Confirmation of exemption of non-regulated status for Petunia's containing the A1 gene of Maize (A1-DFR Petunia's)

Submitted by:

Dümmen NA, Inc. 250 South High Street Suite 650 Columbus, OH 43215 USA

July, 2021

This documentation does not contain confidential business information

Certification

The undersigned submits this documentation under 7 CFR 340.1c to request that the Administrator determines that the varieties requested in the documentation should not be regulated under 7 CFR 340.

This documentation does not contain any confidential business information.

Signature Place, date Keith Cable, President North America <u>K.Cable@DummenOrange.com</u>

 $+1\ 630\ 520\ 8730$

Contacts

Author and primary contacts regarding this document

Tosca Ferber

Research Director Breeding Technology Centre Oudecampsweg 35c 2678 NN De Lier +31681859940 t.ferber@DummenOrange.com

Arjan Koot

Breeding Manager Pot & Bedding Breeding Technology Centre Oudecampsweg 35c 2678 NN De Lier +31612099500 A.Koot@DummenOrange.com

US-based contact

Jonathan Pierre

Operations Director Dümmen NA, Inc. 250 South High Street | Suite 650 Columbus, OH 43215, OH United States of America +1 (614) 638 9859 P.Pierre@DummenOrange.com

Summary (Statement of Grounds)

Dümmen Orange the Netherlands B.V. is requesting the exemption of 3 Genetically Modified (GM) *Petunia hybrida* varieties (*Petunia axillaris* x *Petunia integrifolia*) - also referred to as *Petunia x hybrid Vilm.* – descending from the event described in petition 19-099-01p.

Family	Solanaceae
Genus	Petunia
Species	hybrid
Common name	Petunia
Varieties	40257, 40537, 40794
Events	Reported in Table 1 of petition 19-099-01p
Transformation vector	p35A1

The evidence leading to the conclusion that these 3 GM Petunia varieties follow from the same transformation event as reported in petition 19-099-01p is found in the facts that the GM vector reported in petition 19-099-01p is completely intact in the GM Petunia varieties identified by Dümmen Orange (section 5 of the document "*Data supporting exemption of varieties 40257, 40537 and 40794*") and that the linkage between the transformation and the trait (bright, neon-like orange/red flower color) is fully intact.

Based on the description of exemption written in 7 CFR 340.1c these 3 GM Petunia varieties should not be regulated under 7 CFR 340 as they carry the same modification as described in petition 19-099-01p for which the APHIS Deputy Administrator granted the nonregulated status to the filed Westhoff A1-DFR petunia varieties.

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1) General information about the Petunia genus

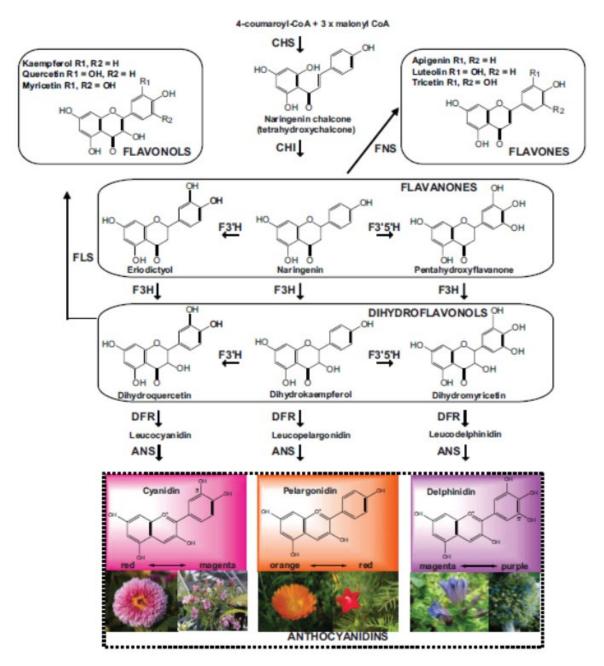
Garden petunia (*Petunia x hybrida*) is a commercially cultivated crop which has been derived from an initial cross between *Petunia integrifolia* and *Petunia axilaris* leading back to 1834 (Sink, 1984). Since this initial hybridization in 1834, private and professional breeders have created a wealth of genetic diversity in this ornamental plant leading to various plant habits, flower colors and flower types. A significant lack in the natural variation present in this crop was a bright orange color (Westhoff, 2019).

Knowledge of pollination of Petunia is based on studies in the parental species of this hybrid and shows that natural pollination can be facilitated through a range of pollinators, however, due to the flower type of *Petunia x hybrida*, it is expected that natural pollination would be most affected by bee pollination (Westhoff, 2019).

