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Dr. Subray Hegde
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USA

Ihr Zeichen, Ihre Nachricht vom	Unser Zeichen, unsere Nachricht vom	+49 (0) 2862/ 58979 -51	Datum
			28.06.2019

Subject: Revised petition 19-099-01p

Dear Dr. Hegde,

Please find enclosed the revised petition 19-099-01p.

Based on your comment, all of the responses previously submitted as an appendix and highlighted by USDA staff, are inserted in the text body of the petition. Due to the insertion of the responses, there is a change in page number.

The enclosed petition contains no CBI information.

Kind Regards,

Manfred Mehring-Lemper

Petition for the determination of non-regulated status for petunias containing the A1 gene of Maize (A1-DFR petunias)

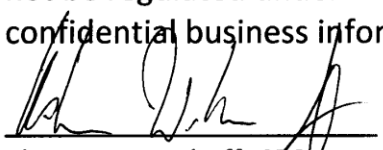
Submitted by
Westhoff Vertriebsgesellschaft mbH
Fresenhorst, 22-24
D-46354 Südlohn-Oeding
Germany

April 3rd 2019

The petition does not contain confidential business information

CERTIFICATION

The undersigned submits this petition under 7 CFR 340.6 to request that the Administrator, make a determination that the article should not be regulated under 7 CFR part 340. The petition does not contain confidential business information.



Südlohn-Oeding, 03.04.2019

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SUMMARY (STATEMENT OF GROUNDS)

Westhoff Vertriebsgesellschaft mbH is petitioning for de-regulation of petunia (*Petunia x hybrida Vilm*.) transformants having a construct containing a dihydroflavonol 4-reductase (DFR) A1 gene from corn (further named A1), events named A1 DFR petunias (GE petunias):

Family	Solanaceae
Genus	*Petunia*
Species	*hybrida*
Common name	petunia
Varieties	Many Varieties
Events	A1 DFR petunias (Lists in Table 1)
Transformation vector	p35A1

The petition is for cultivation in the USA. This petition is made solely on the basis that the articles are regulated because they are genetically modified.

The grounds for petition for de-regulation are:

1. The regulated articles do not become invasive plants.
2. The genetic modification does not present a risk to plant, animal or human health (indirect evidence).
3. The genetic modification is unlikely to pose plant pest risks

1. The regulated articles do not become invasive plants
    - There is a chance for gene dispersal, but petunia has not been reported as invasive crops. Furthermore, petunias are not on the list of noxious weeds even after being in cultivation for more than a century.
    - GE petunias are not different from non-GE petunias in terms of their invasiveness.

2. There is indirect evidence that the genetic modification does not present a risk to plant, animal or human health.

GE petunias do not behave differently from non-GE petunias in the environment and pollinators, in particular, do not show any unusual response to the GE trait. There have been a lot of unintentional GE varieties in the market for more than 10 years without posing any problem for flora, fauna or human health. The GE petunias do not pose a risk in raising major pest risks.

Petunia varieties derived from A1 DFR petunias (Table 1) have an introduced gene from maize and produce the anthocyanin pelargonidin. This modification does not introduce changes that increase the toxicity or allergenicity of the flowers;

- Pelargonidin is a common anthocyanin, present in many other flower species and in commonly consumed foods.
- The introduced, dihydroflavonol 4-reductase (DFR), occur in all plant foods containing pelargonidin
- A flavonoid 3' 5' hydroxylase gene has been introduced into genetically-modified roses earlier. The flowers of these roses have been traded in the USA for several years with no reports of adverse effects.

3. The genetic modification is unlikely to pose plant pest risks

The transgenic plants of the A1 DFR petunias, pose no risk different from that posed by non-GE petunias produced by traditional breeding.

Table 1. List of A1 DFR petunias events carrying the A1 DFR gene of maize.

Name	Alias	Phenotype	Integrated A1 copies	Methylation	References
235/1-15	RP235-15, MPI-15	Red	1	No	Meyer et al., 1987; Linn et al., 1990; Oud et al., 1995
235/1-17	RL01-17, MPI-17, 17	Red	1	No	Meyer et al., 1987; Linn et al., 1990; Meyer et al., 1992; Pröls and Meyer, 1992; Oud et al., 1995
235/1-21		Red	1	Not tested	Meyer et al., 1987; Linn et al., 1990
235/1-24	24	Red	1	No	Meyer et al., 1987; Linn et al., 1990; Pröls and Meyer, 1992
307/10-1		Red	1	No	Meyer et al., 1987; Linn et al., 1990
300/12-7		Red	1	Yes	Meyer et al., 1987; Linn et al., 1990
302/4-3		Red	2	Yes	Meyer et al., 1987; Linn et al., 1990
300/11-4		Red	3	Not tested	Meyer et al., 1987; Linn et al., 1990
235/1-8/2		Variegated	1	Yes	Meyer et al., 1987; Linn et al., 1990
300/11-2/1		Variegated	2	Yes	Meyer et al., 1987; Linn et al., 1990
235/1-9/1		Variegated	5	No	Meyer et al., 1987; Linn et al., 1990
235/1-16/2	16	Variegated	5-6	Yes	Meyer et al., 1987; Linn et al., 1990; Pröls and Meyer, 1992
236/1-1/2		Variegated	8	Yes	Meyer et al., 1987; Linn et al., 1990
235/2-11/2		White	1	Yes	Meyer et al., 1987; Linn et al., 1990
236/1-3/2		White	1	Not tested	Meyer et al., 1987; Linn et al., 1990
235/1-23/2		White	2	Yes	Meyer et al., 1987; Linn et al., 1990
235/1-6/2		White	3	No	Meyer et al., 1987; Linn et al., 1990
235/1-12/1		White	3	Yes	Meyer et al., 1987; Linn et al., 1990
236/1-4/2		White	4	Yes	Meyer et al., 1987; Linn et al., 1990
300/12-2		White	4	Not tested	Meyer et al., 1987; Linn et al., 1990
235/1-2/2		White	7	Yes	Meyer et al., 1987; Linn et al., 1990
302/4-1		White	8	Not tested	Meyer et al., 1987; Linn et al., 1990
235/2-4/2		White	>17	Yes	Meyer et al., 1987; Linn et al., 1990

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Abbreviation[1] and Scientific Terms

A1 gene:	maize dihydroflavonol 4-reductase
ANS:	anthocyanidin synthase
CaMV35S or 35S:	Cauliflower mosaic virus 35S promoter
cDNA:	complementary deoxyribonucleic acid
CHI:	chalcone isomerase
CHS:	chalcone synthase
DFR:	dihydroflavonol 4-reductase
DNA:	Deoxyribonucleic acid
F1:	First filial generation
F2:	Second filial generation
F3'5'H:	flavonoid 3',5'-hydroxylase
F3'H:	flavonoid-3'-hydroxylase
F3H:	flavanone 3-hydroxylase
FLEPPC:	Florida Exotic Pest Plant Council
FLS:	flavonol synthase
FNS:	flavone synthase
GE:	Genetically engineered Organisms
HPLC:	High Performance Liquid Chromatography
Kmr:	Kanamycin resistance.
mAU	milli-Absorbance Unit
mg/g:	Milligrams per gram
mg/L:	Milligram per liter
MPI:	Max-Planck Institute for Plant Breeding research
nm:	nanometer
NPTII:	neomycin phosphotransferase
ocs:	octopine synthase
p35A1:	transformation vector
pnos:	nopaline synthase
RNA:	Ribonucleic acid
Tn	Transposon
US or USA:	United States of America
USDA:	United States Department of Agriculture
ZKBS:	Central Committee on Biological Safety Germany

[1]Note: any terms, words or abbreviation(s) not listed above can be taken to have the "common general meaning" as at the date of this submission.

I. Rationale for the Use of A1 Gene in Petunia Breeding

In 1985 at the Max Planck Institute for Plant Breeding Research (MPI) a petunia line, RL01, was genetically transformed using a plasmid containing a DFR-gene from maize (*Zea mays*). This led to a change in the biosynthetic pathway of flavonoids resulting in flowers showing salmon instead of pale pink color.

This genetic modification, A1 DFR petunias, were transferred probably by crossing unofficially into other petunia background and many varieties were introduced into the market without any permission or deregulation. These varieties were used unintentionally by other breeders, as usually is done with a new variety having a novel trait. In that way, the GE event has spread to the gene pool of most of the petunia breeders worldwide, adding a new color (orange) and brilliance to the color(s) not seen earlier.

Flower color is one of the most important traits in flower breeding. New and brighter colors attract consumers and have a very high commercial value. The varieties bearing the A1 DFR petunia were best sellers in the market before it became known that they are GE petunias.

Therefore, we seek for deregulation of the A1 DFR petunias to allow propagation and commercial production in the USA.

II. The *Petunia* Genus

A. Petunia Cultivation

Origin of the commercial Petunia varieties

The common garden petunia, *Petunia x hybrida* (syn. *P. atkinsiana* D.Don), is derived from *P. integrifolia* and *P. axilaris* (Stehmann et al., 2009), two of many petunia species endemic to South America.
Petunia x hybrida was first obtained by Atkins in 1834 through interspecific crosses in Great Britain (Sink, 1984). Since that time uncountable numbers of crosses have been done by private and professional breeders. Today the garden petunia is popular worldwide. It splits up in different groups according to flower size and number of flowers as grandiflora, multiflora and milliflora.
Petunias are available from seed or propagated vegetatively by cuttings. There are several hundred varieties in the market in a wide range of colors or color patterns. The orange color was missing in commercial varieties until the beginning of the current century.

B. Taxonomy of Petunia

The taxonomy of petunia is shown in Figure 1. The *Petunia x hybrida* (Hook.) Vilm. belongs to the family of Solanaceae. It is a result of interspecies crossings. There are different theories about which species were involved (Stehmann at al., 2009). The most recent work only names *P. integrifolia* and *P. axilaris* as parental species (Wijsman, 1982). In 1990 the Petunia genus was split up into Petunia and Calibrachoa based mainly on the different basic chromosome number of 8 and 9, respectively, and the incompatibility between the two genera (Wijsman, 1990, Stehmann and Semir, 1997). Stehmann at al. (2009) list 14 Petunia species in which *P x hybrida* was not involved.

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants,
Class	Magnoliopsida
Superorder	Asteranae
Order	Solanales
Family	Solanaceae – nightshades, solanacées
Genus	Petunia Juss. – petunia
Direct Children:	
Species	*Petunia x atkinsiana* D. Don ex Louden
Species	*Petunia axillaris* (Lam.) Britton, Sterns & Poggenb. large white petunia
Species	*Petunia x hybrida* Vilm.
Species	*Petunia integrifolia* (Hook.) Schinz & Thell. – violet flower petunia

Figure 1: Taxonomy of Petunia

C. Pollination of Petunia

The genus Petunia features three pollinator syndromes: bee, hawkmoth, and hummingbird (Gübitz et al., 2009). During (co)evolution different flower morphologies were formed and pollinators specialized. Hoballah et al. (2007) observed the pollinator spectrum of *P. axilaris* and *P. integrifolia* and showed that there is a tremendous genetic variation of single genes involved in the pollination syndrome in *P. axilaris*. Dell'Olivo et al. (2011) compared the pollination of the same species but did not analyze commercial hybrids. Kessler et al. (2013) studied the influence of the floral scent of *P. hybrida* on pollinators and herbivores using transgenic plants with silenced special components of the scent. Kessler et al. (2013) finally concluded that the complex blends that comprise floral scents are likely sculpted by the selective pressures of both pollinators and herbivores. The A1 DFR-Petunia were not involved in their trials but the authors mentioned a natural variation in the components of the scent. Furthermore, Gübitz at al. (2009) published a wide range of pollinators observed on the parental species of *P x hybrida* in South America (Table 2). There are no investigations known on commercial Petunia hybrids.

