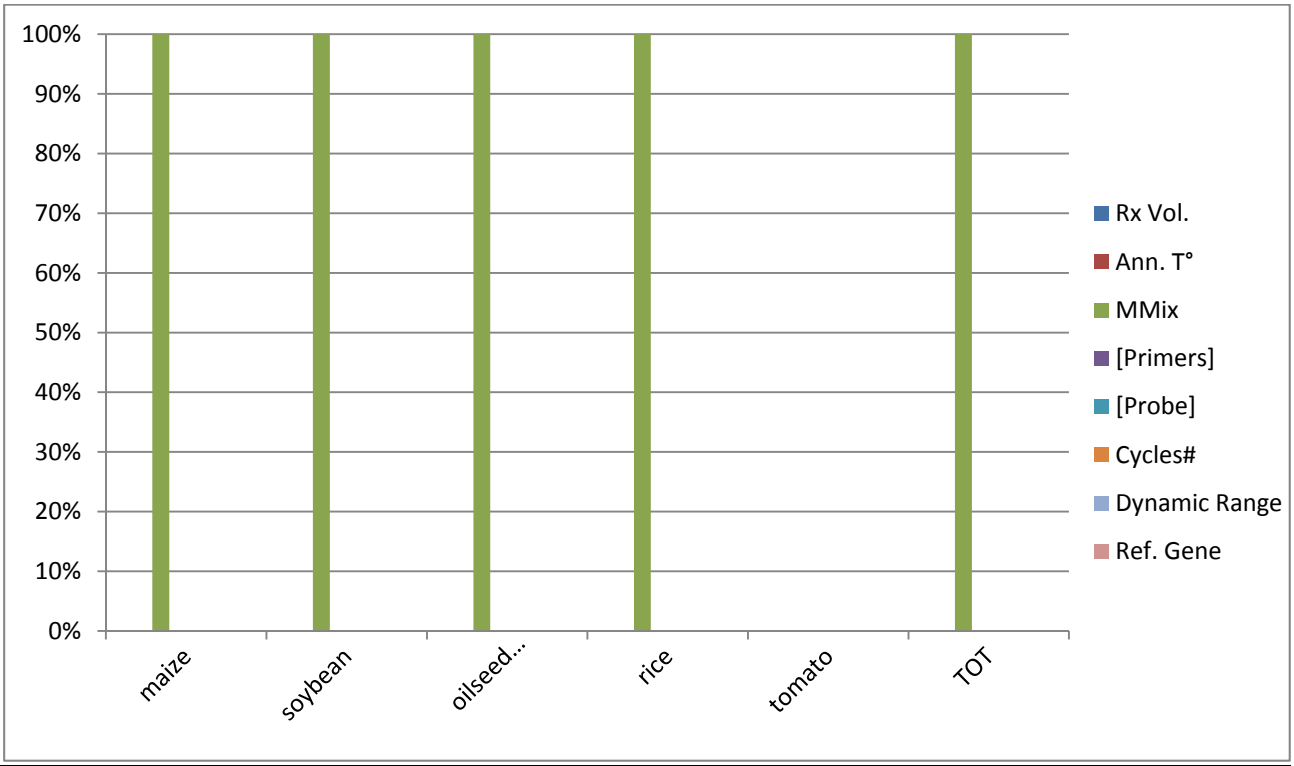
QL ELE Modification	CaMV P-35S 195 bp	CaMV P-35S 82 bp	CaMV P-35S 82 bp duplex	CaMV P-35S 75 bp	nptII 215 bp	nptII 173 bp	T-nos 180 bp
Rx Vol.	0	0	2	0	0	0	0
Ann. T°	0	0	0	0	0	0	0
MMix	0	0	4	0	0	0	0
[Primers]	0	0	2	0	0	0	0
[Probe]	0	0	2	0	0	0	0

QL ELE Modification	CaMV P-35S 195 bp	CaMV P-35S 82 bp	CaMV P-35S 82 bp duplex	CaMV P-35S 75 bp	nptII 215 bp	nptII 173 bp	T-nos 180 bp
Cycles#	0	0	1	0	0	0	0
Dynamic Range	0	0	0	0	0	0	0
Ref. Gene	0	0	0	0	0	0	0
Total Modifications	0	0	11	0	0	0	0
QL ELE Modification	**T-nos 118 bp**	**T-nos 84 bp**	**T-nos 84 bp duplex**	**T-nos 69 bp**	**P-nos 94 bp**	**P-FMV 196 bp**	**P-FMV 78 bp**
Rx Vol.	0	0	1	0	0	0	0
Ann. T°	0	0	0	0	0	0	0
MMix	1	1	3	0	1	1	0
[Primers]	0	1	1	0	1	1	0
[Probe]	0	1	1	0	1	1	0
Cycles#	0	0	0	0	0	1	0
Dynamic Range	0	0	0	0	0	0	0
Ref. Gene	0	0	0	0	0	0	0
Total Modifications	1	3	6	0	3	4	0
QL ELE Modification	**bar 60 bp**	**bar 69 bp**	**cry1Ab/Ac 74 bp**	**CP4-EPSPS 108 bp**	**cry1A(b) 73bp**	**pat 109 bp**	**TOT**
Rx Vol.	0	0	0	0	0	0	3
Ann. T°	0	0	0	0	0	0	0
MMix	5	0	3	0	0	0	19
[Primers]	1	0	1	0	0	0	8
[Probe]	1	0	3	0	0	0	10
Cycles#	0	0	1	0	0	0	3
Dynamic Range	0	0	0	0	0	0	0
Ref. Gene	0	0	0	0	0	0	0
Total Modifications	7	0	8	0	0	0	43

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; P-35S = *Cauliflower Mosaic Virus* 35 S Promoter; nptII = Neomycin phosphotransferase II gene; T-nos = Nopaline synthase terminator from *Agrobacterium tumefaciens*; P-nos = Nopaline synthase promoter from *Agrobacterium tumefaciens*; P-FMV = *Figwort Mosaic Virus* 35S promoter; bar = Phosphinothricin N-acetyl transferase gene from *Streptomyces hygroscopicus;* cryIAb/Ac = Synthetic construct derived from *Bacillus thuringiensis;* CP4-EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4; cry1A(b) = cry1Ab delta-endotoxin from *Bacillus thuringiensis* subsp. Kurstaki; pat = Phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes;* TOT = Type and frequency of modifications for all qualitative element-specific methods; QL = Qualitative; ELE = Element-specific methods

h) Qualitative Taxon-specific Methods

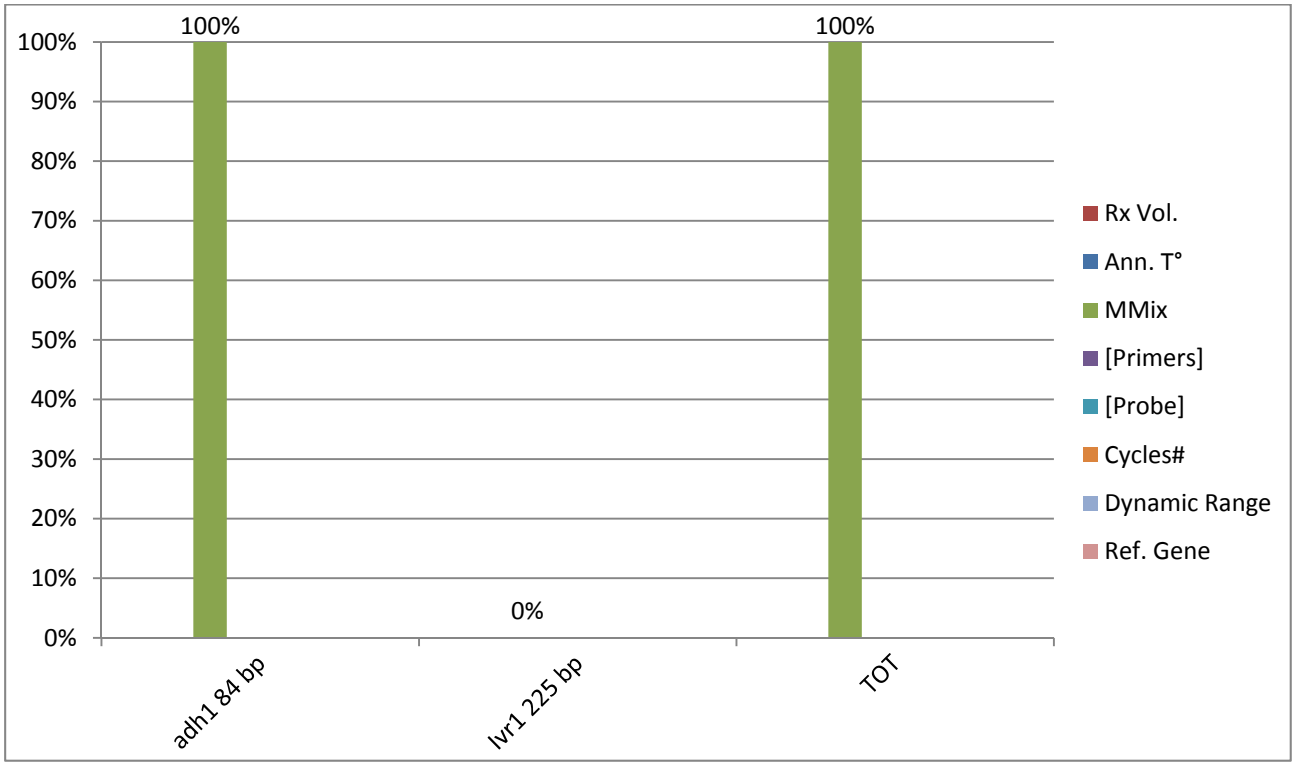
Figure 98 - Type and Frequency of Modifications for Qualitative Taxon-specific Methods According to Species