Distribution of the crop is limited by environmental conditions. Climate regions which include periods of frost are highly unlikely to promote weediness of this somewhat tropical crop. Even in climate regions which resemble the region of origin (South America, on the border of Brazil, Argentina and Uruguay, Turchetto *et al.*, 2019) of Petunia, like Florida, Petunia plants have not become weedy in their distribution. Dispersal of the crop through seeds is, although not impossible, unlikely as seed dispersal of Petunia plants is not a readily seen phenomenon in the US (Westhoff, 2019). Pollen dispersal possess a more likely form of gene flow for Petunia, however, gene flow of traits from Petunia to other (native or non-native) species in the US is deemed unlikely as hybridization with the closest relative of *Petunia* x *hybrida* (i.e. Calibrachoa) is not a hybridization which occurs without extensive human intervention (Westhoff, 2019).

2) Genetic engineering of flower color in Petunia

Flower color, a pivotal trait for ornamental breeding, is determined through a complex biological process which is driven by several biosynthesis pathways. In these pathways, precursor compounds are processed into the floral pigments flavonoids, carotenoids and/or betalains (Westhoff, 2019). During these biosynthesis pathways, multiple enzymes and derivatives play crucial roles in determining the final flower color. In Petunia, anthocyanins, flavonols and carotenoids are the pigments responsible for determining flower color. Anthocyanins consist of three groups of which the cyanidin and delphinidin derivatives are produced in Petunia, while pelargonidin is not (Meyer *et al.*, 1987), see picture 1. This anthocyanin production is responsible for the availability of natural flower colors in Petunia as delphinidins produce variations of blue color (prevalent in Petunia), cyanidins lead to red or pink colors (also prevalent in Petunia), while pelargonidins produce orange or brick red color (lacking in the original commercial Petunia genepool).

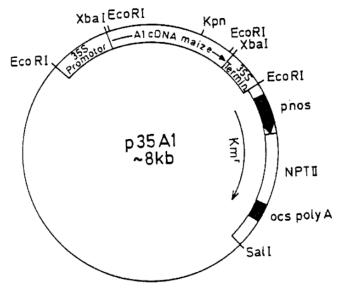


Picture 1: Biosynthesis pathways for anthocyanidins Cyanidin, Pelargonidin and Delphinidin (from Westhoff, 2019).

Meyer *et al.* (1987) studied the anthocyanin pathway in Petunia and traced the lack of orange/brick red flower color back to the dihydroflavonol 4-reductase (DFR) gene. By genetic modification, Mayer *et al.* (1987) introduced the DFR gene from Maize into Petunia. The resulting GM A1-DFR Petunias showed to restore the production of Pelargonidin leading to orange and red flower color phenotypes previously not seen in Petunia.

3) Identification of A1-DFR Petunias on the global commercial market

As described in the Plant Pest Risk Assessment related to petition 19-099-01p (USDA-APHIS, 2020), Petunias resulting from the A1-DFR genetic modification were reported to have been trialed in Florida in 1992 (Oud et al., 1993). After this publication in 1993, no reports on trialing or commercialization of A1-DFR can be identified until early 2017 (Bashandy & Teeri, 2017). In 2017, Bashandy & Teeri published the finding of unauthorized A1-DFR Petunias on the global commercial market. They initially identified plants of the variety "Bonnie Orange" at the railways station in Helsinki to be genetically modified, after which a global scale screen of orange Petunia varieties exposed the unintended commercialization of GM Petunias on the global market. As this release of GM Petunias was unknown to Petunia breeding companies, the unintended sales of GM Petunias came to an abrupt end in 2017. Commercial Petunia breeding companies pulled their GM varieties off the market, substantiated by tests confirming the presence of absence of the p35A1 vector in commercial Petunias (see box 1). Although the direct link between the GM Petunias identified in 2017 and the reports of Meyer et al. (1987)



Box 1: Above the p35A1 vector is presented (taken from Westhoff, 2019) as published by Meyer *et al.* (1987). This vector shows the presence of the CaMV35s promoter, along with the type 2 A1 gene of Zea mays, whose transcription is terminated by the 35S terminator. To allow for the fast selection of transformants, kanamycin resistance through the NPII gene is used. Expression of the NPII gene is governed by the nopaline synthase promoter (pnos) and the ocs poly A gene. Different loci of the vector can be used to screen for the presence or absence of the p35A1 vector.

cannot be made, Westhoff (2019) convincingly presented data claiming the link between their petitioned varieties and the original publication of Meyer *et al.* (1987). Westhoff (2019) provided sequence information of 11 GM Petunias which confirms the decent of these GM varieties reported by Westhoff (2019) from the original publication by Meyer *et al.* (1987). This conclusion was further substantiated by MinION results obtained for various commercial varieties (including Dümmen Orange Variety Potunia Plus Papaya 40257) obtained for the vector region (Fraiture *et al.*, 2019).