Table 2. Species and locations of flower visitors collected from *P. axillaris* and *P. integrifolia* flowers in the natural habitat in Uruguay. Abbreviations for the collection locations: R: Rivera; LC: Las Canas; PV: Puerto Viejo; C: Carmelo; PF: Playa Fomento; M: Minas; PA: Playa Agraciada; ?: precise location unknown

Petunia species	Visitor species (family, subfamily)	Location, month, and year of collection
P. integrifolia	*Calliopsis* sp. (Apidae, Colletinae)	R 01.05, LC 02.07
P. integrifolia	*Calliopsis* sp. (Apidae, Colletinae)	PV 01.05
P. integrifolia	*Halictus* sp. (Apidae, Halictinae)	R 01.05
P. integrifolia	*Lasioglossum* sp. (Apidae, Halictinae)	R 01.05
P. integrifolia	*Lasioglossum* sp. (Apidae, Halictinae)	PV 01.05
P. integrifolia	*Leioproctus* sp. subgen. Hexantheda (Apidae, Colletinae)	? 11.02
P. integrifolia	*Leioproctus enneomera* (Apidae, Colletinae)	R 01.05
P. integrifolia	*Leioproctus enneomera* (Apidae, Colletinae)	PV 01.05
P. integrifolia	*Hylephila phyleus* (Hesperiidae, Hesperiinae)	JI 02.05
P. axillaris	*Halictus* sp. (Apidae, Halictinae)	? 11.02
P. axillaris	indet. panurgine genus (Apidae)	JI 01.04
P. axillaris	*Halictus* sp. (Apidae, Halictinae)	C 02.04
P. axillaris	*Lasioglossum* sp. (Apidae, Halictinae)	C 02.04
P. axillaris	*Manduca diffissa* (Sphingidae, Sphinginae)	PF 11.02, C 02.07
P. axillaris	*M. sexta* (Sphingidae, Sphinginae)	R 02.05, JI 02.07, C 02.07
P. axillaris	*Eumorpha vitis* (Sphingidae, Macroglossinae)	C 04, JI 02.06, 02.07
P. axillaris	*Eumorpha labruscae* (Sphingidae, Macroglossinae)	C 2007,
P. axillaris	*Agrius cingulata* (Sphingidae, Sphinginae)	M 02.05
P. axillaris	*Erinnyis ello* (Sphingidae, Macroglossinae)	C 02.06
P. axillaris	*Hyles lineata* (Sphingidae, Macroglossinae)	C 02.07
P. axilaris	*Diabrotica emorsitans* (Chrysomelidae, Galerucinae)	C 02.04
P. axillaris	*Chrysodina cupricollis* (Chrysomelidae, Eumolpinae)	C 02.04
P. axillaris	*Dahlibruchus* sp. (Chrysomelidae, Bruchinae)	JI 01.04
P. axillaris	harvester ants (unidentified genus)	PA 02.06
P. axillaris	Crabspider (unidentified genus)	

Of the insect species listed in Table 2 the following are documented to exist in the US (Anonymous, 2014): *Calliopsis sp., Halictus sp., Lasioglossum sp., Hylephila phyleus, Manduca sexta, Eumorpha vitis, Eumorpha labruscae, Agrius cingulate, Erinnyis ello* and *Hyles lineata*.

Due to its morphology with more open flowers, the *Petunia x hybrida* will predominantly be pollinated by bees instead of hawkmoths and hummingbirds.
There are male sterile varieties and some double flowering varieties showing a male or female or complete sterility. By far the most varieties are fully fertile and set seed after pollination.

D. Weediness of Petunia

Invasive Species Definition Clarification and Guidance Executive Order 13112 defines an invasive species as "an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Anonymous, 1999).

According to our knowledge, *Petunia x hybrida* is not invasive. Petunias are not listed as federal noxious weed and not listed in the list of invasive plant species (Anonymous, 2019a). Petunia is naturalized in different states (Figure 2)

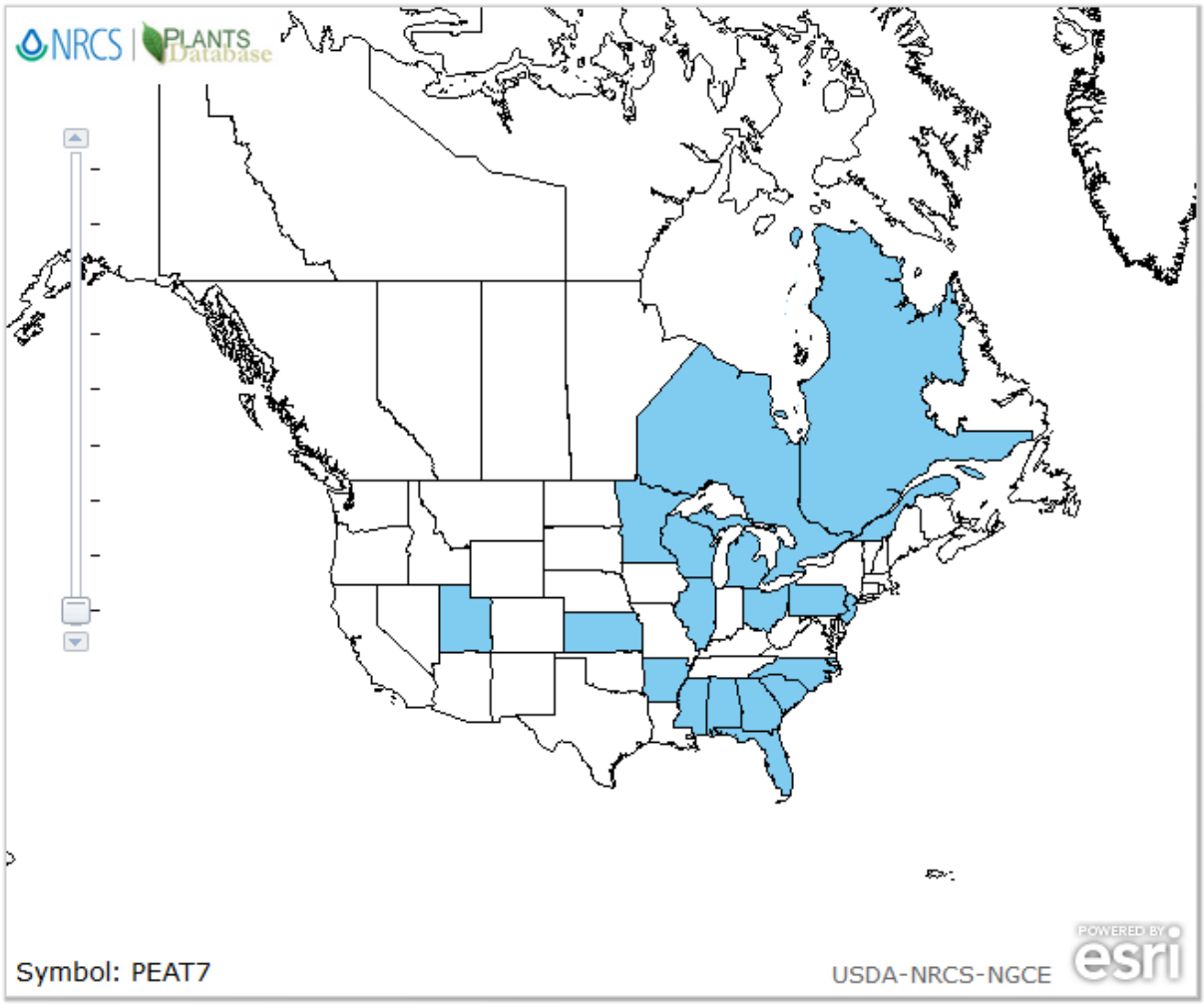


Figure 2: Distribution map of *Petunia ×atkinsiana* D. Don ex Loudon [*axillaris × integrifolia*] across the USA. *Petunia x atkinsiana* is a synonym of *P. x hybrida*. Source: USDA plant database (Anonymous, 1999b).

Even in the thirteen Southern States, where the climate is most similar to the original habitat of petunia (subtropical South America), it has not become a weed (Miller et al.,2018). Furthermore, petunia does not have an impact on endangered species via habitat loss and alteration as can be seen for Florida as an example (petunia not listed in the FLEPPC list of Florida (Anonymous, 2018). Florida is the US state most similar to the conditions of the center of origin of petunia. Florida has also tropical climate with nearly no frost. When the optimum climate of Florida does not favor uncontrolled development of petunia, it can be expected

that in other US states especially with frost, there is less chance of a negative impact due to petunia which are not hardy.
A risk analysis for the Dept. of Health of the Australian Government comes to the conclusion: "Thus, unwanted petunias could be minor weeds in gardens, but probably only if the gardens are in a warm climate and frequently watered" (AUS Dept. of Health, 2017).

E. Potential Modes of Gene Flow in Petunia

There are three theoretical mechanisms for gene dispersal from a cultivated petunia plant:
1. Vegetative spread, leading to the formation of clonal populations.
2. Formation and dispersal of seed as a result of self-fertilization or fertilization with pollen from an external source.
3. Formation of seed by a recipient plant, fertilized by pollen dispersed from transgenic petunia.

The paragraphs below briefly outline why the probability of gene dispersal by some of these routes are likely, but uncritical.

1. Vegetative Spread. petunia does not spread vegetatively, i.e. the plant does not produce organs such as stolons, rhizomes, root borne shoots, tubers, corms, or runners. Roots will not form on discarded parts of a plant under outdoor condition. To promote the development of roots, the cuttings (broken or cut branches) have to be stacked into the soil and kept under humidity at about 73 °F for about two weeks. At cooler temperature the necessary period of high humidity will be prolonged. Very wet conditions will lead to rotting and death of the cuttings. Ideally the rooting takes place in a special greenhouse department. Even if the climatic conditions at outdoor would fit, broken branches would have to be stack into the ground with the stem to the bottom. Just placing a cutting on top of the ground will not aid the successful rooting of the plant part.

2. Formation of seed. Based on a general observation, most petunia plants set seed easily. Very few varieties may show limited fertility or even female sterility and therefore set seed poorly if at all (i.e. double flowering petunias). So, gene dispersal by seed cannot be excluded. However, the fact that petunia did not become a threat or weed in the US shows that the chance of a manifested gene dispersal is limited. Additionally, the trait transformed inherits like a typical flower color gene with no negative side effects so far (Appendixes I-V). For about eight years between 2009 and early 2017, we had extensively used the unintentional GE petunias in our breeding programs at Westhoff. Based on our own observation (breeding note data) of that eight years period, we found no significant difference in seed set, seed viability as well as germination rate between the GE and non-GE petunias. From that observation, we concluded that GE petunias show no selective advantage over non-GE petunias, in terms of seed formation. For example, in a comparison of 35 GE mother petunia clones to 390 non-GE mother petunia clones, seed set scores of 3.11 and 3.10, respectively, were observed, which indicates that no significant difference in seed set scores between GE and non-GE mother plants. Therefore, it is to be expected that they do not change the current status of petunia which has not been in discussion in the past.

3. Pollen dispersal leading to successful hybridization event. The transgenic varieties form viable pollen in general. Dispersal of the transgenes by pollen-mediated gene flow is possible. However, it is important to recognize that petunia does not intercross with other genera. Even with Calibrachoa, the most closely related genus, crosses under natural condition are not successful (Wijsman, 1983; personal experience). As described in Jędrzejuk et al (2017), there are interspecific Petchoas with Petunia and Calibrachoa (commercial name Supercal or Calitunia) on the market. Own experience with hundreds of crossings between Petunia and Calibrachoa and vice versa did not result in seed set. Other companies were not successful with this kind of crosses too. The way to create the hybrids is a company secret. There are two theories how to get the hybrids: A. Embryo rescue, B. a very special ploidy level of one of the parents. The first would mean that the support of a laboratory is essential to get a hybrid and crosses will not appear spontaneously. As to the second option, based on own practical results, would mean that the ploidy level necessary for a successful crossing is very rare in the commercial varieties. Anyway, the interspecific varieties in the market are totally sterile and therefore do not set seed and cannot spread the event (for vegetative spread, see point 1 above). Furthermore, as also stated above, petunia did not become a weed in the US nor somewhere else as far as known, which indicates that the chance of a manifested gene dispersal is limited.

III. Genetic Modification for Flower Color

A. Flower color

Flower color is one of the most important features of the floriculture industry. It determines consumers' preferences and influences commercial value. There exists broad range of flower color in nature, however, some colors are available only in certain ornamental plant species.
The three major floral pigments are flavonoids, carotenoids and betalains. Among these three floral pigments, the flavonoids contribute most to the range and type of colored pigments in plants. Flavonoids consist of several classes of compounds such as anthocyanins, aurones, chalcones, flavones and flavonols. Anthocyanins confer orange, red, magenta, violet and blue colors. Aurones and chalcones are yellow pigments while flavones and flavonols are colorless or very pale yellow. Anthocyanins and flavonols are the pigments responsible for flower color in petunia.

The anthocyanin biosynthesis pathway

The key enzyme at the start of the flavonoid biosynthesis pathway is chalcone synthase, from which the dihydroflavonols are produced after enzymatic reactions catalyzed by chalcone isomerase (CHI) and flavanone 3-hydroxylase (F3H) (Figure 3). The dihydroflavonols are substrates for the biosynthesis of the colored anthocyanin pigments, which are produced as follows. The enzyme dihydroflavonol 4-reductase (DFR) first converts dihydroflavonols to corresponding leucoanthocyanidins (leucocyanidin, leucopelargonidin and leucodelphinidin), which then are oxidized to corresponding anthocyanidins (cyanidin, pelargonidin and Delphinidin) by anthocyanidin synthase (ANS). Anthocyanidins can be further modified in many ways, by addition of glucose, acyl and methyl molecules.
There are three groups of glycosylated anthocyanins (delphinidin, cyanidin and pelargonidin). Delphinidins and their derivatives generally produce blue flower color, cyanidins and their derivatives produce red or pink flower color and pelargonidins and their derivatives produce orange or brick red flower color.
In petunia, cyanidin and delphinidin derivatives are produced as pigments, but no pelargonidin derivatives are produced (Meyer et al., 1987). This is due to the fact that the petunia dihydroflavonol 4-reductase does not accept dihydrokaempferol as substrate, and no conversion into leucopelargonidin. So, the natural color range of petunia flower lacks orange to brick red color, that are products of pelargonidin derivatives.
The A1 DFR petunias have been genetically modified to enable production of pelargonidin and brick red-colored flowers. The gene responsible for the modified trait is the maize dihydroflavonol 4-reductase (A1), which successfully converts dihydrokaempferol into leucopelargonidin, leading to the brick red flower pigment in petunia.
There are several consumed foods containing pelargonidin. In Table 3 below are shown some examples of food groups and their contribution with regard to anthocyanidins and pelargonidins for consumers in north, central and south European regions (Zamora-Ros et al., 2011; Table 3). Strawberry is a typical fruit consumed in the USA with remarkable content of pelargonidin (Anonymous, 2019f).