QL TAX Modification	maize	soybean	oilseed rape	rice	tomato	TOT
Rx Vol.	0	0	0	0	0	0
Ann. T°	0	0	0	0	0	0
MMix	1	1	1	3	0	6
[Primers]	0	0	0	0	0	0
[Probe]	0	0	0	0	0	0
Cycles#	0	0	0	0	0	0
Dynamic Range	0	0	0	0	0	0
Ref. Gene	0	0	0	0	0	0
Total Modifications	1	1	1	3	0	6

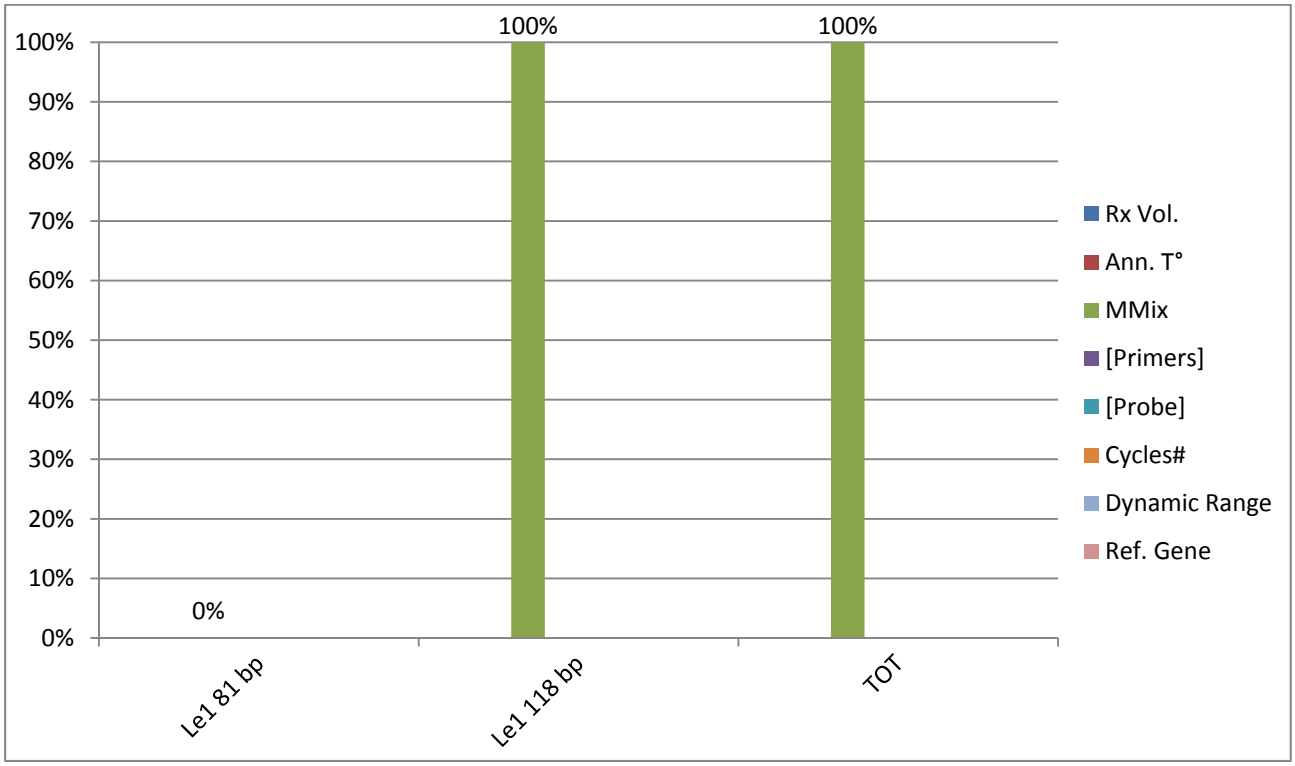
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; TOT = Type and frequency of modifications for all qualitative taxon-specific methods; QL = Qualitative; TAX = Taxon-specific methods;

Figure 99 - Type and Frequency of Modifications for Qualitative Taxon-specific Methods According to Maize Reference Genes



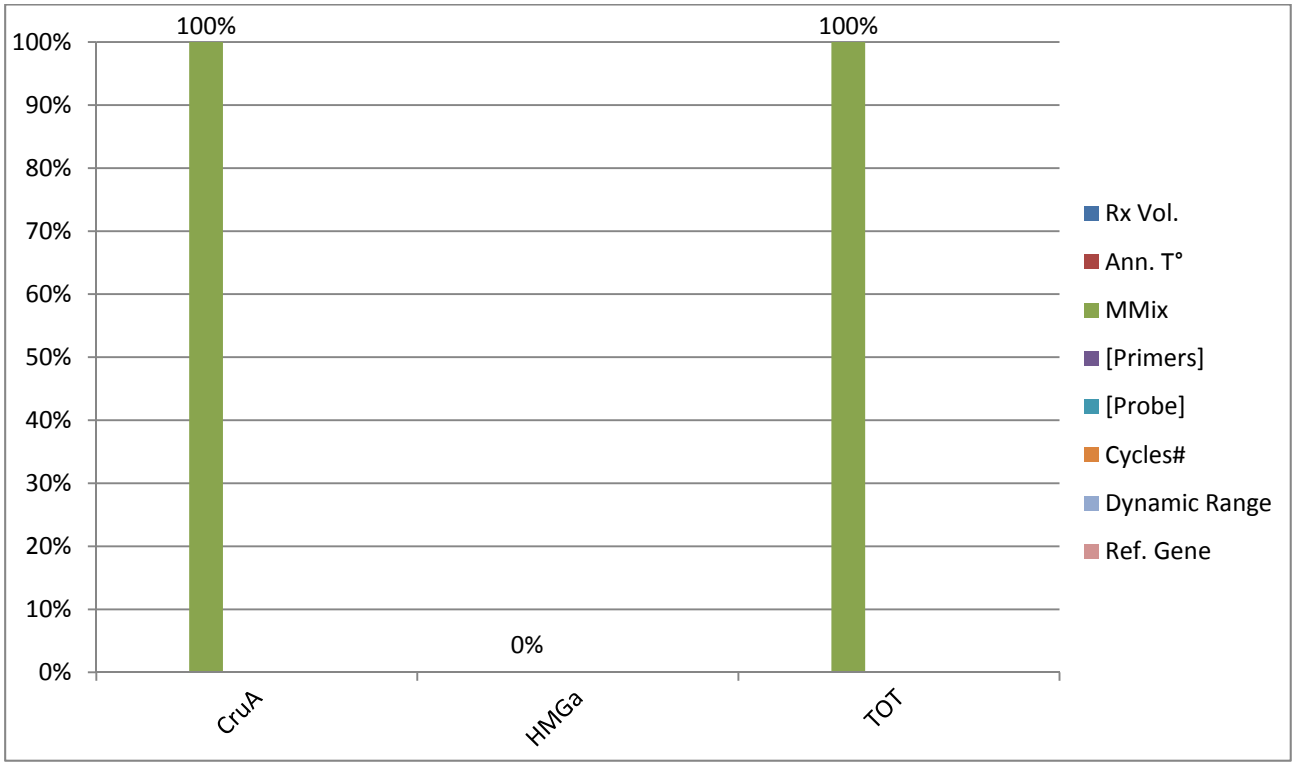
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; adh1 = Alcohol dehydrogenase1 gene; lvr1 = Invertase gene; TOT = Type and frequency of modifications for all qualitative taxon-specific maize methods

Figure 100 - Type and Frequency of Modifications for Qualitative Taxon-specific Methods According to Soybean Reference Genes