4) Granting of nonregulated status to A1-DFR Petunias by APHIS

In their Petition paper, Westhoff (2019) provides data to substantiate the descendance of the GM Petunia varieties from Meyer *et al.*'s A1-DFR Petunias. Additionally, Westhoff provides data showing the limited risk of the previously commercialized GM Petunias to the US market and Flora & Fauna in the US. The potential risks of A1-DFR Petunias were assessed by the USDA (USDA-APHIS, 2020) for impacts on (1) Plant Pests & Diseases, (2) Organisms beneficial to Agriculture, (3) Weediness, (4) Weediness after outcrossing (5) Agriculture or Cultivation and (6) Geneflow. Based on this Risk Assessment, the USDA decided that A1-DFR Petunias will be deregulated under 7 CFR part 340 (USDA-APHIS, 2021).

In this document we provide data that three GM Petunia varieties of Dümmen Orange also decent from the A1-DFR Petunias genetically modified by Meyer *et al.* (1987) and thus should be exempt from regulation under 7 CFR 340.1c.

5) Data supporting exemption of varieties 40257, 40537 and 40794

After the finding of the unintended release of GM Petunias on the global comercial market (Bashandy & Teeri, 2017), Dümmen Orange partnered with Planton® GmbH, a Germany-based testing and biotechnology Company, to test all Dümmen Orange Petunia varieties for the presence or absence of the p35A1

Table 1: The table below shows the tests performed by Planton® to prove the presence or absence of GM constructs in Dümmen Orange petunia plant accessions (both commercial and non-commercial plants). Details of the methods can be found under the test number at the Deutsche Akkreditierungsstelle (www.dakks.de).

Test-Nr. / No.	Method	LOD
PLA-SOP0020	35S, realtime PCR	0.1%
PLA-SOP0022	nptII, realtime PCR	0.1%
PLA-SOP0911	P-NOS, realtime PCR	0.1%
PLA-SOP0912	P-NOS-NPTII, realtime PCR	0.1%

vector used by Meyer *et al.* (1987), see box 1. In total, 730 Petunia accessions were tested by Dümmen Orange in 2017 for the presence of the p35A1 vector based on results for 35S, nptII, P-NOS and P-NOS-NPTII (see table 1). Out of these 730 accessions, 128 accessions were found to be positive for all 4 test of the p35A1 vector (see Appendix 8.4 for 3 certificates). Never were recombinants found in the positive tests: when a positive test was retreived, all 4 tests for that plant accession were positive. In all 128 cases of the presence of the p35A1 vector, the flower phenotype resembled the effect of p35A1: all 128 plant accessions showed a bright orange, red, pink or neon flower color.

Based on the positive test results of Planton®, Dümmen Orange GM Petunias were adequately destroyed except for copies of 40257, 40537 and 40794. Copies of these varieties were maintained in-vitro in a GM certified laboratory (Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Inventigaciones Científicas - CEBAS-CSIC).

To further substantiate the fact that the remaining 3 GM Petunia varieties of Dümmen Orange are derived from the exempt A1-DFR Petunias produced by Meyer *et al.* (1987), we sequenced parts of the p35A1 construct of these 3 varieties. Appendix 8.3 shows the sequence results for each variety for several parts of the p35A1 vector. All retrieved results show 100% sequence identify with the original p35A1 construct.

To test whether additional exogenous sequences may have been introduced into the remaining 3 GM varieties during the modification process, information about the transformation event is required. Since there is no absolute proof which original modification was performed to generate the GM Petunias, and because the 3 requested varieties are the product of generations of conventional breeding, this test for unintended exogenous sequences cannot be performed. If any exogenous sequences were introduced to

GM A1-DFR Petunias, their impact should resemble the level of impact as described in the Plant Pest Risk assessment performed for petition 19-099-01p and thus should be of no significant impact.

6) Conclusion

Based on the facts that no recombinant GM constructs were found while screening the entire Dümmen Orange Petunia genepool (all samples were always either positive for all 4 tested loci or negative for all 4 tested loci) and the fact that sequence data retrieved across the p35A1 vector from the remaining GM Petunia varieties 40257, 40537 and 40794 showed 100% resemblance to the published p35A1 vector for those parts of the vector for which data was obtained, we are convinced that varieties 40257, 40537 and 40794 have the same plant-trait-Mechanism of Action (MOA) combination as as a plant previously reviewed by APHIS and determined to be nonregulated in response to petition 19-099-01p (submitted prior to October 1, 2021), pursuant to § 340.6 of the previous regulations found at 7 CFR part.

7) References

Bashandy H & Teeri TH (2017). Genetically engineered orange Petunias on the market. Planta 246, 277-280. doi: 10.1007/s00425-017-2722-8.

Fraiture MA, Ujhelyi G, Ovesná J, Van Geel D, De Keersmaecker S, Saltykova A, Papazova N & Roosens NHC (2019). MinION sequencing technology to characterize unauthorized GM petunia plants circulating on the European Union market. Scientific Reports. 9. 10.1038/s41598-019-43463-5.

Oud JS, Schneiders H, Kool AJ & van Grinsven MQ (1995). Breeding of transgenic orange Petunia hybrida varieties. Euphytica 84: 175-181.