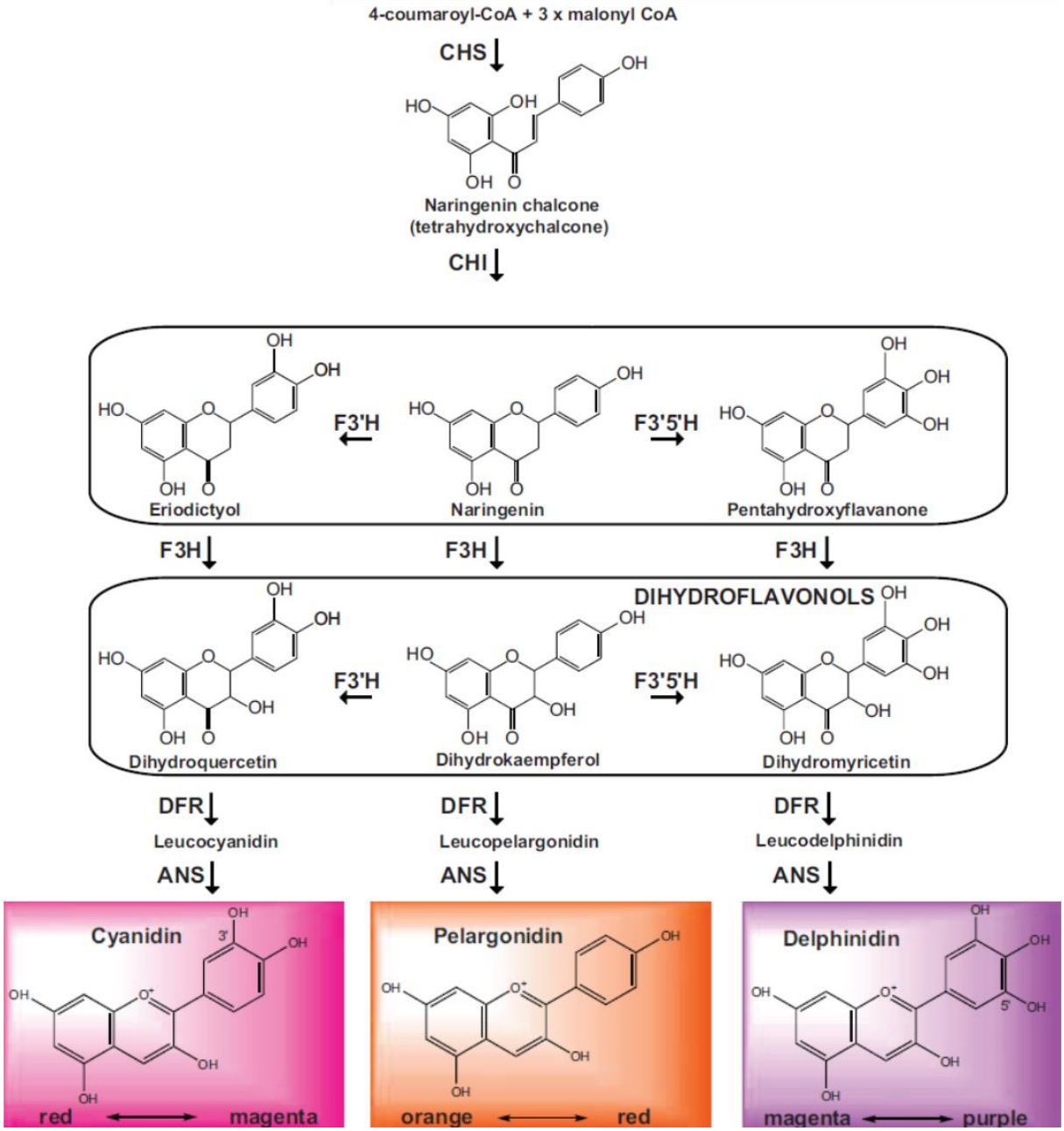


Figure 3: A simplified scheme of the biosynthesis of anthocyanidins- cyanidin, pelargonidin and delphinidin. The enzymes catalyzing each step are indicated in bold. Abbreviations include; CHS- chalcone synthase; CHI- chalcone isomerase; FNS- flavone synthase; F3H- flavanone 3-hydroxylase; F3'H- flavonoid-3'-hydroxylase; F3'5'H- flavonoid 3',5'-hydroxylase; FLS- flavonol synthase; DFR- dihydroflavonol 4-reductase; ANS-anthocyanidin synthase (modified from Falcone Ferreyra et al., 2012).

Table 3. Percentage contribution of food groups and some main foods to the intake of total anthocyanidins and pelargonidins by European region (Zamora-Ros et al., 2011).

	Anthocyanidins (%)			Pelargonidins (%)		
Food groups and foods†	South	Central	North	South	Central	North
Potatoes and other tubers	0·0	0·0	0·0	0·0	0·0	0·0
Vegetables	9·7	8·8	4·8	16·7	19·9	39·4
Leafy vegetables	7·1	3·8	0·4	0·0	0·0	0·1
Fruiting vegetables	0·9	0·4	0·1	0·1	0·0	0·0
Root vegetables	1·2	2·8	2·7	16·5	19·8	39·2
Cabbages	0·4	1·6	1·5	0·0	0·0	0·0
Other and mixed vegetables	0·1	0·1	0·1	0·1	0·0	0·1
Legumes	0·1	0·1	0·1	0·8	0·3	0·6
Fruits, nuts and seeds	61·2	52·9	38·1	73·9	66·3	38·4
Citrus fruits	0·0	0·0	0·0	0·0	0·0	0·0
Apples and pears	14·2	10·0	12·7	0·2	0·1	0·1
Grapes	18·9	13·0	10·6	0·1	0·0	0·1
Stone fruits	14·8	10·0	2·8	1·5	0·6	0·1
Berries	6·3	16·5	10·1	68·1	61·5	34·5
Other and mixed fruits	2·5	2·3	1·1	4·0	4·1	3·7
Olives	4·5	0·8	0·9	0·0	0·0	0·0
Nuts and seeds	0·2	0·1	0·1	0·0	0·0	0·0
Dairy products	0·5	1·5	0·8	6·5	9·3	10·8
Cereal, cakes and confectionery	1·0	6·5	4·5	1·9	2·8	3·4

† Leafy vegetables include red leaf lettuce, red chicory, radicchio and trevise (red Treviso lettuce); fruiting vegetables include aubergines; root vegetables include beetroot, red radish and black radish; cabbages include red cabbage and Chinese cabbage; stone fruits include plums, peaches, nectarines, apricots, mangoes and paraguayos; other and mixed fruits include cherries, red fruit not specified, sour cherries, persimmon, sharon fruit and pomegranate; cereal, cakes and confectionery include fruit cakes, biscuits with jam and plum cake.

B. Genetic Modification and novel flower color

RL01 is the parent line used in the transformation. RL01 is a derivative of line R4 from the Petunia x hybrida collection at Tübingen University (Meyer et al. 1987). RL01 exhibits a pale pink flower phenotype. The genotype of RL01 for flower color is *AnAnhththfhfflfl*, in which *An* stands for anthocyanin, *Ht* for flavonoid-3' hydroxylase, *Hf* for flavonoid-3',5' hydroxylase, *Fl* controls the synthesis of kaempferol and quercetin (Oud et al., 1995). The dominant *Ht* allele leads to an accumulation of cyanidin, where as the dominant *Hf* allele leads to an accumulation of delphinidin. The recessive *ht* and *hf* alleles lead to lack of cyanidin and lack of delphinidin, respectively. But, the RL01 contained a "leaky" *hf* allele which shows that small amounts of delphidin and cyanidin still accumulate (Oud et al., 1995). The dominant *An* allele indicates the presence of cyanidin or delphinidin or pelargonidin-based anthocyanin in the RL01, which is of course influenced by the other alleles (*Ht/ht, Hf/hf, Fl/fl*). The dominant *Fl* allele leads to an accumulation of kaempferol and quercetin, depending on the other allele combinations (*Ht/ht, Hf/hf, Fl/fl*). The genotype description is based solely on the information given in Oud et al (1995). So, the allele demonstration of the RL01 refers back to the state of knowledge at that time.
The genetic modification carried out for A1 DFR petunias used transformation vector p35A1. This transformation vector contains the maize dihydroflavonol 4-reductase (A1) gene.

Pelargonidin derivatives are produced in the genetically modified petunia line, which can be confirmed by the spectrophotometric peak of 512 nm and the formation of brick red-colored flowers, while the non-transgenic parent line produced only pale pink flower (Figure 4) and spectrophotometric peak of 528 nm.
The genetic modification results in the biosynthesis of anthocyanin in the petals of the flower only. This is because the substrates on the pathway (required for pelargonidin biosynthesis in the transgenic plants) are only produced in the flower.

Figure 4: Petunia non-transgenic parental line (left) and the transgenic line RP235-15 (right)

IV. Description of Transformation System

Protoplasts of the petunia line were synchronized into M-phase (Meyer et al 1985). The synchronized M-phase protoplasts were transformed with plasmid p35A1 by direct gene transfer. The transformed microcalli were cultured on regeneration medium containing kanamycin (50 mg/L) as a selection agent. They were placed on Re27/6-medium containing kanamycin (50 mg/L) with benzylaminopurine (2 mg/L) and indoleacetic acid (2 mg/L) for three weeks and transferred to Re17/3-medium containing kanamycin (50 mg/L) with benzylaminopurine (1 mg/L) and indoleacetic acid (1 mg/L). Shoots of the successful transformant were rooted on MS-medium without hormone.

V. Vector and Genes of the Integrated Construct

The transformation vector p35A1 was used to genetically engineer petunia line RL01. The transformation vector was designed at the Max-Planck Institute for Plant Breeding Research in Cologne. The vector map and corresponding elements is shown in Figure 5 and Table 4.
Plasmid p35A1 is derived from plasmid pLGV11 (Meyer et al., 1987). The Plasmid pLGV11 is equivalent to pLGV1103 except for the deleted Tn903 *Sal*I fragment (Hain et al., 1985; Meyer et al., 1987). The plasmid pLGV1103 contains the NPT II gene of Tn5 flanked, at the 5' end, by the NOS promoter and, at the 3' end, the polyadenylation site of the OCS gene (Velten et al., 1984). The plasmid pLGV1103 also contains a second kanamycin resistance gene (APH type I), derived from Tn903, under control of the original prokaryotic promoter (Velten et al., 1984). As indirectly indicated in Velten et al (1984), pLGV1103 is the backbone of a plasmid pAK1003. According to Molecular Biology Vector Sequence Database of the Stanford University, pAK1003 is an *Escherichia coli* plasmid (Misener, 1997). Such indirect evidence indicates that the donor organism of pLGV1103 is an *Escherichia coli* plasmid. Therefore, p35A1 can be confirmed as *Escherichia coli* plasmid.