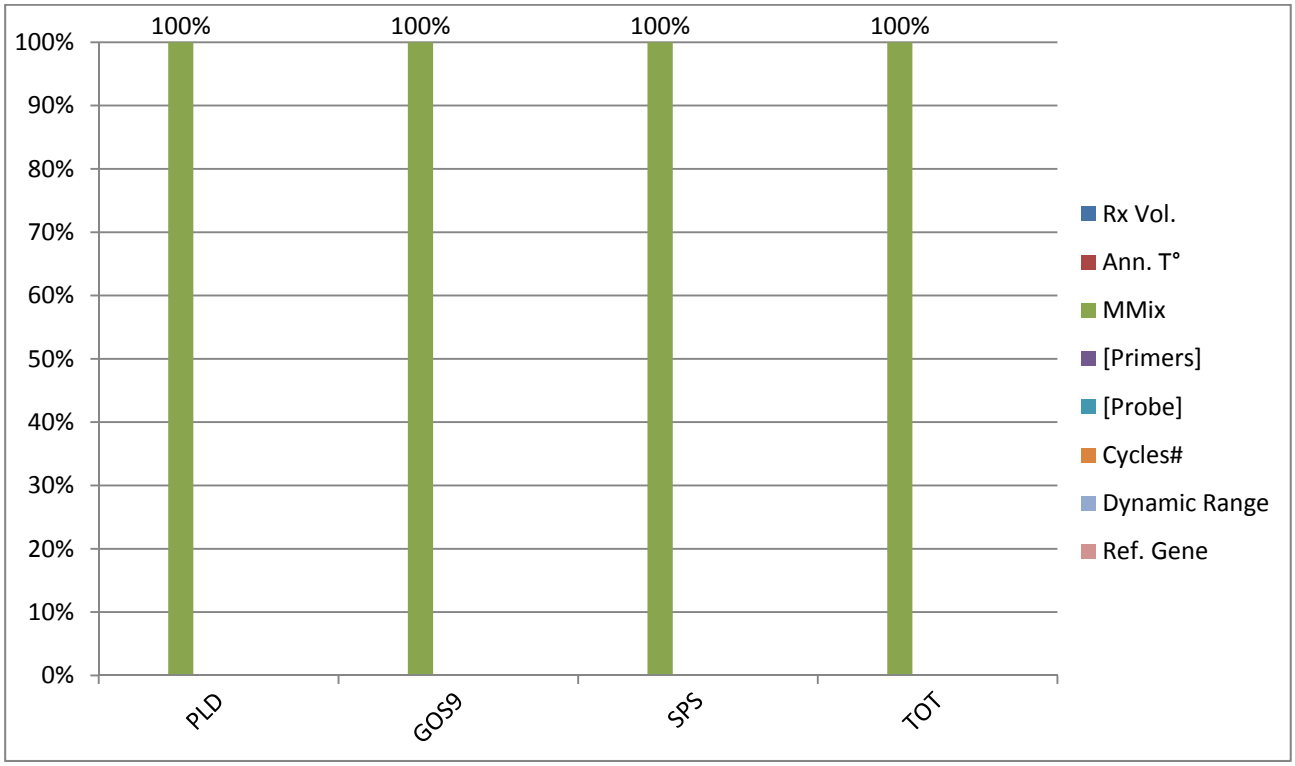
Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; Le1 = Lectin gene; TOT = Type and frequency of modifications for all qualitative taxon-specific soybean methods

Figure 101 - Type and Frequency of Modifications for Qualitative Taxon-specific Methods According to Oilseed Rape Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; CruA = Cruciferin A gene; HMGa = High mobility group protein I/Y gene; TOT = Type and frequency of modifications for all qualitative taxon-specific oilseed rape methods

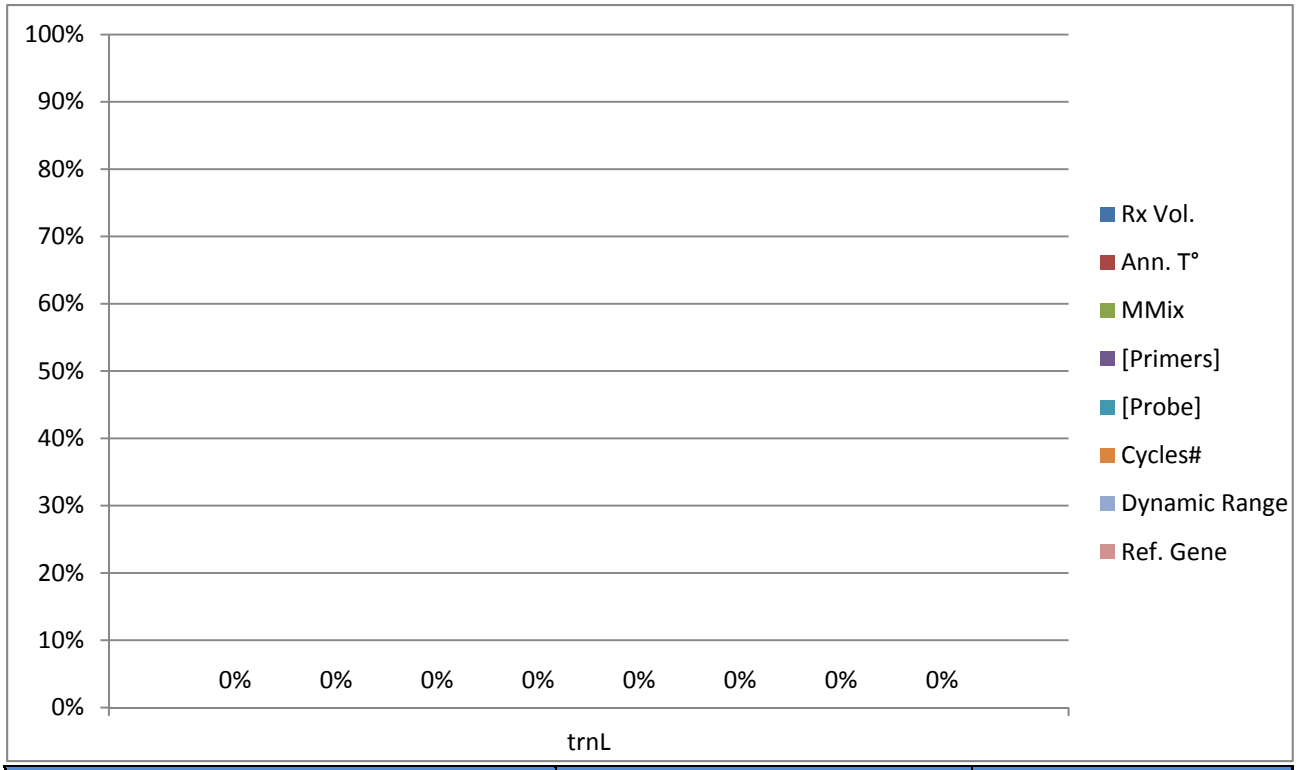
Figure 102 - Type and Frequency of Modifications for Qualitative Taxon-specific Methods According to Rice Reference Genes



Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; PLD = Phospholipase D gene; GOS9 = Rice root-specific GOS9 gene; SPS = Sucrose-phosphate synthase gene; TOT = Type and frequency of modifications for all qualitative taxon-specific rice methods

i) Qualitative Plant-specific Methods

Figure 103 - Type and Frequency of Modifications for Qualitative Plant-specific Methods



QL PLN Modification	trnL	TOT
Rx Vol.	0	0
Ann. T°	0	0
MMix	0	0
[Primers]	0	0
[Probe]	0	0
Cycles#	0	0
Dynamic Range	0	0
Ref. Gene	0	0
Total Modifications	0	0

Legend: Rx Vol. = PCR reaction volume; Ann. T° = Annealing temperature of amplification reaction; MMix = PCR Master Mix; [Primers] = Concentration of primers in the PCR reaction; [Probe] = Concentration of probe in the PCR reaction; Cycles# = Cycles number of the amplification reaction; Dynamic Range = PCR dynamic range; Ref. Gene = Reference gene; QL = Qualitative; PLN = Plant-specific methods

12. Annex 2: Non-EU Reference Methods

In the survey respondents reported to use a total of 65 non-EU reference methods. Information on these methods is combined in Table 5. The respondents did not always include all data requested. When available, references were reviewed to complete and verify the correctness of the characteristics provided. When the information was missing or incorrect the data reported in the corresponding publication (presented in brackets in the Table) was used as a source for the statistical analysis. Eight of the methods supplied in this section were found to be included in the GMOMETHODS database while for another three methods the targets and specificity indicated by the respondent were fully inconsistent and not in line with the information reported in the related reference. The methods already included in the GMOMETHODS database are indicated the table in brackets in the column "Validation" while the inconsistent methods are highlighted in bold letters. All these eleven methods were not included in the statistical analysis of the data displayed in the following charts regarding the assay types, GM events, genetic elements, taxon, reference genes targets and detection chemistry of the non-EU reference methods reported.

Table 5 – Information on non-EU Reference Methods and Relative References Provided by the Respondents

N	Purpose	Specificity	Assay	Target	Reference	Validation
1	N/P (Qualitative and/or Quantitative)	N/P (ELE)	N/P	Rice KMD1 (P-ubi and cry1A(b))	Babekova, R. et al. (2008) Duplex Polymerase Chain Reaction (PCR) for the Simultaneous Detection of Cry1A(b) and the Maize Ubiquitin Promoter in the Transgenic Rice Line KMD1. Biotechnology & Biotechnological Equipment, 22, 2:705-708.	N/P (Single Laboratory Validation)
2	N/P (Qualitative and/or Quantitative)	N/P (EVE)	N/P	LLRICE601	Bayer CropScience (2006) Grain testing method for detection of Rice GM event LLRICE601 using RT-PCR protocols PGS0505 and PGS0476. (http://gmo-crl.jrc.ec.europa.eu/LLRice601update.htm)	N/P
3	N/P (Quantitativeand/or Qualitative)	N/P (CON)	N/P	Bt11 (P-35S/IVS2)	Brodmann, P.D. et al. (2002) Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. Journal of AOAC International, 85 (3): 646-653.	N/P (Single Laboratory Validation)
4	N/P (Qualitative and/or Quantitative)	N/P (ELE)	N/P (Tetraplex Real-time PCR)	N/P (P-35S, T-nos, T-35S and P-FMV)	Eugster, A. et al. (2014) Development and Validation of a P-35S, T-nos, T-35S and P-FMV Tetraplex Real-time PCR Screening Method to Detect Regulatory Genes of Genetically Modified Organisms in Food. CHIMIA, 68, (10): 701-704.	N/P
5	N/P (Qualitative and/or Quantitative)	N/P (ELE)	N/P	T35S_pCAMBIA	Fraiture, M-A. et al. (2014) An innovative and integrated approach based on DNA walking to identify unauthorised GMOs. Food Chemistry, 147:60-69.	N/P (Single Laboratory Validation)
6	N/P (Qualitative and/or Quantitative)	N/P (EVE)	N/P	Rice Kefeng 6	Guertler, P. et al. (2012) Development of an event-specific detection method for genetically modified rice Kefeng 6 by quantitative real-time PCR. Journal für Verbraucherschutz und Lebensmittelsicherheit, 7:63-70.	N/P (Single Laboratory Validation)