Sink, KC (1984). Petunia. In: K.C. Sink (Ed.), Petunia: Monographs on Theoretical and Applied Genetics 9. Springer -Verlag, Berlin, 3-9.

Turchetto C, Segatto ALA, Silva-Arias GA, Bedushci J, Kuhlemeier C, Bonatto SL & Freitas LB (2019). Contact zones and their consequences: hybridization between two ecologically isolated wild Petunia species. Botanical Journal of the Linnean Society 190, 421–435.

USDA-APHIS (2020). Plant Pest Risk Assessment: Westhoff Petition (19099-01p) for Determination of Non-regulated Status of Petunias Containing the A1 gene of Maize (A1-DFR Petunias).

USDA-APHIS (2021). Finding of No Significant Impact: Regarding Deregulation of a Petition (19-099-01p) Under 7 CFR part 340 from: Westhoff Vertriebsgesellschaft mbH A1-DFR Petunias.

Westhoff Vertriebsgesellschaft mbH (2019). Petition for the determination of non-regulated status for Petunias containing the A1 gene of Maize (A1-DFR Petunias). Submitted by C. Westhoff, CEO. Westhoff Vertriebsgesellschaft mbH. Germany.

8) Appendix

8.1) Variety description

Species: *Petunia hybrida* (Petunia axillaris x Petunia integrifolia) Variety: 40257 (Potunia Plus Papaya), *US plant patent file nr:* 22.910, granted 31.07.2012. PI TT08-003356-002 Variety: 40537 (Fortunia Red). PI TT12-001418-002 Variety: 40794 (Surprise Red). PI TT13-004738-013

All 3 Petunia varieties have an orange or bright orange colour.



8.2) Sequence information of the original vector p35A1

Shown below, the DNA sequence of the p35A1 backbone fragments and the A1 gene found integrated in the genome sequence of the GE petunia*. From document: Westhoff Vertriebsges.mbH, revised petition 19-099-01p.

*Description of the highlighted sequence regions:

CaMV 35S promoter

Zea mays A1 gene for dihydroglavonol 4-reductase

Transposon Cin4-1 of maize

35S Terminator

>___> and <___< shows the orientation of used primers to generate PCR-amplicons resulting in data shown in Appendix 8.3

TCCCATGGAGTCAAAGATTCAAATAGAGGA>**CCTAACAGAACTCGCCGTAAAGAC**>TGGCGAACAGTTCA TACAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACGCTTGT CTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTA ATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGG AAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAG TGGTCCCAAAGATGGACCCCCCCCCCCGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCA AAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAG ACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACAGGGTACCCGGGGATCCTCA>**GAGAA** TTCCAGCTGCTCACTCAG>TCCTGCGCAAGAGCTCGCTCTCGGAGAAAAAAACGCGGGAGGCGATAATGC AGGGAGGTGCCGGTGCGAGCGAGAAAGGGACGGTGCTGGTCACGGGGGCGTCGGGCTTCGCCGGCTCCTG GCTCGTCATGAAGCTCCTCCAGGCCGGCTACACCGTCCGGGCGACCGTGCGCGATCCCGCGAACGTTGGG AAGACGAAGCCATTGATGGACCTTCCCGGAGCAACGGAGCGCCTGT<**CCATATGGAAAGCCGACCTG**<GC GGAGGAAGGCAGCTTCCACGACGCCATCAGGGGCTGCACCGGCGTCTTCCACGTCGCCACGCCCATGGAC TTCCTGTCCAAAGACCCTGAGAATGAGGTAATCAAGCCGACGGTGGAAGGGATGATAAGCATCATGCGGG CATGCAAGGAGGCCGGCACCGTGCGGCGCATCGTCTTCACTTCCTCCGCCGGGACGGTCAACCTGGAGGA GCGGCAGAGGCCCGTCTACGACGAGGAAAGCTGGACCGACGTCGACTTCTGCCGTCGCGTCAAGATGACA GGATGGATGTACTTCGTGTCTAAAACCCTGGCGGAGAAGGCGGCCCTGGCGTACGCGGCGGAGCACGGCC TGGACCTGGTCACCATCATCCCGACGCTCGTGGTCGGCCCGTTCATCAGCGCGTCCATGCCGCCCAGCCT CATCACCGCGCTGGCGCTCATCACGGGGAACGCGC>**CGCACTACTCGATCCTCAAGC**>AGGTGCAGCTCA TCCACCTCGACGACCTCTGCGACGCCGAGATCTTCCTCTTCGAGAACCCGGCCGCGGCCGGGCGCTACGT TTGCTCCTCGCACGACGTCACCATCCACGGCCTCGCCGCCATGCTCAGGGATAGGTACCCCGAGTACGAC GTCCCGCAGAGGTTCCCCGGGATCCAGGACGACCTCCAGCCGGTGCG<**CTTCTCGTCCAAGAAGCTCCAG** <GACCTCGGGTTCACCTTCAGGTACAAGACGCTGGAGGACATGTTCGACGCCGCCATCCGGACTTGCCAG GAGAAGGGCCTCATCCCCCTCGCCACTGCCGCCGGAGGGGACGGCTTTGCCTCGGTGCGCGCACCCGGCG AGACGGAGGCGACGATTGGCGCTTAGGCAACGATCCCCCGGCTCTCCCCGTCGATATGATGCAATCAGCT <mark>ATCT</mark>ATCTCTTGTTTGCCAAAAAAAAAAAGGGAGGTCTTGGCATACTCGATCTAGAGCGCTTTGCAAGAG CGTTAAGGCTTAGATGACTATGGCTACGATGGACGAATAGAGACAAAGCATGGACTGGGTTGCAATTAAA ACAAATCTATCTCTCTCTATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCTTATAGGGT AAATTTCTAATT<**CCTAAAACCAAAATCCAGGGGTAC**<CGAGCTC

