Monitoring of Ornamental Plants for Genetic Modifications

Guidance document of the Working Group on Genetic Engineering of the German Federal Government and the *Länder* (LAG)

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Contents

Foreword

At the end of April 2017 – during the high season for petunia trading – the Finnish Food Safety Authority (Evira) reported that it had detected genetically modified (GM) petunias in Finland and had withdrawn them from the market. Testing for genetic modification was prompted by the identification of a flower colour that is unusual for petunias. The petunia varieties of concern found in Finland had originated from breeders in the Netherlands and Germany. Furthermore, in subsequent studies conducted in Germany, genetic modifications were detected in numerous other petunia varieties[1]. As a result of these findings, in the year 2017, breeders, young plant propagators and other operators in the petunia distribution chain were suddenly confronted with huge challenges. No genetically modified petunias are currently authorised for marketing or cultivation in the EU. As a consequence, in 2017, large quantities of plants had to be withdrawn from the market and destroyed.

Early nationwide monitoring at the bottlenecks of ornamental plant production and of the distribution chain (at places where ornamental plants enter the German market) is designed to avoid the propagation of large quantities of GM ornamental plants for commercial purposes and their entry into the market.

In the EU, only five GM carnation varieties may currently be marketed as cut flowers.

At its 53rd session on 18-19 May 2017, the LAG decided to set up an ad-hoc committee of enquiry “Genetically modified ornamental plants” which is tasked with reporting on the current state of affairs regarding genetically modified petunias and developing a concept for the monitoring of ornamental plants.

[1] Synoptic table of the Federal Office of Consumer Protection and Food Safety (BVL) listing GM petunia varieties in which genetic modifications were detected in studies conducted in Germany and the Netherlands (analytical reports have been submitted to the BVL)
https://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/Ereignisse/gv_Petunien_Deutschland_alt.html?nn=9698314

1. Basic considerations for the monitoring of ornamental plants taking into account the results of the self-monitoring activities of the breeders

a. Following the experiences with genetically modified petunias in 2017 and according to information from the petunia industry, future generations of petunia can be expected to be systematically tested for GMO content. This is where a regulatory monitoring strategy can begin. When monitoring ornamental plants for genetic modifications, the results of the companies' own monitoring can be taken into account on a regular basis, provided that the tests have been carried out by an ISO 17025 accredited laboratory and meet the requirements described in Chapter 3.

b. In the current monitoring of seeds of conventionally cultivated varieties of crop and vegetable species used for agriculture, two tests of different samples from the same lot can be expected to differ because of the very low GMO content through contamination. With genetically modified varieties (as in the case of the GM petunia varieties), on the other hand, a sample is either genetically modified or not. For this reason, tests of two different samples of the same variety must always yield the same result.

c. Tests initiated and documented by the breeder can be controlled within the scope of regulatory monitoring. In individual cases, the test results for a particular variety can be controlled by further analysis instigated by the regulatory authority. In this case, the sampling does not have to be carried out at the breeding site but may take place along the distribution chain (e.g. at the young plant production site).

d. Furthermore, imported plants – where it is not known whether self-monitoring has taken place or not – can be tested for the presence of genetic modifications on a random basis.

2. Sampling strategies for ornamental plants

2.1 Sampling strategy for vegetatively propagated ornamental plants

To ensure that the samples being tested are as representative as possible, the objective should be to control the variety at the beginning of the distribution chain. In the ideal situation, this is described by the variety name used by the breeding company. Varieties which are subject to plant variety protection rights have a variety denomination which has been designated at the request of the breeder by the Community Plant Variety Office (www.cpvo.europa.eu) or by the German Federal Variety Office (www.bundessortenamt.de). However, frequently the varieties are not marketed under that name but have been given a trade name. In addition, not all varieties have been registered for variety protection by the breeder so that only a portion of the traded varieties have a designated variety denomination.

Very often breeders will grant licenses for propagation of their varieties to other breeders and young plant growers. These companies frequently market the acquired varieties under different trade names than those used by the breeder so that they match their own brands. This means that the actual identity and origin of the variety is no longer transparent to the consumer (horticultural businesses) or to the monitoring bodies. Thus, the same variety can be marketed by multiple young plant growers and under different trade names.

Therefore, GMO testing should preferably be carried out immediately before licenses are granted at the actual breeding site of new varieties, as this could avoid genetically modified varieties being sent to the licensee and propagated or placed on the market by them.