N	Purpose	Specificity	Assay	Target	Reference	Validation
7	N/P (Qualitative and/or Quantitative)	N/P (TAX)	N/P	N/P	Hernandez, M. et al. (2003) Real-time and conventional polymerase chain reaction systems based on the metallo-carboxypeptidase inhibitor gene for specific detection and quantification of potato and tomato in processed food. Journal of Food Protection, 66, 6:1063-1070.	N/P (Single Laboratory Validation)
8	N/P (Quantitative and/or Qualitative)	N/P (EVE)	N/P	N/P (MON810)	Holck, A. et al. (2002) 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified Mon810 MaisGard maize. European Food Research and Technology, 214, p449–453.	N/P (Single Laboratory Validation)
9	N/P (Qualitative and/or Quantitative)	N/P (CON)	N/P	Rice Bt63 (cry1A(b)-cry1A(c)/DNA spacer)	Maede, D. et al. (2006) Detection of genetically modified rice: a construct-specific real-time PCR method based on DNA sequences from transgenic Bt rice. European Food Research and Technology, 224:271-278.	N/P
10	N/P (Qualitative and/or Quantitative)	N/P (CON)	N/P	P-nos/nptII	Reiting, R. (2010) Real-time PCR methods for the detection of DNA constructs with the nptII gene for the detection of genetically modified plants in food, feed and seed. Journal für Verbraucherschutz und Lebensmittelsicherheit, 5:377–390.	N/P (Single Laboratory Validation and transfer to 4 Labs)
11	N/P (Quantitative and/or Qualitative)	N/P (ELE)	N/P	nptII	Weng, H. et al. (2004) Estimating Number of Transgene Copies in Transgenic Rapeseed by Real-Time PCR Assay With HMG I/Y as an Endogenous Reference Gene. Plant Molecular Biology Reporter 22:289-300.	N/P (Single Laboratory Validation)
12	N/P (Qualitative and/or Quantitative)	N/P (EVE)	N/P	Topas 19/2	Wu, G. et al. (2009) Event-specific qualitative and quantitative PCR detection of genetically modified rapeseed Topas 19/2. Food Chemistry, 112, 1, p232-238.	N/P (Single Laboratory Validation)
13	N/P (Quantitative and/or Qualitative)	N/P (ELE) (TAX)	N/P	N/P	Zeitler, R. et al. (2002) Validation of real-time PCR methods for the quantification of transgenic contaminations in rape seed. European Food Research and Technology, 214:346–351.	N/P (2 Lab. Valid.)
14	Qualitative	N/P	N/P	N/P	N/P	N/P
15	Qualitative	N/P	Single End-point PCR	N/P	N/P	N/P
16	Qualitative	N/P	Multiplex End-point P	N/P	N/P	N/P
17	Qualitative	CON	Single End-point PCR	N/P	N/P	N/P

N	Purpose	Specificity	Assay	Target	Reference	Validation
18	Qualitative	ELE	Simplex Real-time PCR (incorrect according targets prov	pat, cry1A(b), P-FMV, maize P-ALS	N/P	National Collaborative Study
19	Qualitative	EVE	Simplex Real-time PCR	Bt11	N/P	N/P
20	Qualitative	CON (according to the targets should be ELE)	Single End-point PCR (incorrect according targets prov	P-35S, P-gbss and nptII	N/P	Single Laboratory Validation
21	Qualitative	ELE TAX	Multiplex End-point PCR	P-35S and T-nos maize zein soybean Le1	N/P	Single Laboratory Validation
22	Qualitative	TAX	Single End-point PCR (incorrect according to targets provided)	maize zein and soybean Le1	N/P	Single Laboratory Validation
23	Qualitative	ELE	Simplex Real-time PCR	bar	Not published	Single Laboratory Validation
24	Qualitative	ELE	Simplex Real-time PCR	T-nos	Not published	Single Laboratory Validation
25	Qualitative	ELE (CON)	Single End-point PCR (incorrect according to targets provided)	bar, P-35S, cry1A(b)/Ac, CTP2/CP4epsp, pat, P-nos and T-nos	N/P	Single Laboratory Validation
26	Qualitative	ELE	Simplex Real-time PCR	CP4epsps (the bar and nptII targets additionally reported by the respondent are not covered by the reference provided)	Alexander, T.W. et al. (2004) Use of quantitative real-time and conventional PCR to assess the stability of the cp4 epsps transgene from Roundup Ready canola in the intestinal, ruminal, and fecal contents of sheep. Journal of Biotechnology, 9, 112(3):255-266.	National collaborative study

N	Purpose	Specificity	Assay	Target	Reference	Validation
27	Qualitative	CON	Single End-point PCR	P-nos/nptII	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode L 00.00-142 (2013). Untersuchung von Lebensmitteln - Nachweis des DNA-Sequenzübergangs von dem nos-Promotor in das nptII-Gen zum Screening auf Bestandteile aus gentechnisch veränderten Organismen (GVO) in Lebensmitteln mittels real-time PCR - Konstrukt-spezifisches Verfahren. Beuth, Berlin.	National Collaborative Study
28	Qualitative	CON (ELE)	Multiplex Real-time PCR	CTP2/CP4epsp, pat and bar	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode L 00.00-154 (2014). Untersuchung von Lebensmitteln - Nachweis von CTP2-CP4-EPSPS-, pat- und bar-Sequenzen in Lebensmitteln mittels Triplex real-time PCR - Konstrukt-spezifisches und Element-spezifische Verfahren. Beuth, Berlin.	National Collaborative Study
29	Qualitative	ELE (CON)	Multiplex Real-time PCR	CTP2/CP4epsp, pat and bar	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode L 00.00-154 (2014). Untersuchung von Lebensmitteln - Nachweis von CTP2-CP4-EPSPS-, pat- und bar-Sequenzen in Lebensmitteln mittels Triplex real-time PCR - Konstrukt-spezifisches und Element-spezifische Verfahren. Beuth, Berlin.	National Collaborative Study
30	Qualitative	CON (according to reference and target should be ELE)		P-35S and T-nos	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode G 30.40-3 (2013) Nachweis von bestimmten, häufig in gentechnisch veränderten Organismen (GVO) verwendeten DNA-Sequenzen aus dem Blumenkohlmosaikvirus (CaMV 35S-Promotor, P35S) sowie aus Agrobacterium tumefaciens (T-nos) in Pflanzen - Element-spezifische Verfahren (Screening) (Übernahme der amtlichen Methode L 00.00-122, Juni 2008, Band I (Lebensmittel) der Amtlichen Sammlung)	National Collaborative Study (EU Reference Method QL-ELE-00-013/012)
31	Qualitative	CON		CTP2(Arabidopsis thaliana)/CP4epsps	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode G 30.40-5 (2013). Nachweis der CTP2-CP4-EPSPS-Gensequenz zum Screening auf gentechnisch veränderte Organismen (GVO) in Pflanzen - Konstrukt-spezifisches Verfahren (Screening) (Übernahme der amtlichen Methode L 00.00-125, Dezember 2008, Band I (Lebensmittel) der Amtlichen Sammlung) Beuth, Berlin.	National Collaborative Study (EU Reference Method QL-CON-00-008)

N	Purpose	Specificity	Assay	Target	Reference	Validation
32	Qualitative	ELE CON EVE TAX	Simplex Real-time PCR (incorrect according targets prov	bar, P-35S, P-FMV, nptII, P-nos, T-nos, CTP4/CP4epsp, CTP2/CP4epsps GS40/90pHoe6/Ac, GTS-40-3-2 and OXY-235 CaMV, Crucifers, Flax, Oilseed Rape, Rice, Soy Sugarbeet CruA, FatA, GS, invertase, Le1, SAH7, SPS, trnL, UGPase	ASU § 64 LFGB; ASU § 28b GenTG. (N/P)	National Collaborative Study
33	Qualitative	ELE	Duplex Real-time PCR	P-35S and T-nos	Official Collection of Test Methods according to § 64 LFGB (Food and Feed law) (2008). Detection of DNA sequences from CaMV 35S promoter and T-nos for screening of materials derived from genetically modified organisms (GMO) in foodstuffs-screening method. Food Analysis, L 00.00-122. Beuth, Berlin	National Collaborative Study (EU Reference Method QL-ELE-00-013/012)
34	Qualitative	CON		P-35S/pat	Official Collection of Test Methods according to § 64 LFGB (Food and Feed law) (2012). Real-time PCR detection of the P35S-pat–genetic construct to screen for genetically modified plants- Construct-specific method, Food Analysis, G 30.40-1, Beuth, Berlin.	National Collaborative Study (EU Reference Method QL-CON-00-011)
35	Qualitative	CON (according to reference and target should be ELE)	Single End-point PCR	P-nos	Broeders, S. et al. (2013) New SYBR®Green methods targeting promoter sequences used for screening of several GM events pending for authorisation in Europe. European Food Research and Technology, 236, 3:537–547.	Single Laboratory Validation (2 lab transfer)
36	Qualitative	ELE	Single End-point PCR	P-FMV	Broeders, S. et al. (2013) New SYBR®Green methods targeting promoter sequences used for screening of several GM events pending for authorisation in Europe. European Food Research and Technology, 236, 3:537–547.	Single Laboratory Validation (2 lab transfer)
37	Qualitative	ELE	Single End-point PCR	Cry3Bb1	Broeders, S. et al. (2015) New qualitative trait-specific SYBR®Green qPCR methods to expand the panel of GMO screening methods used in the CoSYPS. European Food Research and Technology, 241, 2:275-287.	Single Laboratory Validation