8.3) Sequence information of varieties 40257, 40537 and 40794

Data in Appendix 8.3 were obtained by Sanger sequencing 3 amplicons per sample (primer sites indicated in Appendix 8.2). The sequence below shows the original sequence of p35A1. The highlighted purple are those parts of the vector for which data was obtained for that sample. Highlighted grey regions of the vector did not result in quality data for that sample. In all cases, the highlighted purple sequences resemble the sequence of the vector p35A1 for 100%.

Sample 1 = 40257

TCCCATGGAGTCAAAGATTCAAATAGAGGA<mark>CCTAACAGAACTCGCCGTAAAGACTGGCGAACAGTTCATA</mark> CAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACGCTTGTCT ACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAAT ATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG GTCCCAAAGATGGACCCCCCACCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAA GCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACAGGGTACCCGGGGATCCTCTAGAGAATTC CAGCTGCTCACTCAGTCCTGCGCAAGAGCTCGCTCTCGGAGAAAAAACGCGGGAGGCGATAATGGAGGG AGGTGCCGGTGCGAGCGAGAAAGGGACGGTGCTGGTCACGGGGGCGTCGGGCTTCGCCGGCTCCTGGCTC GTCATGAAGCTCCTCCAGGCCGGCTACACCGTCCGGGCGACCGTGCGCGATCCCGCGAACGTTGGGAAGA CGAAGCCATTGATGGACCTTCCCGGAGCAACGGAGCGCCTGTCCATATGGAAA</mark>GCCGACCTGGCGGAGGA AGGCAGCTTCCACGACGCCATCAGGGGCTGCACCGGCGTCTTCCACGCCACGCCCATGGACTTCCTG TCCAAAGACCCTGAGAATGAGGTAATCAAGCCGACGGTGGAAGGGATGATAAGCATCATGCGGGCATGCA AGGAGGCCGGCACCGTGCGCGCATCGTCTTCACTTCCTCCGCCGGGACGGTCAACCTGGAGGAGCGGCA GAGGCCCGTCTACGACGAGGAAAGCTGGACCGACGTCGACTTCTGCCGTCGCGTCAAGATGACAGGATGG TGGTCACCATCATCCCGACGCTCGTGGTCGGCCCGTTCATCAGCGCGTCCATGCCGCCCAGCCTCATCAC CGCGCTGGCGCTCATCACGGGGGAACGCGCCGCACTACTCGATCCTCAAGCAGGTGCAGCTCATCCACCTC GACGACCTCTGCGACGCCGAGATCTTCCTCTTCGAGAACCCGGCCGCGGCCGCGCGCTACGTTTGCTCCT CGCACGACGTCACCATCCACGGCCTCGCCGCCATGCTCAGGGATAGGTACCCCGAGTACGACGTCCCGCA GAGGTTCCCCGGGATCCAGGACGACCTCCAGCCGGTGCGCTTCTCGTCCAAGAAGCTCCAGGACCTCGGG TTCACCTTCAGGTACAAGACGCTGGAGGACATGTTCGACGCCGCCATCCGGACTTGCCAGGAGAAGGGCC TCATCCCCCTCGCCACTGCCGCCGGAGGGGGGCGCTTTGCCTCGGTGCGCGCACCCGGCGAGACGGAGGC TGTTTGCCAAAAAAATAAGGGAGGTCTTGGCATACTCGATCTAGAGCGCTTTGCAAGAGCGTTAAGGCT CTCTCTCTATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTCATG TGTTGAGCATATAAGAAACCCT TTCCTAAAACCAAAATCCAGGGGTACCGAGCTC