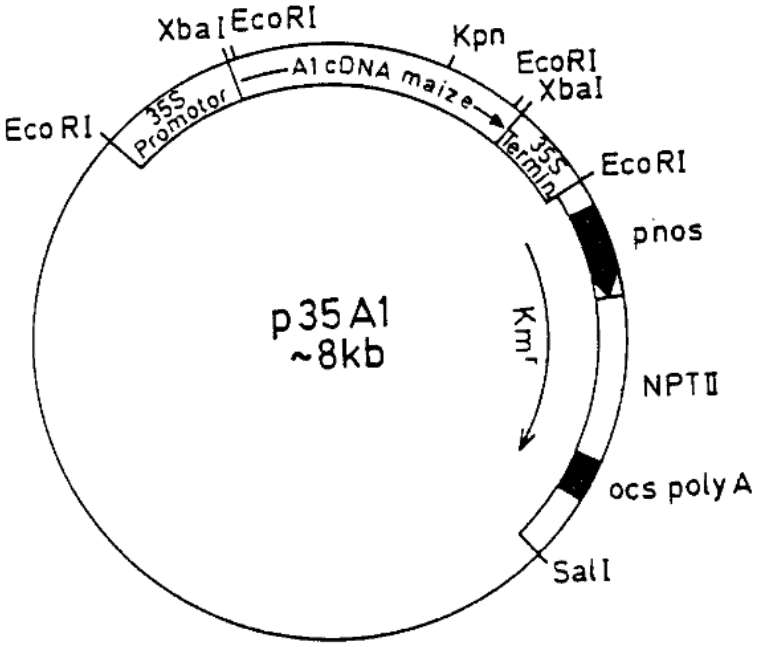


Figure 5: Map of the transformation vector p35A1

The transformation vector is 8 kb long containing the 35S promoter from CaMV (cauliflower mosaic virus), the maize dihydroflavonol 4-reductase (A1) gene, the 35S termination sequence of CaMV, promoter region of nopaline synthase (pnos), neomycin phosphotransferase (NPTII)-conferring resistance to Kanamycin (Kmr), octopine synthase (ocs) poly A- terminator region of nopaline synthase.
CaMV35S promoter is a constitutive promoter used to provide high levels of gene expression. The nptII gene is the most commonly used antibiotic resistance marker gene for the production of genetically modified plants (Goldstein et ai., 2005). It encodes the enzyme neomycin phosphotransferase type II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. NPTII phosphorylates kanamycin and neomycin making them inactive. Genetically modified cells containing the NPTII gene are able to grow in the presence of the antibiotic, while the growth of unmodified cells is inhibited. The NPTII gene functions as a selectable marker during the development of the genetically modified petunia.
A1 cDNA (complementary deoxyribonucleic acid) of maize is a dihydroflavonol 4-reductase gene which converts dihydroquercetin into leucocyanidin leading to the production of cyanidin derivatives in the maize aleurone

Table 4. Elements of the vector p35A1 backbone integrated into the A1 DFR petunias genome sequence, their position on the sequence as depicted in Appendix VI A and their donor organism.

Name of the element	Position in relation to Appendix VI A (the five bases from begin and end of the sequence)	Donor
beta-lactamase	1-620 bp (CAGAA...AGAAT)	*Escherichia coli*
35S Promoter	621-1168 bp (TCCCA...GATCC)	Cauliflower mosaic virus
A1 cDNA maize	1184-2364 bp (CTGCT...TATCT)	*Zea mays*
transposon Cin4-1	2372-2497 bp (GTTTG...CAATT)	*Zea mays*
35S Terminator	2528-2753 bp (GTCGA...AGCTC)	Cauliflower mosaic virus
lacUV5 promoter	2754-2853 bp (GAATT...AACAG)	*Escherichia coli*
pnos	2854-3144 bp (GATCA...CAGAT)	*Agrobacterium tumefaciens*
NPTII	3152-4131 bp (ATGAT...CCCTG)	*Escherichia coli*
ocs	4132-4398 bp (CTTTA...ATGAT)	*Agrobacterium tumefaciens*

VI. Genetic Analyses of Maize A1 DFR Transformed Petunias of the 1980s and 1990s

The initial transformation of petunia carrying the A1 DFR gene of maize was done by Meyer et al. (1987) at the MPI, where the first event line was described as RP235-15. Next, Linn et al. (1990), analyzed 30 transformant petunia plants obtained in the transformation experiments described by Meyer et al. (1987). According to Linn et al. (1990), twenty-three lines of the thirty independent transgenic plants, showed integration of the chimeric A1 DFR gene. Six of the twenty-three transgenic plants contained single copy A1 gene and showed "red" color phenotype displaying a uniform coloration of the whole flower (Linn et al., 1990). Four of the six transgenic plants containing single copy A1 gene, showed non- methylation of the promoter (Linn et al., 1990). One of the four transgenic plant, 235/1-15, corresponds to the RP235-15 plant initially described by Meyer et al. (1987). Later on, Meyer et al. (1992) studied a second of the four transgenic plants, 235/1-17, and renamed it as RL01-17. Southern blot analysis was shown as an example for one of the four transgenic plants, 235/1-24, containing single copy A1 gene and non- methylated promoter (Linn et al., 1990).

Out of the experiment (Meyer et al., 1987; Linn et al., 1990; Meyer et al., 1992; Table 1) material was transferred among others, to a breeding company S & G Seeds B. V. (Oud et al., 1995). The material received by S & G Seeds B. V. were named as MPI-15 and MPI-17 (Oud et al., 1995). The MPI-15 corresponds to 235/1-15 or RP235-15, and the MPI-17 corresponds to 235/1-17 or RL01-17.

Any of these events described above shall potentially be a progenitor for the unintentional GE petunia varieties of Westhoff. The transformants were genetically and morphologically further analyzed in the 1990s (Linn et al., 1990; Meyer et al., 1992 and Oud et al., 1995). These transformants are not available anymore for current morphological and genetic analyses. The morphological information and molecular analysis presented in this section therefore are based on those publications of the 1980s and 1990s (Meyer et al., 1987; Linn et al., 1990; Meyer et al., 1992 and Oud et al. 1995).

A. Southern Blot Analysis

Southern data for the transgenic line is shown in Figure 6. Standard molecular analysis techniques were used for the analysis.
Southern blot analysis of the transgenic petunia line 235/1-24 is presented as an example. After probing with 32p-labeled maize A1 cDNA fragment it revealed a single copy of hybridized band corresponding to one copy of integrated A1 gene in the genome without obvious rearrangements or deletions (Figure 6; Linn et al, 1990).

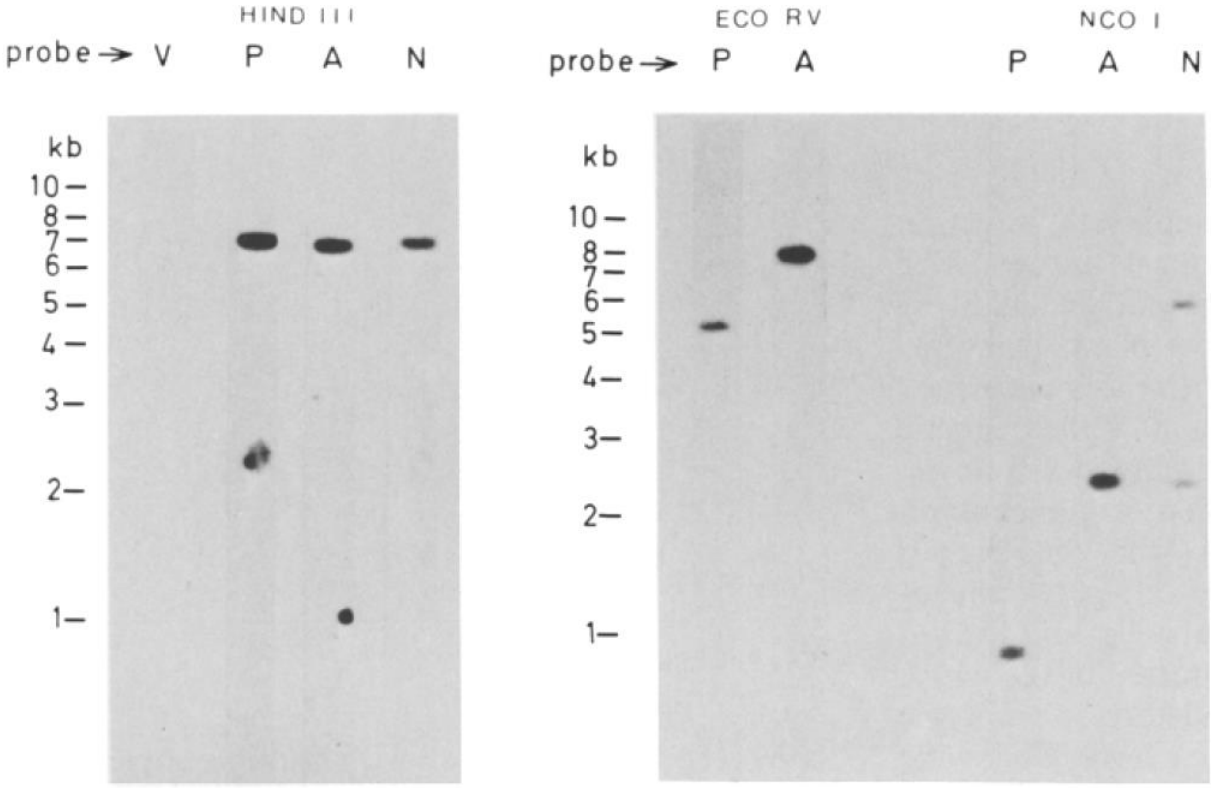


Figure 6: Characteristic integration pattern of the vector p35A1 into the petunia transformant 235/1-24 genome obtained by Southern blot analyzes. According to the *EcoRV* and *HindIII* digest one A1 cDNA copy integrated at one genomic locus. The *NcoI* restriction pattern shows that the A1-neomycin phosphotransferase II (NptII) construct integrated properly without obvious rearrangements or deletions. The genomic DNA was digested with *HindIII*, *EcoRV* and *NcoI* and probed with the ampicillin *EcoRI-PstI* fragment (V), the 35SEcoRI fragment (P), the A1 cDNA *EcoRI* fragment (A) and the *NptII-PstI* fragment (N).

B. Mendelian Inheritance

In the work done by Oud and colleagues (1995), two transformants (MPI-15 and MPI-17) were crossed with S&G Seeds breeding lines L2134 (pink), L2124 (rose), F5 (salmon) and Y1 (red). Recombination using the transgenic line as male as well as female was made with the four selected elite lines. The flowers of the resulting F1 progeny were not orange, due to the presence of dominant allele from the crossing partner. Several F1 plants from each recombinant offspring were self-pollinated and in the F2 populations, plants with an orange flower color were found. The results revealed that the maize dihydroflavonol 4-reductase (A1) gene segregates and follows Mendelian inheritance.

C. Expression of Inserted Genes

Total RNA was extracted from non-transgenic and transgenic petunia leaves. Northern blot hybridization analysis was done with an oligomer-primed maize A1 cDNA probe. The transcript for the maize dihydroflavonol 4-reductase gene was detected in the transgenic petunia line RP235-15 but not in the non-transgenic petunia (Figure 7, Meyer et al., 1987). The expression of the maize dihydroflavonol 4-reductase gene in the line RP235-15 supports the proper

functioning of the gene, which was also revealed by the spectrophotometric peak of 512 nm and the formation of brick red-colored flowers.

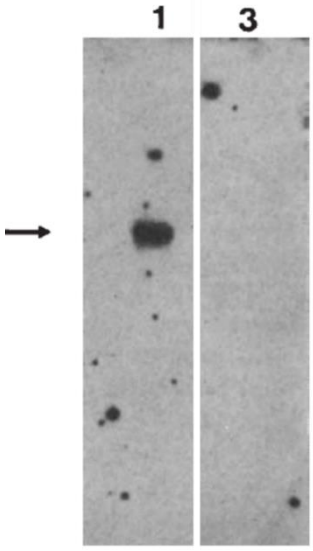


Figure 7: Transcription of the maize dihydroflavonol 4-reductase (A1) gene in transgenic and non-transgenic petunia. The transgenic line RP235-15 shows strong messenger RNA expression of maize A1 gene (lane 1), whereas no A1 transcript is detectable in non-transgenic petunia (lane 3).

VII. Description of Foreign Sequences and Phenotype of the Unintentional GE Petunia

The descriptions reported are those of petunia unintentionally derived from multiple events of the A1 DFR petunias (Table 1). The descriptions of A1 DFR petunias events based on available literatures are given in the previous sections.

A. Foreign sequences

Tests were performed by Eurofins following a USDA validated protocol. The discovery of unintentionally bred transgenic petunias on the market was performed by PCR and Realtime PCR analysis using 35S promoter and NPTII primers.

Furthermore, to complement this petition, eleven unintentional GE petunia varieties of Westhoff were sequenced at the region of integration by Vienna University of Technology (Appendix VI A; Halbwirth unpublished). The sequence finding confirms that, the vector construct that was used to obtain the 23 transformants, is maintained in the unintentional GE petunia varieties discovered in 2017 (Bashandy and Teeri, 2017; Haselmair-Gosch et al., 2018).

At the integration point in the GE A1 DFR petunias, the left end of the vector backbone is a partial sequence of beta-lactamase and the right end is a partial sequence of octopine synthase terminator. Due to the fact that the beta-lactamase on the left end is partial sequence, it is expected to be unfunctional. The sequences of these constructs are listed under Appendix VI A. The amino acid translation of the coding sequences for A1 DFR and NPTII of the GE petunia are listed under Appendix VI B.

At the 3'-end of the A1 DFR sequence, a sequence segment of a non-viral transposable element named Cin4-1 (Schwarz-Sommer et al. 1987) is detected. The Cin4-1 sequence is also a natural gene of maize and does not add any functional advantage with respect to DFR activity (Haselmair-Gosch et al., 2018).

The complete sequence of the construct region of the vector p35A1 integrated into GE petunia is 4398 bp, and found integrated into petunia chromosome bordered with the natural petunia sequence (Appendix VI A; Halbwirth unpublished). This leads to the conclusion that another part of the vector construct was not integrated.