N	Purpose	Specificity	Assay	Target	Reference	Validation
38	Qualitative	CON	Single End-point PCR	gat/tpinII	Broeders, S. et al. (2015) New qualitative trait-specific SYBR®Green qPCR methods to expand the panel of GMO screening methods used in the CoSYPS. European Food Research and Technology, 241, 2:275-287.	Single Laboratory Validation
39	Qualitative	TAX	Simplex Real-time PCR	N/P (CaMV)	Chaouachi, M. et al. (2008) An accurate real-time PCR test for the detection and quantification of cauliflower mosaic virus (CaMV): applicable in GMO screening. European Food Research and Technology, 227:789-798.	Single Laboratory Validation
40	Qualitative	ELE	Multiplex Real-time PCR	P-35S, T-nos and P-FMV	Eurofins GeneScan GMOScreen RT IPC 35S/NOS/FMV Kit.	International Collaborative Study (Commercial Kit)
41	Qualitative	ELE	Multiplex Real-time PCR	P-35S, T-nos and P-FMV	Eurofins GeneScan GMOScreen RT IPC 35S/NOS/FMV Kit.	International Collaborative Study (Commercial Kit)
42	Qualitative	ELE (CON)		bar and CTP2/CP4epsps (the targets P-35S, T-nos, cryIA(b), P35S/pat, duplex P-35S and T-nos additionally reported by the respondent are not covered in the reference provided)	Grohmann, L. et al. (2009) Collaborative Trial Validation Studies of Real-Time PCR-Based GMO Screening Methods for Detection of the bar Gene and the ctp2-cp4epsps Construct. Journal of Agricultural and Food Chemistry, 57:8913–8920.	International Collaborative Study (EU Reference Methods QL-ELE-00-014 and QL-CON-00-008)
43	Qualitative	EVE	Simplex Real-time PCR	Kefeng 6	Guertler, P. et al. (2012) Development of an event-specific detection method for genetically modified rice Kefeng 6 by quantitative real-time PCR. Journal für Verbraucherschutz und Lebensmittelsicherheit, 7:63-70.	Single Laboratory Validation
44	Qualitative	ELE (CON)	Multiplex Real-time PCR	P-35S, T-nos, pat, bar and CTP2/CP4epsps	Huber, I. et al. (2013) Development and Validation of Duplex, Triplex, and Pentaplex Real-Time PCR Screening Assays for the Detection of Genetically Modified Organisms in Food and Feed. Journal of Agricultural and Food Chemistry, 61 (43):10293–10301.	International Collaborative Study (according to reference it is a three laboratories ring-trial)
45	Qualitative	ELE	Simplex Real-time PCR	P-35S	Kuribara, H. et al. (2002) Novel reference molecules for quantitation of genetically modified maize and soybean. Journal of AOAC International, Vol 85, No.5:1077-1089.	Single Laboratory Validation

N	Purpose	Specificity	Assay	Target	Reference	Validation
46	Qualitative	TAX	Simplex Real-time PCR	FMV	Moor, D. et al. (2012) Real-time PCR method for the detection of figwort mosaic virus (FMV) to complement the FMV 34S promoter-specific PCR assay used for screening of genetically modified plants. European Food Research and Technology, 235, 5:835-842.	Single Laboratory Validation
47	**Qualitative (according to reference and validation status should be quantitative)**	**ELE** (CON)		**bar, and nptII (The targets indicated by the respondent are not covered by the reference provided (P-35S and P-35S/epsps)**	**Pauli, U. et al. (2001) Quantitative detection of genetically modified soybean and maize: Method evaluation in a swiss ring trial. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 92, 2:145-158.**	**National Collaborative Study**
48	Qualitative	ELE	Simplex Real-time PCR	pat (the bar and nptII targets additionally reported by the respondent are not covered by the reference provided)	Permingeat, H.R. et al. (2002) Detection and quantification of transgenes in grains by multiplex and real-time PCR. Journal of Agricultural and Food Chemistry, 31, 50(16):4431-4436.	National Collaborative study
49	Qualitative	CON	Simplex Real-time PCR	CpTI/T-nos,	Reiting, R. et al. (2010) A testing cascade for the detection of genetically modified rice by real-time PCR in food and its application for detection of an unauthorized rice line similar to KeFeng6. Journal für Verbraucherschutz und Lebensmittelsicherheit, 5(2):185-188.	Single Laboratory Validation
50	Qualitative	CON	Simplex Real-time PCR (incorrect according targets prov	CpTI/T-nos, P-35S/hpt and P-ubi/cry	Reiting, R. et al. (2010) A testing cascade for the detection of genetically modified rice by real-time PCR in food and its application for detection of an unauthorized rice line similar to KeFeng6. Journal für Verbraucherschutz und Lebensmittelsicherheit, 5(2):185-188.	Single Laboratory Validation
51	Qualitative	ELE	Simplex Real-time PCR	CP4epsps (the bar and nptII targets additionally reported by the respondent are not covered by the reference provided)	Vaïtilingom, M. et al. (1999) Real-time quantitative PCR detection of genetically modified Maximizer maize and Roundup Ready soybean in some representative foods. Journal of Agricultural and Food Chemistry, 47(12), p5261-5266.	National Collaborative study

N	Purpose	Specificity	Assay	Target	Reference	Validation
52	Qualitative	ELE (according to reference and target should be CON)		P-35S/pat and CTP2/CP4epsps (the specificity and targets P-35S, T-nos, Cry1A(b), and duplex P-35S,/T-nos additionaly reported by the respondent are not covered by the reference provided)	Waiblinger, H.U. et al. (2005) Die Untersuchung von transgenem Rapspollen in Honigen mittels real-time PCR. Deutsche Lebensmittel-Rundschau, 101 (12):543-549.	International Collaborative Study (EU Reference Methods QL-CON-00-011 and QL-CON-00-008)
53	**Qualitative**	**ELE** (CON)		**bar (the specificity and target reported by the respondent are not covered by the reference provided)**	**Waiblinger, H.U. et al. (2005) Die Untersuchung von transgenem Rapspollen in Honigen mittels real-time PCR. Deutsche Lebensmittel-Rundschau, 101 (12):543-549.**	**Single Laboratory Validation**
54	Qualitative	CON		P-35S/pat	Waiblinger, H.U. et al. (2010) A practical approach to screen for authorised and unauthorised genetically modified plants. Analytical and Bioanalytical Chemistry, 396:2065-2072.	National Collaborative Study (EU Reference method QL-CON-00-011)
55	Qualitative	TAX	Simplex Real-time PCR	papaya CHY (chymopapain)	Wei, J. et al. (2013) Collaborative Ring Trial of the Papaya Endogenous Reference Gene and its Polymerase Chain Reaction Assays for Genetically Modified Organism Analysis. Journal of Agricultural and Food Chemistry, 61: 11363-11370.	National Collaborative Study (according to reference should be international)
56	Qualitative	EVE	Simplex Real-time PCR	CBH-351	Windels, P. et al (2003) Qualitative and event-specific PCR real-time detection methods for StarLink maize. European Food Research and Technology, 216:259-263.	Single Laboratory Validation
57	Qualitative	ELE	Single End-point PCR	pat	Zeitler, R. et al. (2002) Validation of real-time PCR methods for the quantification of transgenic contaminations in rape seed. European Food Research and Technology, 214:346–351.	Single Laboratory Validation (2 Laboratories Validation)