Sample 2: 40537

TCCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAA<mark>CTCGCCGTAAAGACTGGCGAACAGTTCATA</mark> CAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACGCTTGTCT ACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAAT ATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG GTCCCAAAGATGGACCCCCCACCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAA GCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACAGGGTACCCGGGGATCCTCTAGAGAATTC CAGCTGCTCACTCAGTCCTGCGCAAGAGCTCGCTCTCGGAGAAAAAACGCGGGAGGCGATAATGGAGGG AGGTGCCGGTGCGAGCGAGAAAGGGACGGTGCTGGTCACGGGGGGCGTCGGGCTTCGCCGGCTCCTGGCTC GTCATGAAGCTCCTCCAGGCCGGCTACACCGTCCGGGCGACCGTGCGCGATCCCGCGAACGTTGGGAAGA CGAAGCCATTGATGGACCTTCCCGGAGCAACGGAGCGCCTGTCCATATGGAAAGCCGACCTGGCGGAGGA AGGCAGCTTCCACGACGCCATCAGGGGGCTGCACCGGCGTCTTCCACGTCGCCACGCCCATGGACTTCCTG TCCAAAGACCCTGAGAATGAGGTAATCAAGCCGACGGTGGAAGGGATGATAAGCATCA<mark>TGCGGGCATGCA</mark> AGGAGGCCGGCACCGTGCGCGCGCATCGTCTTCACTTCCTCCGCCGGGACGGTCAACCTGGAGGAGCGGCA GAGGCCCGTCTACGACGAGGAAAGCTGGACCGACGTCGACTTCTGCCGTCGCGTCAAGATGACAGGATGG TGGTCACCATCATCCCGACGCTCGTGGTCGGCCCGTTCATCAGCGCGTCCATGCCGCCCAGCCTCATCAC CGCGCTGGCGCTCATCACGGGGAACGCGCCGCACTACTCGATCCTCAAGCAGGTGCAGCTCATCCACCTC CGCACGACGTCACCATCCACGGCCTCGCCGCCATGCTCAGGGATAGGTACCCCGAGTACGACGTCCCGCA GAGGTTCCCCGGGATCCAGGACGACCTCCAGCCGGTGCGCTTCTCGTCCAAGAAGCTCCAGGACCTCGGG TTCACCTTCAGGTACAAGACGCTGGAGGACATGTTCGACGCCGCCATCCGGACTTGCCAGGAGAAGGGCC TCATCCCCCTCGCCACTGCCGCCGGAGGGGGGCGCGCTTTGCCTCGGTGCGCGCACCCGGCGAGACGGAGGC TGTTTGCCAAAAAAAAAAAGGGAGGTCTTGGCATACTCGATCTAGAGCGCTTTGCAAGAGCGTTAAGGCT CTCTCTCTATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTCATG **TGTTGAGCATATAAGAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAATAAAATTTCTAA** TTCCTAAAACCAAAATCCAGGGGTACCGAGCTC

Sample 3: 40794

TCCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAAAGACTGGCGAACAGTTCATA CAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACGCTTGTCT ACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAAT ATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG GTCCCAAAGATGGACCCCCCACCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAA GCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACAGGGTACCCGGGGATCCTCTAGAGAATTC CAGCTGCTCACTCAGTCCTGCGCAAGAGCTCGCTCTCGGAGAAAAAAACGCGGGAGGCGATAATGGAGGG AGGTGCCGGTGCGAGCGAGAAAGGGACGGTGCTGGTCACGGGGGGCGTCGGGCTTCGCCGGCTCCTGGCTC GTCATGAAGCTCCTCCAGGCCGGCTACACCGTCCGGGCGACCGTGCGCGATCCCGCGAACGTTGGGAAGA CGAAGCCATTGATGGACCTTCCCGGAGCAACGGAGCGCCTGTCCATATGGAAAGCCGACCTGGCGGAGGA AGGCAGCTTCCACGACGCCATCAGGGGGCTGCACCGGCGTCTTCCACGTCGCCACGCCCATGGACTTCCTG TCCAAAGACCCTGAGAATGAGGTAATCAAGCCGACGGTGGAAGGGATGATAAGCATCATGCGGGCATGCA AGGAGGCCGGCACCGTGCGCGCGCATCGTCTTCACTTCCTCCGCCGGGACGGTCAACCTGGAGGAGCGGCA GAGGCCCGTCTACGACGAGGAAAGCTGGACCGACGTCGACTTCTGCCGTCGCGTCAAGATGACAGGATGG ATGTACTTCGTGTCTAAAACCCTGGCGGAGAAGGCGGCCCTGGCGTACGCGGCGGAGCACGGCCTGGACC TGGTCACCATCATCCCGACGCTCGTGGTCGGCCCGTTCATCAGCGCGTCCATGCCGCCCAGCCTCATCAC CGCGCTGGCGCTCATCACGGGGAACGCGCCGCACTACTCGATCCTCAAGCAGGTGCAGCTCATCCACCTC GACGACCTCTGCGACGCCGAGATCTTCCTCTTCGAGAACCCGGCCGCGGCCGCGCGCTACGTTTGCTCCT CGCACGACGTCACCATCCACGGCCTCGCCGCCATGCTCAGGGATAGGTACCCCGAGTACGACGTCCCGCA GAGGTTCCCCGGGATCCAGGACGACCTCCAGCCGGTGCGCTTCTCGTCCAAGAAGCTCCAGGACCTCGGG TTCACCTTCAGGTACAAGACGCTGGAGGACATGTTCGACGCCGCCATCCGGACTTGCCAGGAGAAGGGCC TCATCCCCCTCGCCACTGCCGCCGGAGGGGGCCGCGCTTTGCCTCGGTGCGCGCACCCGGCGAGACGGAGGC TGTTTGCCAAAAAAAAAAAGGGAGGTCTTGGCATACTCGATCTAGAGCGCTTTGCAAGAGCGTTAAGGCT CTCTCTCTATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTCATG <mark>TGTTGAGCATATAAGAAACCCTTAGTATGTA</mark>TTTGTATTTGTAAAATACTTCTATCAATAAAATTTCTAA TTCCTAAAACCAAAATCCAGGGGTACCGAGCTC