We also found lacUV5 promoter sequence between the 35S termination sequence of CaMV and promoter region of nopaline synthase (pnos). The lacUV5 promoter is a mutant of the lactose operon promoter from *Escherichia coli* that also functions as an efficient transcription terminator (Bogosian and Kane, 1987).

In its statement from December 2008, the ZKBS came to the conclusion that events of horizontal gene transfer from genetically modified plants to other organisms, if they did occur at all, would be negligibly rare as compared to the natural processes of transmission and new formation of resistance genes and the natural presence of the resistance genes in question in natural communities of microorganisms (ZKBS, 2008). In its statement the ZKBS also stated that especially the NPTII gene would be widely spread in soil bacteria and Enterobacteriaceae. Therefore, it can be assumed that the presence of the NPTII gene in the genome of the genetically modified petunias will not take effect on the spread of this antibiotic resistance gene in the environment. In an experimental protocol for assessing the invasiveness of plants, Crawley et al. (1993) found no indication that genetic engineering for kanamycin tolerance (conferred by NPTII) increased the invasive potential of oilseed rape. In terms of seed survival on burial, Crawley et al. (1993) observed that transgenic lines were less invasive and less persistent than their conventional counterparts.

Even in the event of a transfer of the detected p35A1 regulatory sequences into other organisms, no relevant increase of the overall frequency of the corresponding DNA segments in the environment would occur. The regulatory sequences of the p35A1 originate from the Cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*. CaMV is a plant-infecting, double-stranded DNA virus which is widely distributed in plants. *Agrobacterium tumefaciens* is a widely distributed soil bacterium.
The plasmid p35A1 also contains a fragment necessary for bacterial replication and selection, and an origin of replication. However, a gene product which is functional in plants is not encoded by these sequences, as the regulatory elements originate from bacteria. Furthermore, the probability of a spread of these nucleic acid fragments by transmission among bacteria, is by far greater than the probability of a spread by means of a horizontal gene transfer from the genetically modified plants to microorganisms.

B. Phenotype

Petunias containing the DFR A1 allele originating from the A1 DFR petunias are very variable in the phenotype due to multiple crosses done over the years. It is not clear when the event was crossed into commercial varieties, nor where it happened. But there is a high probability that it was done in the 1990s or early 2000s. For at least the past 15 years of crossing work the event spread into most breeding programs worldwide due to the common use of competitor varieties to broaden the own genetic pool and adding new traits.
The DFR A1 allele of the DFR event was present in more than 50 varieties created by many different breeders of petunia (Anonymous, 2017). They show a wide range of phenotypes, i.e., different habits from upright mounding to trailing, different flower sizes and different flower colors and color patterns. Some of Westhoff unintentional GE varieties are listed in Table 5 including the year of introduction (USA and Europe). These unintentional GE petunia varieties were in cultivation since 2011/12 including in the USA, until the GE petunia report in 2017, after which they were destroyed.

Table 5. List of the 15 unintentional GE petunia varieties of Westhoff†.

Genus	Series	Variety	Year of crossing	Year of introduction
Petunia	WFL®Perfectunia®	Orange	2009	2011
Petunia	WFL®Hells®	Glow	2009	2011
Petunia	**WFL®Perfectunia®**	**Citrus Wheel**	**2009**	**2011**
Petunia	**WFL®Crazytunia®**	**Cherry Cheesecake**	**2009**	**2012**
Petunia	WFL®Hells®	Bells Orange	2009	2012
Petunia	**WFL®Hells®**	**Fruit Punch**	**2009**	**2014**
Petunia	**WFL®Crazytunia®**	**Star Jubilee**	**2010**	**2013**
Petunia	**WFL®Perfectunia®**	**Red impr,**	**2010**	**2016**
Petunia	WFL®Perfectunia®	Mandarin	2011	2015
Petunia	**WFL®Crazytunia®**	**Swiss Dancer**	**2011**	**2015**
Petunia	**WFL®Perfectunia®**	**Orange Morn**	**2011**	**2014**
Petunia	**WFL®Crazytunia®**	**Sparky Impr.**	**2012**	**2016**
Petunia	**WFL®Crazytunia®**	**Maniac Pink**	**2013**	**2016**
Petunia	WFL®Crazytunia®	Citrus Twist	2013	2016
Petunia	WFL®Big Deal	Freaky Fuchsia	2013	2016

Petunia	**WFL®Crazytunia®**	**Firecraker**	**2013**	**2016**
Petunia	**WFL®Big Deal**	**Salmon Shimmer**	**2012**	**2016**

† Bolded lines indicate the 11 unintentional GE petunia of Westhoff for which sequencing of the maize A1 DFR construct region was done.

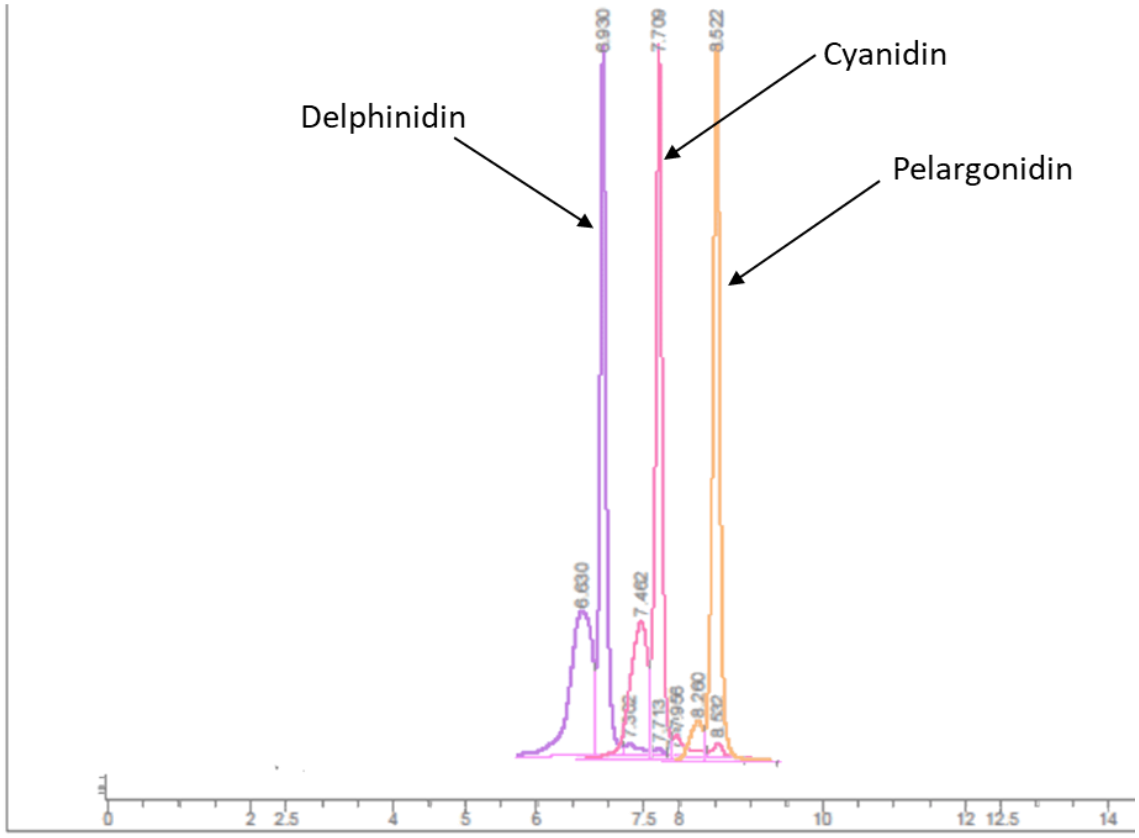
GE and non-GE petunias show the same diversity in all traits but differ in flower color if at all. Outdoor trial in 2016 and 2017 by Ohio State University, Colorado State University and Horticultural trial station Bad Zwischenahn (Germany) showed no difference between non-GE and GE petunias for all traits studied (Appendix VII-IX). There are GE petunias with white, red, orange, salmon and purple flower color or color shades, and others showing a star pattern in red and white or orange and yellow. Morn types (with different colors and having a white center) were detected too. It is not possible to identify GE petunia by traits other than flower color and here only orange is currently a clear indication for the presence of the event. As a good example some petunia varieties from Westhoff are shown in Figure 8 including GE petunias.

The GE Petunias name (from left to right and top to bottom):
- Crazytunia Cherry Cheesecake
- Crazytunia Citrus Twist
- Crazytunia Star Jubilee
- Crazytunia Maniac Pink
- Crazytunia Sparky imp.
- Crazytunia Swiss Dancer

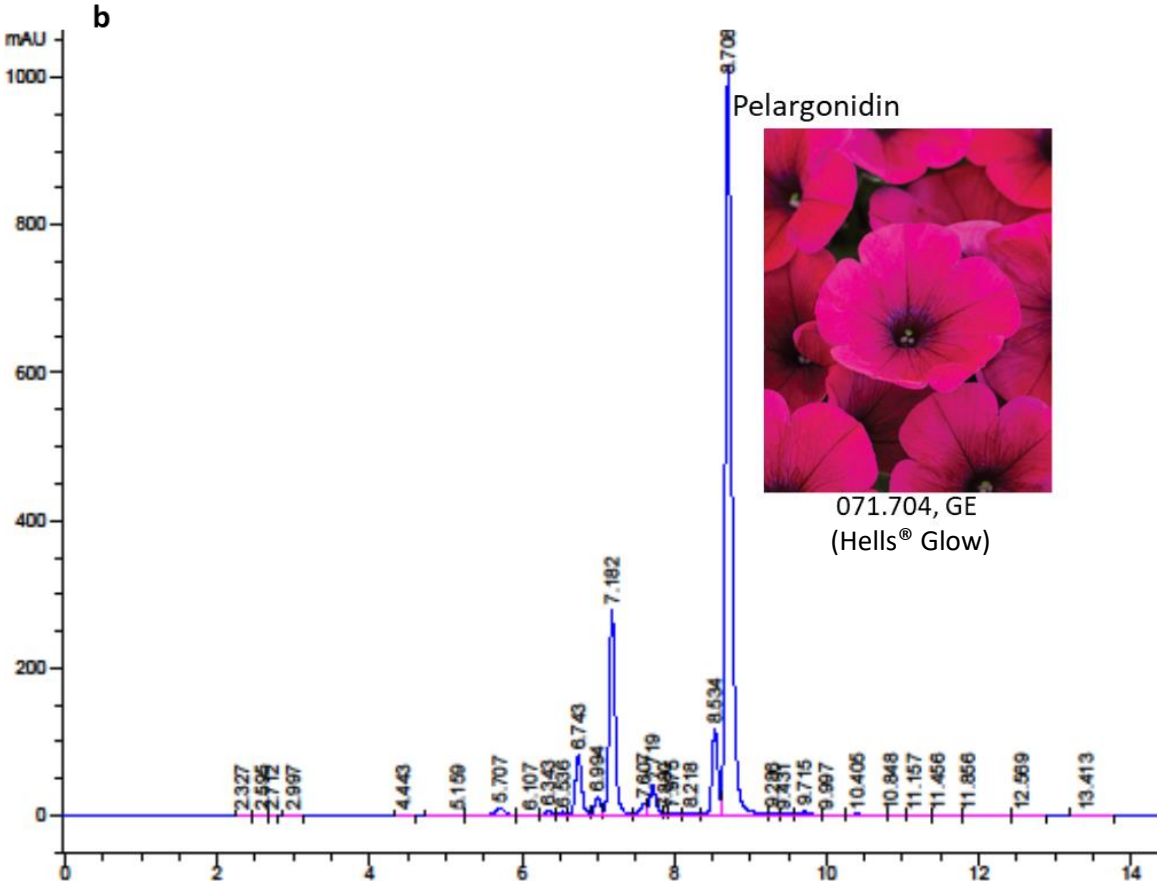
Figure 8: The Crazytunia collection as an example of the range of flower colors. GE petunias are marked with GMO in white box.

As previously described, the natural range of petunia flower colors lacks orange hues typical to pelargonidin derivatives. But petunias which were unintentionally derived from multiple events of the A1 DFR petunias, containing the DFR A1 allele, do not show substrate specificity and had a successful pathway to pelargonidin. Pelargonidin analysis of some of Westhoff petunia varieties revealed the presence of plenty of pelargonidin (Figure 9), supporting that the varieties were originated from the A1 DFR petunias events containing the DFR A1 allele.