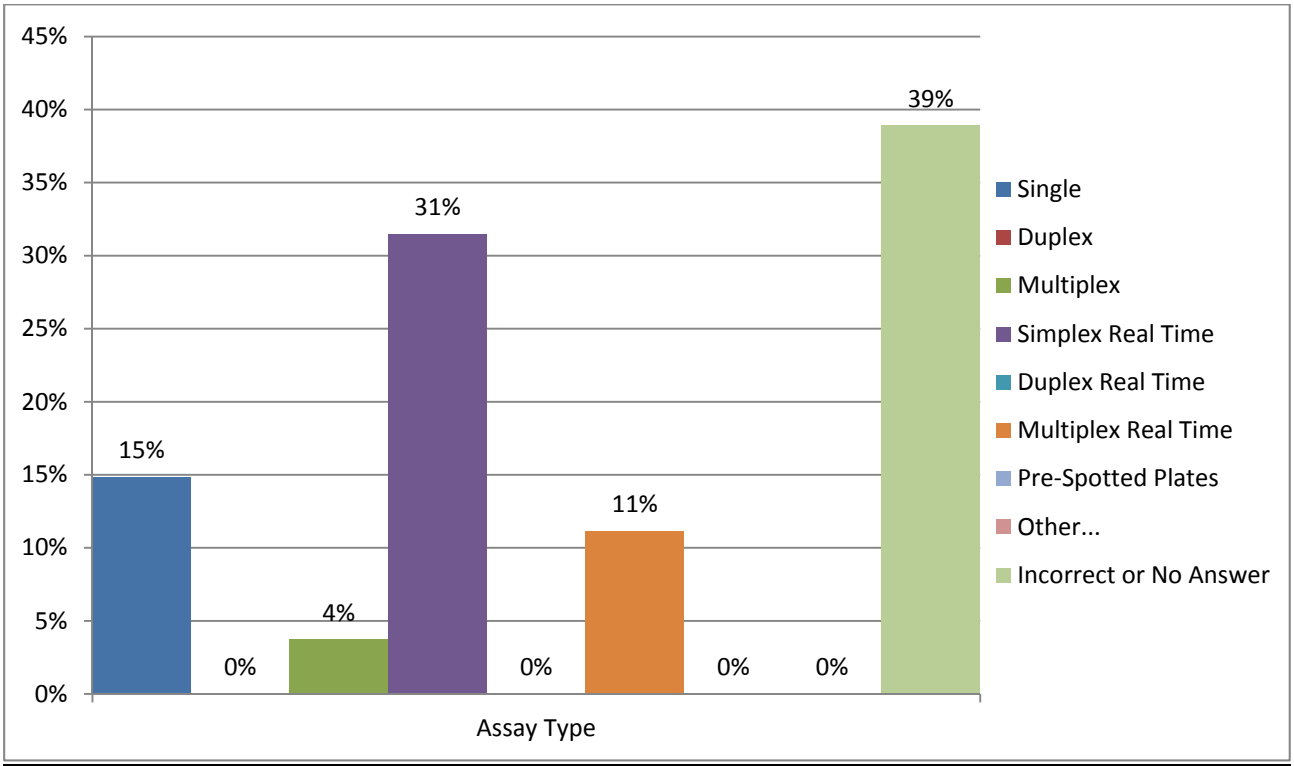
N	Purpose	Specificity	Assay	Target	Reference	Validation
58	Quantitative	CON (according to reference and target should be ELE)		P-35S	Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB Methode L 00.00-105 (2014). Untersuchung von Lebensmitteln - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Quantitative auf Nukleinsäuren basierende Verfahren (Übernahme der gleichnamigen Norm DIN EN ISO 21570, Ausgabe August 2013).	International Collaborative Study (EU Reference Method QT-ELE-00-004)
59	**Quantitative**	**EVE (according to the reference should be CON)**		**GA21 (target and specificity indicated by respondent not covered by the reference provided)**	**Brodmann, P.D. et al. (2002) Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. Journal of AOAC International, 85 (3):646-653.**	**International Collaborative study (according to reference should be Single Laboratory Validation**
60	Quantitative	CON	Simplex Real-time PCR	PEP-C/CryIA(b) Bt176	Brodmann, P.D. et al. (2002) Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. Journal of AOAC International, 85 (3) :646-653.	Single Lab Validation
61	Quantitative	CON	Simplex Real-time PCR	P-35S/IVS2 Bt11	Brodmann, P.D. et al. (2002) Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. Journal of AOAC International, 85 (3) :646-653.	Single Lab Validation
62	Quantitative (?) (according to reference, assay type and validation status should be Qualitative)	CON	Single End-point PCR (incorrect quantitative	P-35S/CTP4epsps (GTS-40-3-2)	DIN/EN ISO 21569 (2005) Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organsimen und ihren Produkten - Qualitative auf Nukleinsäuren basierende Verfahren. C.1 Konstrukt-spezifisches Verfahren GTS 40-3-2 (Roundup Ready-Sojabohnen).	International Collaborative Study)
63	Quantitative	EVE	Simplex Real-time PCR (Incorrect according targets prov	MIR162 (?), T25 (?) and TC 1507	Eurofins GeneScan	Commercial Kit
64	Quantitative	CON	Simplex Real-time PCR	CTP2/CP4epsps	Grohmann, L. et al. (2009) Collaborative Trial Validation Studies of Real-Time PCR-Based GMO Screening Methods for Detection of the bar Gene and the ctp2-cp4epsps Construct. Journal of Agricultural and Food Chemistry, 57:8913–8920.	National Collaborative Study

N	Purpose	Specificity	Assay	Target	Reference	Validation
65	Quantitative	EVE	Simplex Real-time PCR	MON810	Holck, A. et al. (2002) 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified Mon810 MaisGard maize. European Food Research and Technology, 214, p449–453.	Single Laboratory Validation

Legend: N/P = Not provided; EVE = Event-specific methods; CON = Construct-specific methods; ELE = Element-specific methods; TAX = Taxon-specific methods; CpTI (cow pea trypsin inhibitor gene); Cry3Bb1 (*Bacillus thuringiensis* (Bt) δ-endotoxin encoding gene *cry3Bb1)*; CruA: Cruciferin A gene; FatA: acyl-ACP thioesterase gene; Gat (glyphosate N-acetyltransferase of *Bacillus licheniformis*); GS: glutamine synthetase gene; Hpt (hygromycin phosphotransferase gene); Le1: lectin gene; PEP-C (phosphoenolpyruvate carboxylase promoter); P-ubi (ubiquitin promoter); SAH7: IVS of the putative Sinapis Arabidopsis Homolog 7 protein gene; SPS: Sucrose-phosphate synthase gene; tpinII (terminator of the *Solanum tuberosum* proteinase inhibitor); trnL: chloroplast tRNA-Leu intron; UGPase: UDP-glucose pyrophosphorylase gene

12.1 Assay Types of non-EU Reference Methods

Figure 104 – Percentage of Analytical Approaches Used with non-EU Reference Methods

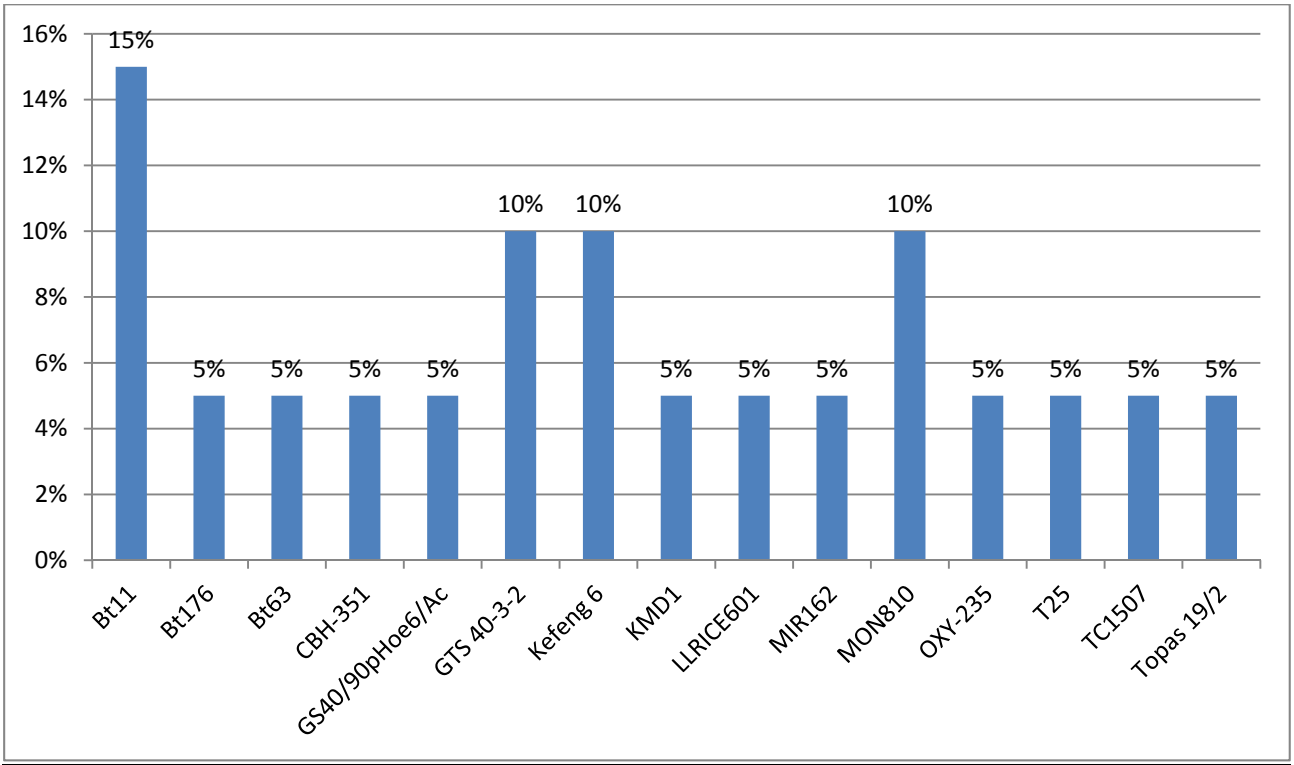


Assay Type	Non-EU Reference Methods using the Assay Type	%
Single	8	15 %
Duplex	0	0 %
Multiplex	2	4 %
Simplex Real Time	17	31 %
Duplex Real Time	0	0 %
Multiplex Real Time	6	11 %
Pre-Spotted Plates	0	0 %
Other	0	0 %
Incorrect or No Answer	21	39 %
TOT	54	100 %