8.4) GM test certificates

Variety 40257



PLANTON GmbH Tel.: +49(0)431 / 38015 0 Fax: +49(0)431 / 38015 11 www.planton.de E-Mail: info@planton.de Am Kiel-Kanal 44 D-24106 Kiel, Germany

Auftraggeber / Customer		Seite / Page: 1 / 1 Datum / Date:	24.05.2017
Firma / Company: Dümmen Orange		Kundennummer / Customer No.:	1227
Ansprechpartner / Contact person: Mrs. Tosca Ferber		Auftragsnummer / Order No.:	1227-005
Straße / Adress: Oudecampsweg 35c		Auftragsdatum / Date of Order:	16.05.2017
PLZ / ZIP-code: 2678NN	Ort / City: De Lier	Land / Country: Netherlands	
Telefon / Phone:	Fax:	E-Mail:	

Probe / Sample

Probennummer / Sample no.:	DU0170517-039_G	Probeneingang / Sample arrival:	16.05.2017	
Prüfung eingeleitet am / Start of analysis:	16.05.2017	Prüfung beendet am / End of analysis:	24.05.2017	
Probennummer Auftraggeber / Client sample ID:				

Petunia plant leaves, sample code 132

Probenmenge / Sample size:	37,0 g	Verpackung / Packaging: PE-Bag
Vermahlene Gesamtmenge / Size of grinde	d sample: 37 g	Probentyp / sample type: Petunia plant leaves
Menge für DNA-Isolation / Sample size for I	DNA extraction:	1,2 g

Angewendete Analysen und Prüfergebnisse / Conducted Tests and Results

Test-Nr. / No.	Methode / Method	NWG / LOD	Ergebnis / Result
PLA-SOP0020	35S, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0022	nptII, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0911	P-NOS, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0912	P-NOS-NPTII, qualitative analysis, realtime PCR	0,1 %	positive

Ergebnis von skkreditierten Untersuftragnehmern geliefert / Analysis delivered by accredited subcontractor
 Nicht skkreditiertes Verfahren / Not accredited test

NWG / LOD: Nachweisgrenze / Limit of detection

Meinung und Interpretation / Opinion and Interpretation:

Beurteilung / Appraisal:

Positiv/e = GVO-Anteil gleich oder oberhalb der LOD / GMO content equal or above LOD. Negativ/e = GVO-Anteil unter der LOD / GMO content below LOD.

Chisting Hengel

Datum / Date

24.05.2017

Unterschrift / Subscription: Dipl.-Biol.

Dipl.-Biol. Christina Hempel (Gruppenleiterin)

Die vorliegenden Ergebnisse beziehen sich ausschließlich auf die untersuchte Probe. Auszüge aus dem Prüfbericht dürfen nicht ohne die schriftliche Zustimmung der PLANTON GmbH veröffentlicht werden. / Results refer solely to the analysed sample. It is not permitted to publish or transfer any parts of this report to third parties without a written consent of PLANTON GmbH.

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AG Kiel HR B 5568 · USt-IdNr. / VAT: DE212977228

F0020 / Vers. 09 / 18.11.13



Variety 40537

PLANTON"		PLANTON GmbH Tel.: +49(0)431 / 38015 0 Fax: +49(0)431 / 38015 11 www.planton.de
Prüfbericht / Repor	t	E-Mail: info@planton.de
PB_DUO180517-03	2_G	Am Kiel-Kanal 44 D-24106 Kiel, Germany
Auftraggeber / Customer		Seite / Page: 1 / 1 Datum / Date: 26.05.2017
Firma / Company: Dümmen Ora	ange	Kundennummer / Customer No.: 1227
Ansprechpartner / Contact pers	on: Mrs. Tosca Ferber	Auftragsnummer / Order No.: 1227-006
Straße / Adress: Oudecampswe	eg 35c	Auftragsdatum / Date of Order: 18.05.2017
PLZ / ZIP-code: 2678NN	Ort / City: De Lier	Land / Country: Netherlands
Telefon / Phone:	Fax:	E-Mail:
Probe / Sample		
Probennummer / Sample no.:	DU0180517-032_G	Probeneingang / Sample arrival: 18
Prüfung eingeleitet am / Start of	analysis: 18.05.2017	Prüfung beendet am / End of analysis: 26