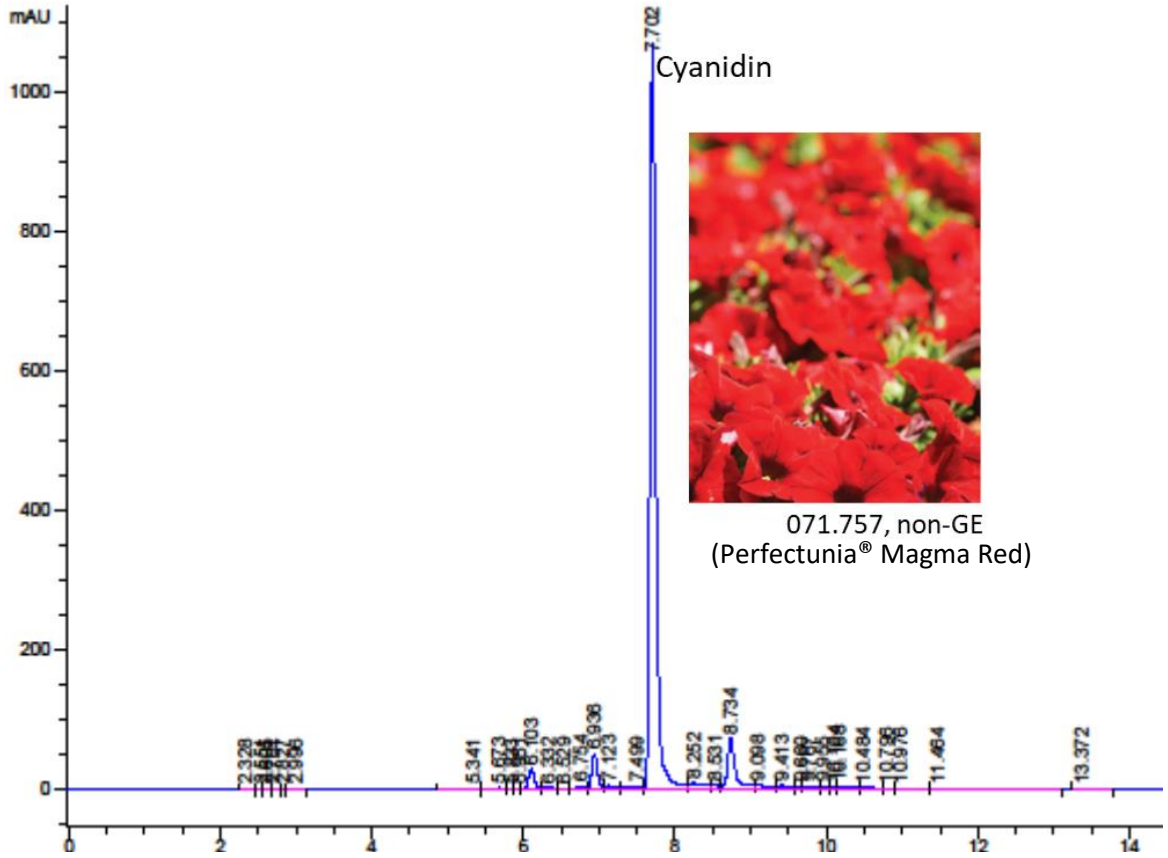
a


Delphinidin
Cyanidin
Pelargonidin
6.930
7.709
8.522
6.630
7.462
7.713
8.200
0
2
2.5
4
5
6
7.5
8
10
12
12.5
14

b

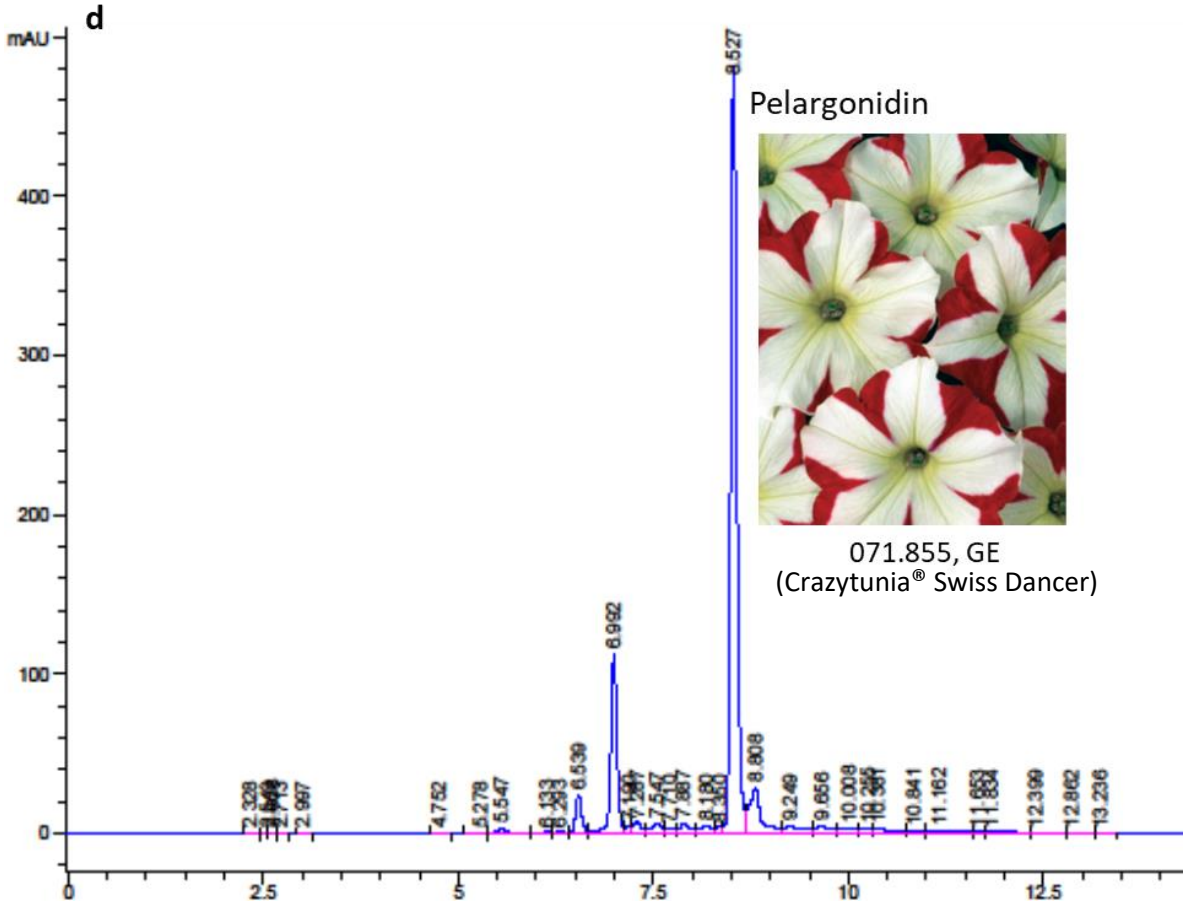

mAU
1000
800
600
400
200
0
Pelargonidin
8.708
7.182
8.534
6.743
071.704, GE
(Hells® Glow)
0
2
4
6
8
10
12
14

c



071.757, non-GE
(Perfectunia® Magma Red)

d



071.855, GE
(Crazytunia® Swiss Dancer)

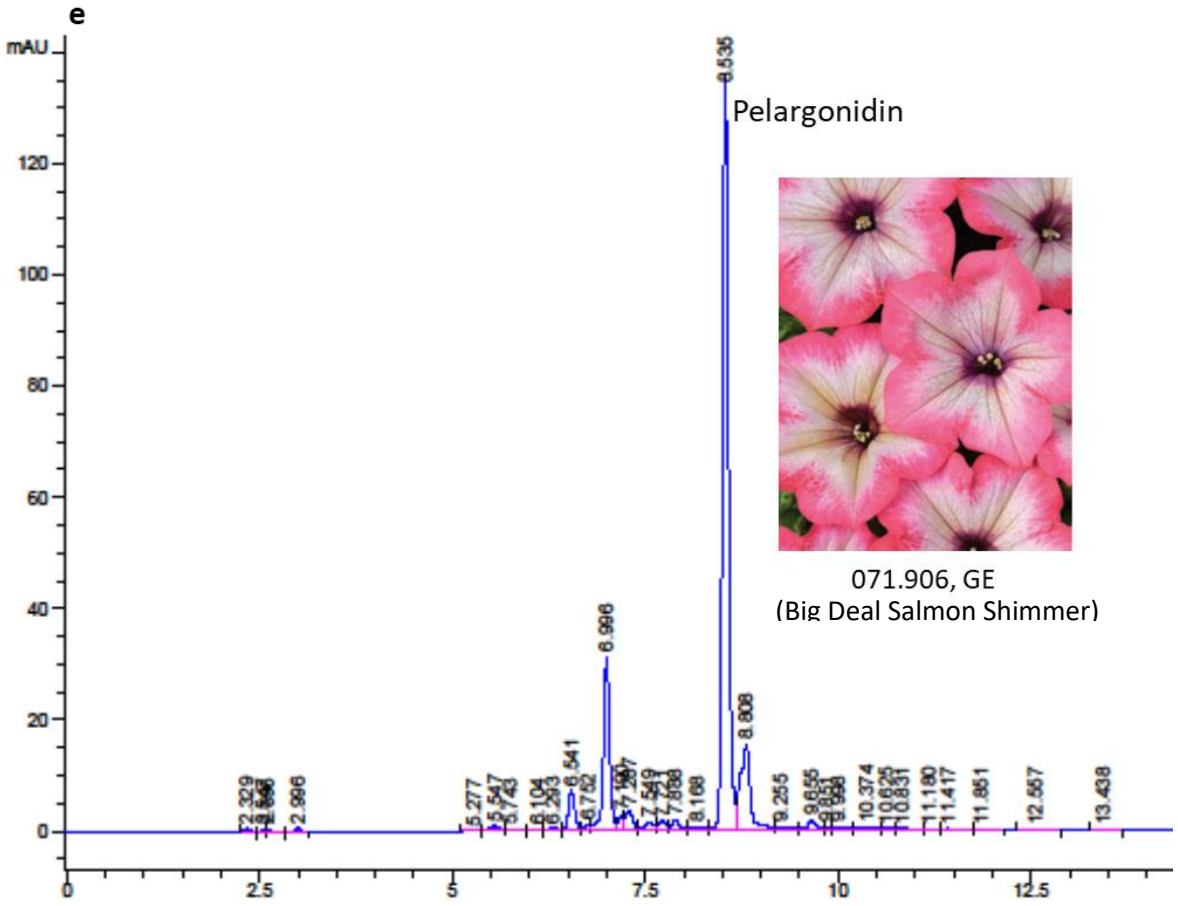


Figure 9: HPLC chromatograms of anthocyanidins extracted from four Westhoff varieties of petunia flowers.
(a). Authentic standards for delphinidin (left, 6.90 min), cyanidin (middle, 7.80 min) and pelargonidin (right, 8.64 min).
(b). The GE variety "071.704, Hells® Glow" containing pelargonidin derived anthocyanidins
(c). The non-GE variety "071.757, Perfectunia® Magma Red", containing cyanidin derived anthocyanidins.
(d). The GE variety "071.855, Crazytunia® Swiss Dancer", containing pelargonidin derived anthocyanidins.
(e). The GE variety "071.906, Big Deal Salmon Shimmer", containing pelargonidin derived anthocyanidins.

VIII. Environmental Consequences of Introduction

In flowers, color is the trait people always look for something different and new. So, it cannot surprise that flower breeders always search for new colors. New colors can appear in nature or during breeding due to natural mutations in nearly all species. Examples for very new colors are Orange pansies introduced by Ernst Benary in Germany in the 1970s or the first true red Primroses in the 1960s. Current examples are the new Echinacea (*Echinacea purpurea*) with a range of new colors different to the natural purple (as the species name implies). The range of new colors can be seen on the website of Terranova nurseries (Anonymous, 2019c). This new Echinacea varieties with the non-natural flower colors are famous for attracting butterflies and other pollinators as the purple ones are.
Petunias were discovered in South America in the mid-1700s and early 1800s. White-flowered Petunia axillaris and purple-flowered *Petunia integrifolia* (syn. *Petunia violacea*) are the parent species of current hybrids. Therefore, the color range of the early petunia was very narrow. During about 200 years of breeding, a huge range of new colors came to the market with blue and red as the most popular. But also star pattern, burgundy, magenta, pink, almost black and yellow, white edge colors (picotee), veined types and so on. Different flower sizes and forms (fringed types) have also been developed (example Figure 8 in this petition). The first commercialized truly red petunia, a multiflora called 'Comanche' and bred by PanAmerican Seed was brought out in 1953. The first yellow petunia, called 'Summer Sun', was bred by Claude Hope and introduced in 1977 by a relatively new company, Goldsmith Seeds (Anonymous, 2019d).
Most if not all new colors in the past (before GE petunias) go back to natural mutations. One example, the green corolla 1-1 (gc1-1) gene was found in inbreeding lines developed for F1-seed varieties by PanAmerican Seed. In 2007 Ball Horticulture Co applied for a patent for the gc1-1 gene (US Grant App Number: US11734924). This gene or more precisely allele of a gene led to a huge broadening of the color range including black, mauve, star pattern with yellow or crème, “dusty” colors with purple or almost black fading center and more.
In spite of all these new colors being introduced to the market and gardens there has never been any complaint or hint that pollinators are harmed by the flower color or for any other environmental problem. The situation did not change with the unintended introduction of the A1 DFR petunias.
Seed set is the same in GE and non-GE petunia, indicating that pollinators have no preference (our observations looking back to notes taken in previous years).
The genetic modifcation does not have any effect on pests and diseases. Unfortunately slugs, thrips and aphids show the same preference to modified and non-modified petunia. Petunias are in general sensitive to mildew. There are differences between varieties but regardless the presence or absence of the GE modification.
Kessler et al. (2013) showed that transgenic petunias with an altered scent profiles can have an effect on florivores(beetle and cricket). Natural variation of elements (traits) of the pollination syndrome does exist in the ancestor species of *P. hybrida* (Hoballah et al., 2007; Dell’Olivo et al., 2011). The scent of GE petunia has not been tested scientifically. An unintended alteration of the scent cannot be excluded and there might be a theoretical chance for an impact on a plant pest risk. In commercial non-GE varieties, there is variability in the sensitivity to diseases (especially mildew) and there is rumor about aphid preference for special varieties. This has not been confirmed scientifically. It can be stated that during the years of commercial distribution of unintended GE petunia there have never been complaints

about problems in that regard. We have not detected difference between the GE and non-GE petunias in their scent in our observation prior to 2017.
GE and non-GE petunia have been in different trials in the greenhouse as well as outdoor all over the world in different years. Up to now there have not been any remarks about different reaction to or performance in the environment. The declarations of the following trial sites supporting this observation are attached (Appendixes I-V):

- Mast Young Plants, Grand Rapids, MI 49544
- Colorado State University, Fort Collins, CO 80523
- Centro Sperimentale Ortofloricolo, 45010 Rosolina (RO), Italy
- Horticultural Trial Station (LVG), Bad Zwischenahn, Germany
- Trial Station for Viticulture and Horticulture (LWG), Veitshöchheim, Germany
- Ohio State University 2016 outdoor trial results

Further details of the biology of petunia and a discussion of possible routes of gene dispersal have been provided in Section II of this petition, and are summarized here.