Legend: TOT= Total non-EU Reference methods reported

12.2 GM Event Targets of non-EU Reference Methods

Figure 105 – Percentage of GM Events Detected with non-EU Reference Methods

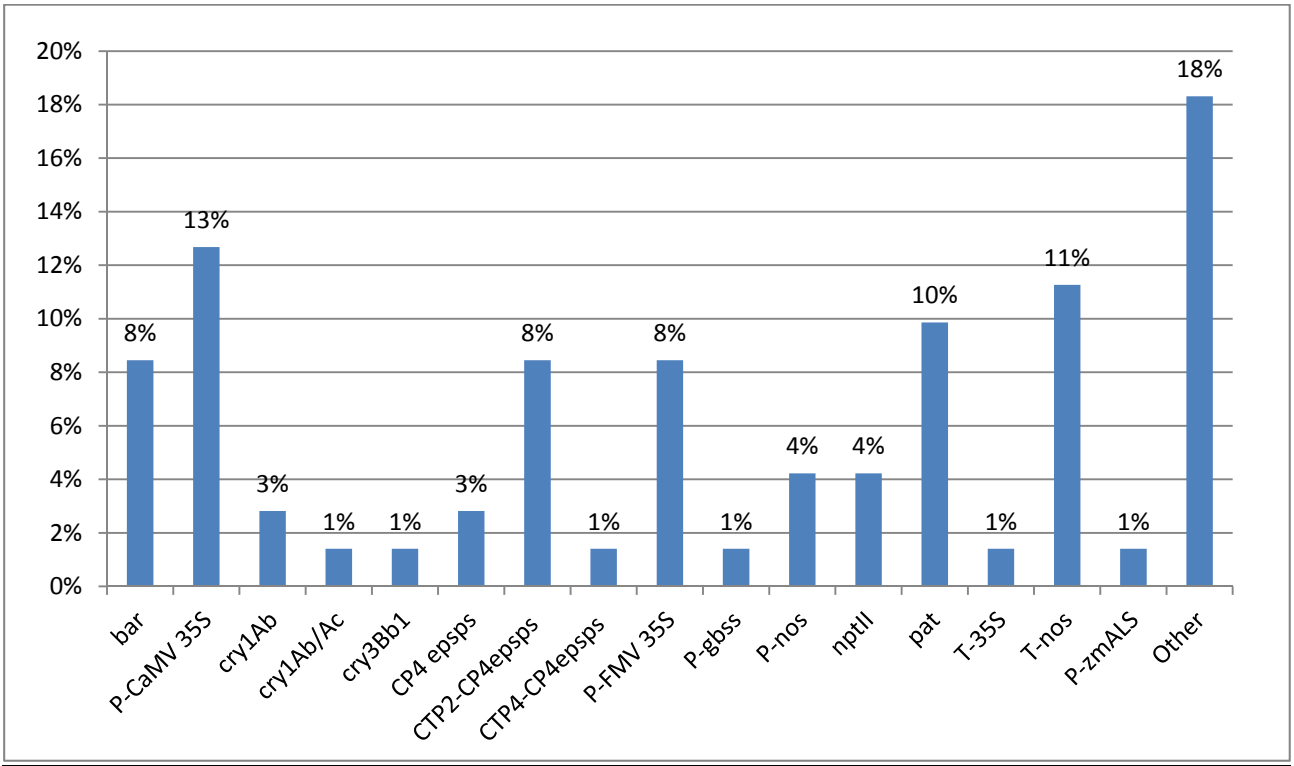


Target GM Event	Non-EU Reference Methods Detecting the GM Event	%
Bt11	3	15 %
Bt176	1	5 %
Bt63	1	5 %
CBH-351	1	5 %
GS40/90pHoe6/Ac	1	5 %
GTS 40-3-2	2	10 %
Kefeng 6	2	10 %
KMD1	1	5 %
LLRICE601	1	5 %
MIR162	1	5 %
MON810	2	10 %
OXY-235	1	5 %
T25	1	5 %
TC1507	1	5 %
Topas 19/2	1	5 %
TOT	20	100 %

Legend: TOT= Total

12.3 Construct/Element-specific Targets of non-EU Reference Methods

Figure 106 –Percentage of Genetic Elements Detected with non-EU Reference Methods

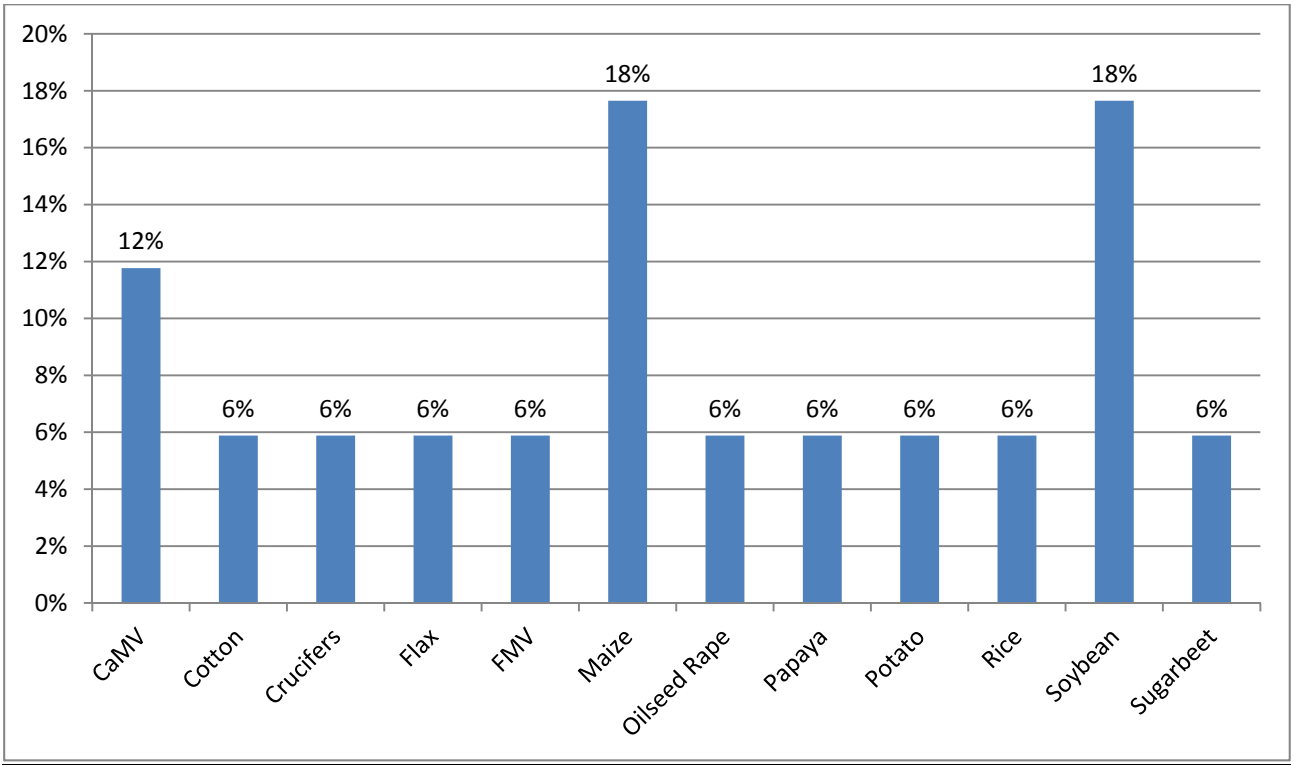


Target Genetic Element	Non-EU Reference Methods Detecting the Genetic Element	%
bar	6	8 %
P-CaMV 35S	9	13 %
cry1Ab	2	3 %
cry1Ab/Ac	1	1 %
cry3Bb1	1	1 %
CP4 epsps	2	3 %
CTP2-CP4 epsps	6	8 %
CTP4-CP4epsps	1	1 %
P-FMV 35S	6	8 %
P-gbss	1	1 %
P-nos	3	4 %
nptII	3	4 %
pat	7	10 %
T-35S	1	1 %
T-nos	8	11 %
P-zmALS	1	1 %
Other	13	18 %
TOT	71	100 %

Legend: bar = Phosphinothricin N-acetyl transferase gene from Streptomyces hygroscopicus; P-CaMV 35S = Cauliflower Mosaic Virus 35 S Promoter; Cry1A(b) = Cry1Ab delta-endotoxin from Bacillus thuringiensis subsp. Kurstaki; CryIAb/Ac = Synthetic delta endotoxin construct derived from Bacillus thuringiensis; cry3Bb1 = Delta endotoxin encoding gene cry3Bb from Bacillus thuringiensis subsp. kumamotoensis; CP4 epsps = 5-enolpyruvylshikimate-3-phosphate synthase gene from Agrobacterium tumefaciens strain CP4; CTP2-CP4 epsps = Chloroplast transit peptide 2 sequence from Arabidopsis thaliana epsps gene; CTP4-CP4epsps = Chloplast transit peptide coding sequence from Petunia hybrida epsps gene; P-FMV 35S = Figwort Mosaic Virus 35S promoter; P gbss = Granule-bound starch synthase gene Promoter from Solanum tuberosum; nptII = Neomycin phosphotransferase II gene; pat = Phosphinothricin N-acetyltransferase gene from Streptomyces viridochromogenes; P-nos = Nopaline synthase promoter from Agrobacterium tumefaciens; T-35S = Cauliflower Mosaic Virus 35S Terminator; T-nos = Nopaline synthase terminator from Agrobacterium tumefaciens; P zmALS = Actolactase synthase gene Promoter from Zea mays; TOT= Total