Sampling sites:

a. **for varieties from German breeders [2]:**
   Sampling in the "nuclear stock"[3] and testing in an ISO 17025 accredited laboratory should take place, if possible, in the context of self-monitoring by the breeder.

b. **for varieties from breeders outside Germany for which a German plant grower has purchased a propagation license directly or through a European licensing agency:**
   Sampling and testing of the new variety by an ISO 17025 accredited laboratory should take place, if possible, prior to setting up the stock of mother plants or at the start of that process
   - either in the context of the young plant grower's self-monitoring
   - or in the context of the self-monitoring of the licensing agency or the breeder, if the young plant grower has received a copy of the test report of an ISO 17025 accredited laboratory on the respective plants.

c. **for plants introduced into Germany via certain ports of entry (e.g. airports):**
   Random sampling can be carried out for regulatory testing of cuttings, e.g. at Frankfurt Airport. If the competent authorities are aware that test reports from an ISO 17025 accredited laboratory which certify that these varieties are not genetically modified are available for the variety in question, those cuttings can be exempted from inclusion in the random sampling.

d. **for commercially traded plants which were not produced in Germany:**
   When conducting random sampling for the official control of plants available on the market (e.g. from auction houses, distribution outlets, mail-order businesses, wholesale chains such as supermarkets, garden centres, furniture stores, etc.) care should be taken to ensure that the origin of the plants can be researched so that these plants can be assigned to a specific breeder or a specific variety.
   If test reports are presented from ISO 17025 accredited laboratories certifying that no genetic modification was detected in the plants in question, these plants can be excluded from the random sampling.

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[2] Many breeders and young plant growers are organised nationwide in the Expert Group on Young Plants (*Fachgruppe Jungpflanzen*): http://www.youngplants.de/content/firmen_index.php

[3] NS = new, selected crossbreeds or varieties for maintenance breeding, which were rendered free of pathogens by meristem culture. These plants and the plants of the propagation stock (PS) form the basis for further propagation and the establishment of the mother plants (frequently in operations in southern Africa or, in the case of *Solanaceae,* in Europe or in Israel).

e. **Random control testing of varieties which have already been tested within the scope of the economic operators' self-monitoring**:
These varieties, which were already analysed within the scope of the economic operators' self-monitoring, can be randomly sampled for official testing along the distribution chain. In the process, care must be taken to ensure that the sampled plants, which may be traded under a different name, can be clearly assigned to the variety in question.
(Note: If analytical reports from an accredited laboratory are available and no mistake has been made, these control tests must verify the breeder's tests; see Chapter 1.)

2.2 Sampling strategy for generatively propagated ornamental plants

Ornamental plants are sometimes also propagated by generative methods. For example, petunias that have been grown from seed are available on the market as well as petunia seeds.
Since relevant ornamental plant species such as petunias are currently propagated mainly by vegetative methods through cuttings, monitoring should initially cover vegetatively propagated ornamental plants in particular.
In justified cases, seeds which are on the market can be sampled randomly and where there is reason to do so.

3. Sample preparation and analysis of ornamental plants

3.1 Sample preparation and analysis of petunias

Sampling and analysis are based on the official collection of methods of sampling and analysis published by the Federal Office of Consumer Protection and Food Safety. This is in accordance with §28b GenTG regarding the collection and analysis of samples conducted or applied in the context of the monitoring of genetic engineering operations, genetic engineering facilities, deliberate releases of genetically modified organisms and placing on the market.

The testing of petunias for GMO content can be performed on seeds as well as on vegetatively or generatively propagated plants. When collecting samples and when

transporting samples, the requirements and instructions set out in the official procedures G 00.00-3[4] and G 30.10-1[5] must be taken into consideration. The analysis focuses primarily on assessing whether the variety in question is a GMO variety or not. This means that the purpose of the test is to verify the identity of the sampled plants. Consequently, the collection of individual samples as described under point 6.2.2 of the official procedure G 30.10-1[5] is appropriate. For seed testing, at least 10 mg should be used. According to the circumstances described under 1.b., in the case of vegetation samples, the sampling of a plant or plant material of a plant is sufficient.
When extracting DNA from the test sample, the general instructions and requirements of the official procedure G 00.00-4[6] must be taken into consideration.

For molecular biological analysis of petunias for genetic modifications, the following methods of volume VI – Genetic Engineering – of the official collection of sampling and analysis methods are available:

- G 30.40-3[7] for the detection of P-35S and T-nos (element-specific methods),
- G 30.40-7[8] for the detection of P-nos (element-specific method) and
- G 30.40-8[9] for the detection of P-nos - nptII (construct-specific method).