A. Vegetative spread

Petunia can be vegetatively propagated for commercial production but the plant does not produce organs such as stolons, rhizomes, root borne shoots, tubers, corms, or runners. Roots will not form on discarded parts of a plant under outdoor condition.

B. Gene dispersal by pollen dissemination

Petunia does not intercross with other genera. Even with Calibrachoa, the most closely related genus, crosses are not successful (Wijsman, 1983; personal experience). Therefore there is no possibility of the dispersal of the transgenes by pollen-mediated gene flow to other genera.

C. Gene dispersal by seed formation

Most petunia plants set seed. Very few varieties may show limited fertility or even female sterility and therefore poorly set seed. Despite these conditions, petunia did not become a weed in the US nor elsewhere as far as known.

D. Related weeds

We have not found petunia related weeds in North America. Therefore, there is no risk for hybridization to weeds. Unwanted petunias could be minor weeds in gardens, “but probably only if the gardens are in a warm climate and frequently watered” and “neither the altered flower color trait conferred by the A1 gene, nor the antibiotic resistance conferred by the nptII gene are expected to increase the weediness potential of the GE petunias” (AUS Dept. of Health, 2017).

E. Potential impact on threatened and endangered species

The GE petunias will be cultivated by a limited number of experienced growers inside a horticultural greenhouse setting. The mature plants are then delivered to end consumers, which usually grow on balcony and isolated beds. In case of discarded material for composting, there is negligible opportunity to become established in natural habitats. Therefore, these factors limit potential impact.

Some consumers like to grow them from seed and very few take the effort to harvest seed themselves or take cuttings. Petunia is sensitive to a lot of viruses. That is the reason why growers start with clean material every year. Cuttings of vegetatively propagated varieties are produced in highly specialized nurseries with a very high hygienic standard whick keep a mother stock. If consumers do their own propagation from cutting there is a high risk of infection and sooner or later losing the plants. Very few viruses are seed transmissible and this kind of propagation gives a higher chance of successful propagation. However, 99% of the varieties on the market are F1-hybrids. The important traits of such F1-hybrids will be maintained by propagation using cuttings only. The descendance of the F1-hybrids will segregate very much. This is the reason for many consumers not to harvest their own seed in order not to miss the important traits of the F1-hybrids. A very small group of hobbyists does its own breeding or propagation. And they can do it protected or outdoor depending on the time of the year and the region.
We have described that GE petunia do not behave different than non-GE Petunia. Therefore, production or growing of GE petunias outside greenhouses shall not cause any additional risk.

IX. Theoretically Adverse Consequences of Introduction

The maize dihydroflavonol 4-reductase gene, that was introduced into petunia has led to the production of pelargonidin. Pelargonidin is a common anthocyanin that occurs in several food plants including strawberry (Lopes-da-Silva et al., 2002; Zamora-Ros et al., 2011). In fact, pelargonidin is THE main pigment in strawberry. An adverse consequence of pelargonidin is not yet known to us.
Pelargonidin content was tested in the petal and leaf parts of petunia. No pelargonidin is detected in the leaf parts of the unintentional GE petunia varieties and non-GE petunias (Halbwirth, personal communication). A summary of the pelargonidin content in the petal of those analyzed unintentional GE petunia varieties and non-GE are presented in Table 6. The difference in the pelargonidin absorbance (mAU) between the different GE petunias can be explained by the difference in their genetic backgrounds. The value of the mAU is a relative figure. The mAU value can reduce with more dilution factors and increase with less dilution factors. The pelargonidin content of strawberry, a typical fruit consumed in the USA and all over the world, is included for comparison (Lopes-da-Silva et al., 2002; Table 6).
Therefore, the level of pelargonidin content, as the product of dihydroflavonol 4-reductase (DFR) in the GE petunias do not differ from those that occur in nature in other foods, example strawberry.

Table 6. Pelargonidin content of certain GE and non-GE petunia varieties and strawberry.

Variety Code	GE petunia	Pelargonidin absorbance (mAU)	Petal /fruit color	Reference
071.704	Yes	1000	Purple	Own data
071.757	No	70	Red	Own data
071.855	Yes	480	Red white star	Own data
071.906	Yes	135	Salmon	Own data
071.802	Yes	1300	Red	Own data
071.886	Yes	975	Magenta yellow spot	Own data

071.897	Yes	95	White rosa star	Own data
071.902	Yes	310	White purple star	Own data
071.933	Yes	215	Orange yellow star	Own data
Aladdin Orange	No	45	Orange red (in fact Salmon)	Bashandy and Teeri, 2017
Strawberry	No	~630	Red fruit color	Lopes-da-Silva et al., 2002

In Canada, the Canadian Food Inspection Agency has determined that GE petunias pose no more risk to the environment than conventional petunias. Therefore, they are not considered to be a plant with novel traits and will not be regulated in Canada (Anonymous, 2019g). So far, no country has identified specific environmental risks of GE petunias.

A. Toxicity

Petunia are not used for food and feed. Therefore, no studies were set up to check for differences between GE and non-GE petunia. However, petunia is described as a no-poison plant (Anonymous, 2019e). To be no-poison does not mean that no organism can be harmed but reflexes the impact on humans. Thurston (1970) has shown that Petunia trichomes contain ingredients that are harmful to larvae of the tobacco horn worm, Manduca sexta (Johannson). Thurston (1970) tested species and not commercial hybrids. Of the species tested by Thurston (1970), P. axilaris is one of the ancestors of the current commercial varieties. Therefore, commercial varieties might be as poisonous to some insects as this species is. As shown by Levin (1973) there is variance between genotypes in the number and shape of trichomes (in other genera) which can influence the level of poisonousness/defense. There is no scientific information on the variation between commercial GE and non-GE varieties in that traits. Growers know and unfortunately experience that aphids, thrips and white fly are not harmed by the Petunia trichomes so that insecticides have to be used to protect the crop. If endangered insect species are harmed by the petunia trichomes it obviously was not critical in the past for Petunias are used in official and private plantings for more than 150 years. A1 DFR-Petunia were developed with older commercial varieties and it is to be expected that they do not differ in that regard.

The construct integrated into the modified petunias (A1 DFR events) does not contain any part that could let expect a direct poisonous effect by itself. The genes are selected for changing the flower pigments or have a function in integration of the construct or in the selection process to find successful transformants. As shown above the Pelargonidin content of GE petunia is of about the same dimension as in for example strawberries which are not poisonous even in larger quantities.

GE petunia containing the A1 DFR event have been in the market for at least 10 years without rising any problems. Petunia is one of the most important bedding plants and grown worldwide for more than 150 years with millions of plants every year and without any reports about being poisonous to humans or animals in a critical way. No special trials have been run to test poisonous of GE petunia but results shown in Appendices I to V together with the indirect general observations over the years in the market and gardens give a strong hint that GE petunia do not behave differently to non-GE petunias in any other regard than coloration of the flowers.

B. Allergenicity

Petunias are recommended for low-allergen gardens (Anonymous, 2013). Australian Dept. of Health states that: “The maize DFR protein [in the GE petunia] is not expected to be toxic or allergenic to people or other organisms, including insect pollinators.” And regulatory agencies in Australia and other countries have found no evidence that the NPTII protein is toxic or allergenic to people and other organisms, including insect pollinators (Australian Government, Dept. of Health: Risk Analysis, 2017). There are no results or hints for adverse consequences of an (re-) introduction of GE petunia in any regard. Observation in other crop, for example, a study on seven-year-old orchard grown transgenic citrus trees, indicated a stable expression of NPTII without causing unexpected effects on crop characteristics (Pons et al., 2012). Comparisons between the GE citrus lines with their non-GE counterparts across three years of study showed that the expression of these transgenes did not cause alterations of the main phenotypic and agronomic plant and fruit characteristics including fruit weight, fruit volume, fruit color index, juice content, total soluble solids, titratable acidity and maturity index (Pons et al., 2012). Similarly, it can be assumed that the presence of the NPTII gene in the genome of the GE petunias does not cause unexpected effects on the crop characteristics, which may lead to an adverse effect.

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Appendix I. Declaration of trial site, Mast Young Plants, MI 49544 Grand Rapids

Laura Robles, PH:(616) 784-0583 251 | FAX: (616) 784-3136
Laura.Robles@mastyoungplants.com

To whom it may concern,

Over the last seven years, I have been involved in trialing new varieties of petunias from Westflowers, many of which were then picked up into our finished or young plant programs. A number of these petunias were recently discovered to be unknowingly descended from genetically engineered petunias, and taken off the market.

During our time trialing and growing these plants, there was never anything about them that would have indicated that they were any different from other petunias. Growth both inside the greenhouse and outside in a garden setting was always within the range of what is considered normal for a petunia, as was the flowering. There were no significant differences in heat, cold, rain, disease or insect tolerance, and there was never any apparent difference in attractiveness of these flowers to pollinators.

Below are some examples of varieties supplied by Westflowers (left), which have since been put on the GE list, compared to other non-GE petunia varieties (right).

Sincerely,
Laura Robles
Innovating Manager
Neal Mast Greenhouses/Mast Young Plants

Appendix II. Declaration of trial site, Colorado State University, Fort Collins, CO 80523

Department of Horticulture and
Landscape Architecture

1173 Campus Delivery

MEMORANDUM

DATE: October 29, 2017

TO: Colorado State University Trial Gardens Committee

FROM: Sean Markovic, Annual Trial Garden Coordinator

SUBJECT: 2017 Trial: Hells Series petunia Evaluation

REMARKS: The Colorado State University Trial Garden evaluated all the petunia varieties entered for the 2017 trial year. We had over 100 total entries from seed and vegetative and spreading and mounded types. The Hells series, H. Glow and H. Fruit Punch (GE) and H. Flamin´ Rose (non-GE) from Westhoff performed well and was commented on for having deep, rich colors. The average height and width was taken in July and again in September. A flower color rating was taken every 10 days on average starting in July and ending in September. The averages are all similar to the Hells series petunias.

The overall growth and habit of the Hells series was similar to most petunias entered this year. I did not see any peripheral factors that would suggest abnormal qualities of this series in comparison to other petunia series entered this year. Furthermore, we did not notice any difference in the effect to the environment or pollinators as in the sensitivity to pests and diseases.

If you have any further questions, or need more data please feel free to contact me at Sean.Markovic@colostate.edu or by phone at (630) 639-9160.

Sincerely,

Sean Markovic

Appendix III. Declaration of trial site, Experimental Centre Po di Tramontana, 45010 Rosolina

Giovanna Pavarin, Agenzia Veneta per l'Innovazione nel Settore Primario
Horticultural Experimental Centre Po di Tramontana, Via Moceniga 7, 45010 ROSOLINA (RO) Italia, phone: 049/8293950 Fax: 046/8293959

REPORT ABOUT 3 YEARS VARIETY TRIALS ON PETUNIA INCLUDING GM VARIETIES

I'm Giovanna Pavarin and work as a floricultural technician at the Experimental Centre Po di Tramontana at Rosolina RO (northern Italy).
Our main activity deals with variety trials, and during the period 2014-2016 we tested more than 400 petunia varieties, both in greenhouse and outside all summer long.
Tha aim of our work is to give both breeders and growers information about both greenhouse and garden performance of the varieties, above all new and experimental ones.