12.4 Taxon Targets of non-EU Reference Methods

Figure 107 –Percentage of Species Detected with non-EU Reference Methods

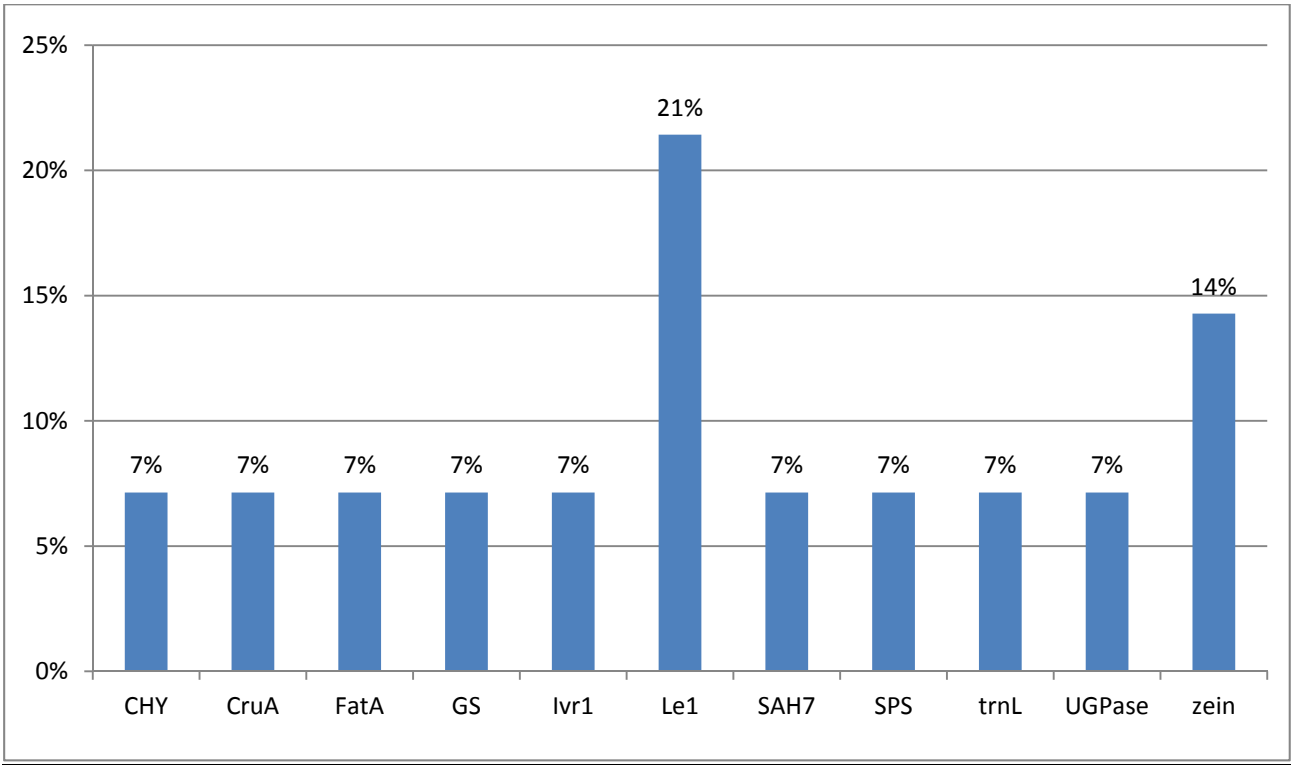


Species	Non-EU Reference Methods Detecting the Species	%
CaMV	2	12 %
Cotton	1	6 %
Crucifers	1	6 %
Flax	1	6 %
FMV	1	6 %
Maize	3	18 %
Oilseedrape	1	6 %
Papaia	1	6 %
Potato	1	6 %
Rice	1	6 %
Soybean	3	18 %
Sugarbeet	1	6 %
TOT	17	100 %

Legend: CaMV = Cauliflower Mosaic Virus; FMV = Figwort Mosaic Virus, TOT=Total

12.5 Taxon-specific Gene Targets of non-EU Reference Methods

Figure 108 –Percentage of Reference Genes Detected with non-EU Reference Methods

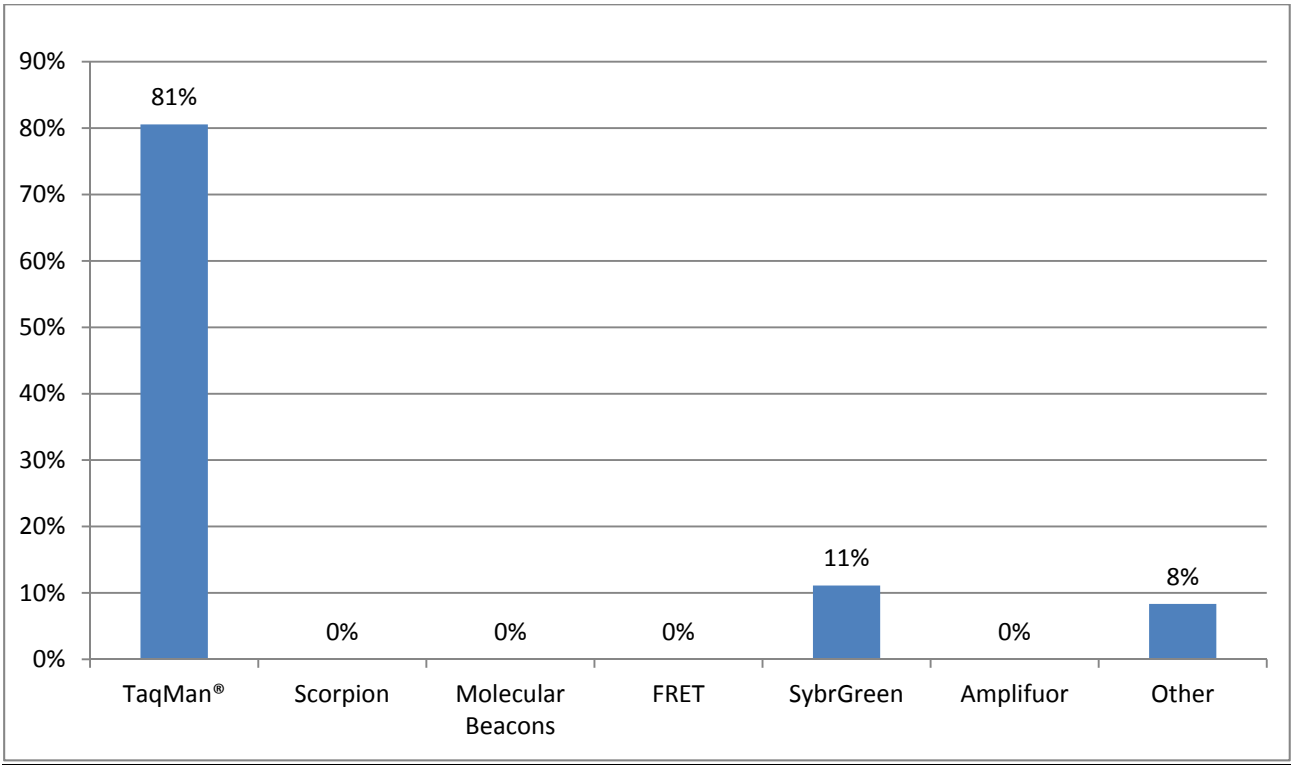


Reference Gene	Non-EU Reference Methods Detecting the Reference Gene	%
CHY	1	7 %
CruA	1	7 %
FatA	1	7 %
GS	1	7 %
Ivr1	1	7 %
Le1	3	21 %
SAH7	1	7 %
SPS	1	7 %
trnL	1	7 %
UGPase	1	7 %
zein	2	14 %
TOT	14	100 %

Legend: CHY = Papaia chymopapain; CruA = Oilseed rape cruciferin A gene; FatA = Oilseed rape acyl-ACP thioesterase gene; GS = Sugar beet glutamine synthetase gene; Ivr1 = Maize invertase gene, Le1 = Soybean lectin gene; SAH7 = Cotton IVS of the putative *Sinapis Arabidopsis Homolog 7* protein gene; SPS = Rice sucrose-phosphate synthase gene; trnL = Chloroplast tRNA-Leu intron; UGPase = Potato UDP-glucose pyrophosphorylase gene; TOT=Total

12.6 Detection Chemistry of non-EU Reference Methods

Figure 109 – Percentage of Detection Chemistries Used with non-EU Reference Methods



Detection Chemistry	Non-EU Reference Methods Using the Detection Chemystry	%
TaqMan	29	81 %
Scorpion	0	0 %
Molecular Beacons	0	0 %
FRET	0	0 %
SybrGreen	4	11 %
Amplifuor	0	0 %
Other	3	8 %
TOT	36	100 %

Legend: TOT= Total

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