In addition, other as yet non-official element-specific (e.g. T-35S, T-ocs, T-g7) or construct-specific (e.g. P-35S – A1) detection methods may be used for GMO screening (see Annex 3).
A list of the genetically modified petunia varieties known to date, including the elements and/or constructs that were introduced by genetic engineering methods, is presented in Annex 3.

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[4] Official collection of methods of analysis according to § 28b of the German Genetic Engineering Act (GenTG), volume VI (G), G 00.00-3: Sampling Procedures – General Instructions and Requirements
[5] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 30.10-1: Sampling of Plant Material
[6] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 00.00-4: Nucleic Acid Extraction Methods – General Instructions and Requirements
[7] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 30.40-3: Detection in plants of specific DNA sequences frequently used in genetically modified organisms (GMOs) from cauliflower mosaic virus (CaMV 35S promoter, P35S) as well as from *Agrobacterium tumefaciens* (T-nos) – element-specific methods (screening)
[8] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 30.40-7: Detection of the P-nos sequence for the purpose of screening plant material for components from genetically modified organisms (GMOs) by means of real-time PCR – element-specific method
[9] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 30.40-8: Detection of the DNA sequence at the transition from the nos promoter to the nptII gene to screen for components from genetically modified organisms (GMOs) in plant material by means of real-time PCR - construct-specific method

When drawing up the analytical report, the general instructions and requirements described in the official methods G 00.00-1[10] und G 00.00-5[11] are to be taken into account.

4. Exchange of information

Information provided by the EU Commission and EU Member States as well as third countries concerning suspected or detected genetically modified ornamental plants is immediately passed on by the BVL to the competent regional authorities under genetic engineering law.

In the case that genetically modified ornamental plant varieties are detected within the scope of the monitoring activities of the *Länder*, the competent authorities of the other affected *Länder* should be informed immediately if it cannot be verified that the plant lot is limited to the operation where the sampling took place.

The following information should be communicated:

- the analytical report on the variety in question
- the official variety denomination of the variety in question (if available), the variety name used by the breeder as well as the trade names
- origin/supplier of the plants
- customer lists for the relevant varieties
- description or photos of the tested plants for possible identification of uncharacterized plants

Additionally, the *Land* responsible for the commercial operation from which the plants concerned originate should ask the operation of origin to inform its customers directly.

[10] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 00.00-1: Sampling and analysis methods for monitoring according to Genetic Engineering Act – General Instructions and Requirements

[11] Official collection of methods of analysis according to § 28b GenTG, volume VI (G), G 00.00-5: Methods for detection of nucleic acid sequences by polymerase chain reaction (PCR) – General Instructions and Requirements

If there are any indications that other EU Member States or third countries are affected (whether as countries of origin or as receiving countries), the BVL must be informed. The BVL will then assume its statutory tasks resulting from this and will inform the affected Member States, the EU Commission, the Biosafety Clearing-House (BCH) and/or the affected third countries[12].

5. Ornamental plants relevant for risk-oriented monitoring

Due to the massive presence of genetically modified petunias on the market in 2017, the focus will continue to be on monitoring petunias for genetic modifications. In addition, samples of other ornamental plants may be tested on a random basis or as warranted.

Other ornamental plant species may become relevant for monitoring in the future. This applies especially to plants of which it is known that genetically modified varieties of these plants have been produced in a closed contained system (e.g. pelargonias, begonias, carnations, roses, chrysanthemums, orchids, lilies).

[12] In the case that GM plants from Germany have been delivered to third countries, the BVL, as the competent national authority, may have an obligation under Art. 17 and Art. 25 of the Cartagena Protocol on Biosafety to inform those states about the unintentional transboundary movement of GMOs.

Annex 1 - The value chain of vegetatively propagated ornamental plants (example: petunia)

	Stages of Production	Duration	Remarks
1	**Breeding - crossbreeding**	**3-5 years**	In Germany, in the EU, or outside the EU
2	**Breeding - selection**		
3	**Production of the source material**	**1 year**	Establishment of meristem cultures, serological and virological testing in the laboratory
4	**Re-testing of the variety**	**1-3 years**	Field tests under consumer conditions
5	**Propagation**		
6	**Registration for variety protection, where applicable**		The breeder **may** register new varieties with the German Federal Variety Office (*Bundessortenamt*) or the Community Plant Variety Office (CPVO) to have the variety protected.
7	**Mother plant cultivation and market introduction**	**8 months**	The production of mother plants and cuttings generally takes place abroad, often outside the EU (because the import of *Solanaceae* is prohibited, petunias/petunia cuttings may only be imported from EU Member States, other European countries, and countries in the Mediterranean region).
8	**Shipment of cuttings**	**5 months**	The cuttings are cut in other EU countries and shipped to young plant growers, sometimes also directly to the production facilities (from around calendar week (CW) 45 of the previous year up to CW 14; the peak weeks for shipment of cuttings are between CW 1 and CW 10).