This year I heard that many petunia varieties had been found GM positive, and realized that we had had in our trials most of them.
They were destroyed after the trial so nothing remained, but I have photos and evaluations made during the trials; according to them, I can say by sure that I never found in those varieties anything that could indicate they came from a different source, if compared to Gm-free ones.
Thay showed only the normal differences that can be seen among different varieties, regard vigour, earliness, habit or wather sensitivity. They didn't seem attract insects more than the Gm free varieties, or cause insects any kind of damage.

Giovanna Pavarin

Appendix IV. Declaration of trial site, Horticultural Trial Station (LVG), Bad Zwischenahn

Landwirtschaftskammer Niedersachsen

Lehr- und Versuchsanstalt für Gartenbau

LWK Niedersachsen •Hogen Kamp 51 • 26160 Bad Zwischenahn

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Ihr Zeichen	Unser Zeichen	Ansprechpartner | in	Durchwahl	E-Mail	Datum
		Dr. Elke Ueber	- 15	elke.ueber@lwk-niedersachsen.de	September 6, 2017

Observations of Horticulture Trial Station (LVG) Bad Zwischenahn at Bad Zwischenahn (NW Germany) on GMO-Petunia

The semi-official trial station LVG Bad Zwischenahn runs trials of bedding plants every year. One of the genera tested is Petunia. Retrospectively we learned that some of the varieties in trial did contain elements of genetic modification (GMO). The table below shows examples of what has been trialed since 2011 at Bad Zwischenahn based on the varieties of the company Westhoff. GMO-varieties are highlighted in yellow.
During the observations over the years we never noticed differences between non-GMO and GMO-petunias in regard of the influence by or onto the environment. The behavior of pollinating and other insects did not show any difference too. Furthemore we could not find more dead pollinators around GMO-petunias compared to "normal" petunias.
Of course there have been differences in the phenotype between petunias as usual will be seen in a wide assortment. The only extraordinary trait was a special flower color in certain varieties.

With kind regards

Dr. Elke Ueber
Horticultural engineer

Appendix V. Declaration of trial site, Trial Station for Viticulture and Horticulture (LWG)

Bayerische Landesanstalt für
Weinbau und Gartenbau

Bayerische Landesanstalt für Weinbau und Gartenbau
An der Steige 15, 97209 Veitshöchheim

J. + H. Westhoff
Fresenhorst 22 – 24
46354 Südlohn
GERMANY

Mobil
0173/89 02 427

Name
Gerd Sander
Telefon
0931/9801-318
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0931/9801-300
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Ihr Zeichen, Ihre Nachricht vom	Unser Zeichen	Veitshöchheim
	Sand/Vä	12.09.17

GM-Petunia: Observation results on test plants at the Governmental Research Station for Viticulture and Horticulture (Bayerische Landesanstalt für Weinbau und Gartenbau Veitshöchheim, LWG Veitshöchheim)

LWG Veitshöchheim

One of the duties of the Governmental Research Station for Viticulture and Horticulture, an institution of the state of Bavaria for applied research in horticulture, is the trialing of a broad range of bedding plants for their value or usefulness from the point of view of the grower and the consumer.

One of the genera investigated is Petunia with a wide assortment of varieties. As we had to learn this spring some of the varieties being tested in the past and some currently in observation were tested positive for genetic modification (GM).

GM-Petunias have no permission for the European market. Therefore the GM-varieties had to be destroyed after the first period of growth in May 2017.

During our extensive and systematical evaluation and observations of the test samples until the end of summer in earlier years and this year ending in spring we could not find any differences between GM-Petunias and GM-free-Petunias except the differences which usually are seen between varieties. GM-Petunias did neither show a different environmental performance nor did they differ in their effect on pollinating or other insects compared to GM-free Petunias.

A far as it was possible to evaluate the genetically modified Petunias behave as any other Petunia without genetic modification at our location in Veitshöchheim.

Gerd Sander
Chief Director Horticultural Research Department

Eva-Maria Geiger
Director Department Ornamental Plants

Bayerische Landesanstalt für
Weinbau und Gartenbau
An der Steige 15
97209 Veitshöchheim

Telefon 0931 9801-0
Fax 0931 9801-100
E-Mail poststelle@lwg.bayern.de
Internet www.lwg.bayern.de

Öffnungszeiten
Mo–Do: 7:30-16:00 Uhr
Fr: 7:30-12:00 Uhr
und nach Vereinbarung

Appendix VI A. DNA sequences of the p35A1 backbone fragments and the A1 gene found integrated in the genome sequence of the GE petunia (A1 DFR petunias)*.

*Description of the highlighted sequence regions:
PGS//= Natural petunia genome sequence left margin of the integration
//PGS= Natural petunia genome sequence right margin of the integration
Partial sequence of beta-lactamase
CaMV 35S promoter
Zea mays A1 gene for dihydroflavonol 4-reductase
transposon Cin4-1 of maize
35S Terminator
lacUV5 promoter
Nopaline synthase promoter
Neomycin phosphotransferase II
Octopine synthase terminator

PGS//CAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATG
CCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCG
AGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGA
AAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCA
CCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA
AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTAT
CAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACA
TTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATC
ACGAGGCCCTTTCGTCTTCAAGAATTCCCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAA
AGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGC
ACGACACGCTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAAC
AAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAA
AGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTA
TATAAGGAAGTTCATTTCATTTGGAGAGGACAGGGTACCCGGGGATCCTCTAGAGAATTCCAGCTGCTCACTCAG
TCCTGCGCAAGAGCTCGCTCTCGGAGAAAAAAACGCGGGAGGCGATAATGGAGGGAGGTGCCGGTGCGAGCGAGA
AAGGGACGGTGCTGGTCACGGGGGCGTCGGGCTTCGCCGGCTCCTGGCTCGTCATGAAGCTCCTCCAGGCCGGCT
ACACCGTCCGGGCGACCGTGCGCGATCCCGCGAACGTTGGGAAGACGAAGCCATTGATGGACCTTCCCGGAGCAA
CGGAGCGCCTGTCCATATGGAAAGCCGACCTGGCGGAGGAAGGCAGCTTCCACGACGCCATCAGGGGCTGCACCG
GCGTCTTCCACGTCGCCACGCCCATGGACTTCCTGTCCAAAGACCCTGAGAATGAGGTAATCAAGCCGACGGTGG
AAGGGATGATAAGCATCATGCGGGCATGCAAGGAGGCCGGCACCGTGCGGCGCATCGTCTTCACTTCCTCCGCCG
GGACGGTCAACCTGGAGGAGCGGCAGAGGCCCGTCTACGACGAGGAAAGCTGGACCGACGTCGACTTCTGCCGTC
GCGTCAAGATGACAGGATGGATGTACTTCGTGTCTAAAACCCTGGCGGAGAAGGCGGCCCTGGCGTACGCGGCGG
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GCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACA
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AAAATGCTCCACTGACGTTCCATAAATTCCCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAGATC
CGGCCCATGATCATGTGGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTC

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TTAAAATGAAAACAATATATTGTGCTGAATAGGTTTATAGCGACATCTATGAT//PGS

Appendix VI B. The coding sequence translation into amino acid sequences of the A1 DFR and NPTII found integrated in the genome sequence of the GE petunia (A1 DFR petunias).

>A1 DFR
MEGGAGASEKGTVLVTGASGFAGSWLVMKLLQAGYTVRATVRDPANVGKTKPLMDLPGATERLSIWKADLAEEG
SFHDAIRGCTGVFHVATPMDFLSKDPENEVIKPTVEGMISIMRACKEAGTVRRIVFTSSAGTVNLEERQRPVYDEES
WTDVDFCRRVKMTGWMYFVSKTLAEKAALAYAAEHGLDLVTIIPTLVVGPFISASMPPSLITALALITGNAPHYSILK
QVQLIHLDDLCDAEIFLFENPAAAGRYVCSSHDVTIHGLAAMLRDRYPEYDVPQRFPGIQDDLQPVRFSSKKLQDLG
FTFRYKTLEDMFDAAIRTCQEKGLIPLATAAGGDGFASVRAPGETEATIGA

> NPTII-neomycin phosphotransferase II
MIMWIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRPVLFVKTDLSGALNELQDEAARLSWL
ATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDLLSSHLAPAEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERAR
TRMEAGLVDQDDLDEEHQGLAPAELFARLKARMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRYQ
DIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF

Appendix VII. Ohio State University 2016 outdoor trial results of the comparison between non-GE and GE petunias.

Trait†	Non-GE Basket (Average)*	GE Basket (Average)*	Non-GE Container (Average)*	GE Container (Average)*
Flower Qnty1	4.6	4.8	4.7	4.9
Flower Qnty2	4.1	4.3	4.3	4.8
Flower Qnty3	3.3	3.2	4.0	4.1
Flower Qnty4	2.9	2.4	3.2	3.5
Average Flower Quant.	3.7	3.7	4.0	4.3
Two-Sample t-Test	p-value = 0.9426 > 0.05, no difference		p-value = 0.5687 > 0.05, no difference	
Flower Qual1	5.0	5.0	5.0	5.0
Flower Qual2	4.9	4.8	4.9	5.0
Flower Qual3	5.0	4.8	4.9	5.0
Flower Qual4	5.0	4.8	4.9	5.0
Average Quality Flowers	5.0	4.8	4.9	5.0
Two-Sample t-Test	p-value = 0.08274 > 0.05, no difference		p-value = 0.05767 > 0.05, no difference	
Veg Vigor1	4.9	5.0	4.9	4.9
Veg Vigor2	3.9	3.9	4.1	4.5
Veg Vigor3	3.1	3.1	3.5	3.6
Veg Vigor4	2.6	2.3	3.0	3.2
Average Vigor	3.6	3.6	3.9	4.0
Two-Sample t-Test	p-value = 0.95 > 0.05, no difference		p-value = 0.768 > 0.05, no difference	
Overall1	4.5	4.8	4.6	4.9
Overall2	3.9	4.0	4.2	4.7
Overall3	3.3	3.1	3.9	3.9
Overall4	2.7	2.3	3.0	3.2
Overall Average	3.6	3.5	3.9	4.2
Two-Sample t-Test	p-value = 0.9429 > 0.05, no difference		p-value = 0.6465 > 0.05, no difference	
*rating 1-5, 5= best				

†FlowerQnty= Quantity of flowers open; Flower Qual= Quality of the flowers (stability, shape, color attributes like brightness), VegVigor= vegetative vigor (Growth habit including branching), Overall= the general impression of the plants including all different trait from the view of a grower and a consumer, 1 to 4= represent different dates of evaluation to get information for the whole growth period.

Appendix VIII. Colorado State University 2017 and 2016 outdoor trial results of the comparison between non-GE and GE petunias.

A) 2017 Trial		
Trait*	Non-GE Petunia (Average)	GE Petunia (Average)
FCR 7/12	3.9	4.4
FCR 7/26	3.6	4.1
FCR 8/3	3.4	3.4
FCR 8/14	3.4	3.4
FCR 8/21	3.4	3.4
FCR 9/5	3.2	3.0
FCR 9/19	3.2	3.0
Average FCR	**3.5**	**3.6**
H 7/12	9.5	10.0
H 9/19	12.0	11.3
Average FCR	**9.8**	**10.6**
W1 7/12	22.3	20.7
W2 7/12	21.3	22.8
W1 9/19	27.8	28.0
W2 9/19	27.8	25.7
Average FCR	**24.8**	**24.3**

B) 2016 Trial		
Trait*	Non-GE Petunia (Average)	GE Petunia (Average)
FCR 7/5	3.3	3.8
FCR 7/17	3.6	4.1
FCR 7/27	3.8	4.2
FCR 8/8	3.7	3.6
FCR 8/22	3.3	3.8
FCR 9/6	3.3	3.8
FCR 9/14	3.3	3.8
Average FCR	**3.5**	**3.9**
H 7/1	6.6	7.7
H 9/14	10.3	7.3
Average FCR	**8.5**	**7.5**
W 7/1	9.7	9.2
W 9/15	20.9	17.2
Average FCR	**15.3**	**13.2**

*FCR, flower count records, 1-5, 5= best; H, height in centimeter; W, width in centimeter.

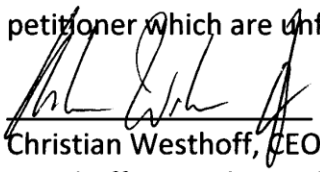
Appendix IX. LVG Bad Zwischenahn (Germany) 2016 outdoor trial results of the comparison between non-GE and GE petunias.

Observations/Trait*	non-GE Petunia (Average)	GE Petunia (Average)
Flower quantity May - June	5.3	5.5
Flower quantity July - August	4.9	4.5
Average flower quantity	**5.1**	**5**
Total Performance May - June	6.3	6.1
Total Performance July - August	4.6	4.3
Total Performance Week 18/19/20	7.3	7.2
Average total performance	**6**	**5.9**
Result Jury	5	4.8

*rating 1-9, 9= best; Result Jury is the general impression figure of a group of specialists.

Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.



Südlohn-Oeding, 03.04.2019

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