Petunia plant leaves, sample code: 29			
Probenmenge / Sample size:	12 leaves	Verpackung / Packaging: PE Bag	
Vermahlene Gesamtmenge / Size of grinded sample: 12 leaves Probentyp / sample type: Petunia plant leaves			
Menge für DNA-Isolation / Sample size for DNA extraction:		12 leaves	

Angewendete Analysen und Prüfergebnisse / Conducted Tests and Results

Unterschrift / Subscription:

Test-Nr. / No.	Methode / Method	NWG / LOD	Ergebnis / Result	
PLA-SOP0020	35S, qualitativer Nachweis, realtime PCR	0,1 %	positive	
PLA-SOP0022	nptll, qualitativer Nachweis, realtime PCR	0,1 %	positive	
PLA-SOP0911	P-NOS, qualitativer Nachweis, realtime PCR	0,1 %	positive	
PLA-SOP0912	P-NOS-NPTII, qualitativer Nachweis, realtime PCR	0,1 %	positive	
 Erosbris von skkreditierte 	Ergebris von skkreditierten Untersuftragnehmern geliefert / Anslysis delivered by accredited subcontractor			

-: Nicht skizeditiertes Verfahren / Not accredited test

NWG / LOD: Nachweisgrenze / Limit of detection

Meinung und Interpretation / Opinion and Interpretation:

Beurteilung / Appraisal:

Positiv/e = GVO-Anteil gleich oder oberhalb der LOD / GMO content equal or above LOD. Negativ/e = GVO-Anteil unter der LOD / GMO content below LOD.

Chisting Hengel

26.05.2017

Datum / Date

Dipl.-Biol. Christina Hempel

(Gruppenleiterin)

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Geschäftsführer / Managing director: PD Dr. Michael Kleine

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DAkkS Deutsche Akireditierungsstelle D-PL-19148-01-00

18.05.2017 26.05.2017 Variety 40794



Prüfbericht / Report PB_DUO170517-063_G

PLANTON GmbH Tel.: +49(0)431 / 38015 0 Fax: +49(0)431 / 38015 11 www.planton.de E-Mail: info@planton.de Am Kiel-Kanal 44 D-24108 Kiel, Germany

Auftraggeber / Customer		Seite / Page: 1 / 1 Datum / Date:	24.05.2017
Firma / Company: Dümmen Orange		Kundennummer / Customer No.:	1227
Ansprechpartner / Contact person: Mrs. Tosca Ferber		Auftragsnummer / Order No.:	1227-005
Straße / Adress: Oudecampsweg 35c		Auftragsdatum / Date of Order:	16.05.2017
PLZ / ZIP-code: 2678NN	Ort / City: De Lier	Land / Country: Netherlands	
Telefon / Phone:	Fax:	E-Mail:	

Probe / Sample

Probennummer / Sample no.:	DU0170517-083_G	Probeneingang / Sample arrival:	16.05.2017
Prüfung eingeleitet am / Start of analysis:	16.05.2017	Prüfung beendet am / End of analysis:	24.05.2017
Probennummer Auftraggeber / Client sample	e ID:	ł	

Petunia plant leaves, sample code 197

Probenmenge / Sample size:	39,0 g	Verpackung / Packaging: PE-Bag	
Vermahlene Gesamtmenge / Size of grinded sample: 39 g		Probentyp / sample type: Petunia plant leaves	
Menge für DNA-Isolation / Sample size for	DNA extraction:	1,28 g	

Angewendete Analysen und Prüfergebnisse / Conducted Tests and Results

Test-Nr. / No.	Methode / Method	NWG / LOD	Ergebnis / Result
PLA-SOP0020	35S, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0022	nptII, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0911	P-NOS, qualitative analysis, realtime PCR	0,1 %	positive
PLA-SOP0912	P-NOS-NPTII, qualitative analysis, realtime PCR	0,1 %	positive

Ergebnis von skizeditierten Untersuftragnehmern geliefert / Anslysis delivered by scon
 Nicht skizeditiertes Verfahren / Not accredited test

NWG / LOD: Nachweisgrenze / Limit of detection

Meinung und Interpretation / Opinion and Interpretation:

Beurteilung / Appraisal:

Positiv/e = GVO-Anteil gleich oder oberhalb der LOD / GMO content equal or above LOD. Negativ/e = GVO-Anteil unter der LOD / GMO content below LOD.

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24.05.2017 Datum / Date

Dipl.-Biol. Christina Hempel (Gruppenielterin)

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Unterschrift / Subscription:

Geschäftsführer / Managing director: PD Dr. Michael Kleine AG Klei HR B 5568 · USt-IdNr. / VAT: DE212977228

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