	Stages of Production	Duration	Remarks
9	**Rooting takes place in horticultural operations**	**3- 4 weeks**	The unrooted cuttings are put to root in young plant nurseries, sometimes also in plant production operations.
10	**Production of the finished plants**	**6- 8 weeks**	Rooted cuttings are sent to horticultural farms for further cultivation; sometimes they are further cultivated up to the finished product in the young plant nurseries. In the horticultural farms, these young plants are potted and cultivated until they are ready for sale (the main sales period is between CW 14 and CW 22).
11	**Marketing through wholesale distributors**	**1 day**	Distribution of the plants via wholesale distributors such as traders, auction platforms, cash and carry stores, wholesale markets or tele-marketers to garden centres, florists, hardware stores and supermarkets. Sometimes the plants are also sent directly from the producer to garden centres or markets on the basis of a contract growing agreement (from CW 14 to CW 22).
12	**Sale to the final consumer / retail sector**	**1 - 3 days**	Marketing to the final consumer takes place in sales outlets such as florists, garden centres, hardware stores and supermarkets usually without a variety or trade name, but e.g. under the term "petunia" or "bedding plant" (from CW 14 to CW 22).

Annex 2 - The relationship between variety name, variety denomination, brand name and trade name

When a variety is placed on the market by a breeder, it has an in-house variety name and a trade name. The trade name is generally composed of the name of the series and that of the respective variety within the series. The series name may be protected by trademark.

Example trade names:

Crazytunia® Maniac Pink, Crazytunia® Cherry Cheesecake

Crazytunia® is the name of the series; it is protected under trademark law as indicated by the "®". Maniac Pink and Cherry Cheesecake are names of varieties within the series.

The trade name (possibly the brand name) is not necessarily linked to a specific variety. Different trade names may be used for the same variety, not only by different licensees but also in different countries. Conversely, different varieties may also be sold from year to year under the same trade name. As a result, only the breeder and/or the respective licensee know which variety is behind the trade name used.

If a plant variety has variety protection, then it has a variety denomination registered in the variety register of the Community Plant Variety Office (CPVO) or the German Federal Variety Office. If this variety denomination has been used in the marketing of the variety (i.e. it appears on the label attached to the variety material), the plant material can be clearly assigned to the protected variety.

Example:

variety name breeder	designated variety denomination CPVO	trade name breeder	trade name licensee 1	trade name licensee 2
Salmon Ray	Draysalmon	Salmon Ray	Pegasus Orange Morn	Viva Orange

Annex 3 – Information on GM petunia with a modified flower colour

Information based on publications

As of 19-04- 2018

GM petunien lines	genetic elements									Selection marker	Transgene	Reference	Name of the plasmid used for transformation (starting plasmid)
	P-35S	T-35S	T-nos	T-ocs	T-g7	P-nos	nptII	P-nos/nptII	P-35S/A1				
RL01-15, RL01-17, RL01-21, RL01-24 u.a.	+	+	-	+	-	+	+	+	+	ampR, kanR	maize A1 gene (dfr)	Meyer et al. (1987); Linn et al. (1990)	p35A1 (wih parts of pLGV11, Hain et al., 1985)
G1, G12, G23, G24, G27, G35, G37, G120, G143, G149, G151, G154	+	-	-	+	+	+	+	+	-	ampR, kanR	gerbera dfr gene	Elomaa et al. (1995)	pHTT294, pHTT372 (with parts of pLGV, Hain et al., 1985)
A19, A41, A42, A45, A47, A48, A49, A52, A54	+	-	-	+	+	+	+	+	+	ampR, kanR	maize A1 gene (dfr)	Elomaa et al. (1995)	pHTT294, pHTT372 (with parts of pLGV, Hain et al., 1985)
PT13-4, PT14-10, PT84-73, PT103-26	+	-	+	-	-	+	+	+	-	tetR, kanR	several transgenes (sense or antisense): petunia F3'5'H, FLS, F3'H, 3RT, AR–AT, F3H; rose DFR und FLS; torenia FNS	Tsuda et al. (2004)	(pBIN19, pBinPlus, van Engelen et al., 1995)
No. 13, 18, 33, 60, 96, 102, 106, 110	+	-	+	-	-	+	+	+	-	tetR, kanR	F3'5'H genes : TG1 (prairie gentian), AK14 (petunia, sense or antisense)	Shimada et al. (1999); Shimada et al. (2001)	pB853 (pBI121)
CG1, CG3	+	-	+	-	-	+	+	+	-	tetR, kanR	chalcone synthase (chsA)	Li et al. (2001)	pBI121-chsA (pBI121)
218.11, 218.38, 218.41, 218.43, 218.56	+	-	+	+	-	+	+	+	-	tetR, kanR	petunia chs gene (sense and antisense)	Napoli et al. (1990)	pFLG5972, pFLG7010 (pJJ3942 based on pRK290, Ditta et al., 1980)
M3011-104-1, M3011-104-2, M3011-104-13, M3011-104-18, M3011-104-30, M3011-104-35, M3011-104-38	+	-	+	-	-	+	+	+	-	tetR, kanR	petunia antisense chs gene	van der Krol et al. (1988)	VIP104 (pBIN19)
104-9, 104-28, 104-31, 104-4A, 104-8A, 104-32A, 104-21A	+	-	+	-	-	+	+	+	-	tetR, kanR	petunia antisense chs gene	van der Krol et al. (1990a)	VIP104 (pBIN19)
RHSCC 64C, RHSCC 67C, RHSCC 71B/C, RHSCC 71D, RHSCC 78A	+	-	-	-	-	-	+	-	-	gentR, kanR	petunia 3RT sense and antisense gene	Brugliera et al. (1994)	pCGP810, pCGP811 (pCGN1559, McBride & Summerfelt, 1990)
#3759, #3760, #3768	+	-	-	-	-	-	+	-	-	gentR, kanR	rose dfr gene	Tanaka et al. (1995)	pCGP645 (pCGN1559, McBride & Summerfelt, 1990)
B18-4, B18-12, B58-9, B58-23	(+)?						(+)?		-	not specified	cineraria CYP75B18v4 and CYP75B58 genes	Tanaka & Brugliera (2013)	not specified

Information based on sequence[1] or experimental data[2]

RL01-17[1]	+	+	-	-*	-	+	+	+	+	ampR*, kanR	maize A1 gene (dfr)	Meyer (2017) pers. comm. sequence in www.euginius.org	p35A1?
African Sunset/ Bonnie Orange cultivar[1 2]	+	+	-	+	-	+	+	+	+	ampR*, kanR	maize A1 gene (dfr)	Bashandy & Teeri (2017)	p35A1?
Sanguna Salmon[2]; Go!Tunia Orange[2]	+	+	-	+	?	+	+	+	+	ampR, kanR	maize A1 gene (dfr)	analytical reports (DE, NL)	p35A1?
Raspberry Blast[2]; Mini Blast Rose[2]; Supertunia Flamingo; Lipstick	+	-	+	-	?	+	+	+	-	kanR	petunia F3'5'H gene (AK14, see Shimada et al., 1999)	analytical reports (DE)	pB853?

*only partly present in sequence of integration site

Bashandy and Teeri (2017), Planta 246, 277-280		
Brugliera et al. (1994), Plant Journal 5, 81-92		
Ditta et al. (1980), Proc Natl Acad Sci USA 77, 7347-7351		
Elomaa et al. (1995), Mol Gen Genet 248, 649-656		
Hain et al. (1985), Mol Gen Genet 199, 161-168		
Li et al. (2001), Science in China 44, 661-668		
Linn et al. (1990), Mol Gen Genet 222, 329-336		
McBride and Summerfelt (1990), Plant Mol Biol 14, 269-276		
Meyer et al. (1987), Nature 330, 677-678		
Napoli et al. (1990), Plant Cell 2, 279-289		
Shimada et al. (1999), FEBS Lett 461, 241-245		
Shimada et al. (2001), Plant Cell Rep 20, 456-462		
Tanaka et al. (1995), Plant Cell Physiol 36, 1023-1031		
Tanaka and Brugliera (2013), Phil Trans R Soc B 368, 20120432		
Tsuda et al. (2004), Plant Biotechn 21, 377-386		
van der Krol et al. (1988), Nature 333, 866-869		
van der Krol et al. (1990), Mol Gen Genet 220, 204-212		
van Engelen et al. (1995), Transgenic Research 4, 288-290		