FLORIGENE PTY LTD
1 PARK DRIVE
BUNDOORA
VICTORIA 3083
AUSTRALIA
TEL +61 3 9243 3800
FAX +61 3 9243 3888

John Cordts
USDA
Unit 147 4700 River Rd
Riverdale MD 20737-1236
USA

DATE: 23/12/09

Subject: Petition number 08-315-01p for a determination of non-regulated status for Rosa X hybrida varieties IFD-52401-4 and IFD-52901-9 – ADDENDUM 1.

Dear John,

As requested Florigene is submitting "Addendum 1: pests and disease for roses" to add to petition 08-315-01p. This addendum outlines some of the common pest and diseases found in roses. It also includes information from our growers in California and Colombia stating that the two transgenic lines included in the petition are normal with regard to susceptibility and control.
This addendum contains no CBI information.

Kind Regards,

Katherine Terdich
Regulatory Affairs

ADDENDUM 1: PEST AND DISEASE ISSUES FOR ROSES

Roses like all plants can be affected by a variety of pests and diseases. Commercial production of plants free of pests and disease requires frequent observations and strategic planning. Prevention is essential for good disease control. Since the 1980's all commercial growers use integrated pest management (IPM) systems to control pests and diseases. IPM forecasts conditions which are favourable for disease epidemics and utilises sprays only when necessary.

The most widely distributed fungal disease of roses is Powdery Mildew. Powdery Mildew is caused by the fungus *Podosphaera pannosa.* It usually begins to develop on young stem tissues, especially at the base of the thorns. The fungus can also attack leaves and flowers leading to poor growth and flowers of poor quality. Control of the fungus is achieved by protective sprays such as Benomyl, Captan, and Triforine. Other preventive measures include; removal of infected shoots and lowering night humidity (in glasshouse conditions).

Table 1 outlines a variety of other pest and diseases that can affect roses and describes treatment.

Table 1: Some common pest and diseases of Roses and the recommended treatment (Horst, 1995; Stroom *et al*., 2008; Roberts et al, 2003)

Pest/disease	**Treatment**
Two spotted mite	ETI miticides, fenpyroximate (Dynamite) and tebufenpyrad (Pyranica) for adult stages and Ovicides, clofentezine (Apollo) for eggs. Introduce predatory mite *Phytoseiulus persimilis*
Downy mildew	Lowering humidity and keeping temperature constant. Removal of infected tissues and spray with fungicidal sprays such as Metiram, Fosetyl-aluminium or Propamocarb.
Rust	Removal of infected tissues and spray with fungicidal sprays such as Thiram, Maneb or Zineb at ten day intervals
Verticullium wilt	Sterilise soil before planting
Botrytis blight	Lowering humidity and keeping temperature constant. Removal of infected tissues and spray with fungicidal sprays such as Clorthalonil or Fenhexamide
Black spot (minor problem in glasshouse grown roses)	Removal of infected tissues and spray with Mancozeb, Ziram, Triforine, Dodine or Kresoxim-methyl.
Aphids	Removing them by hand, high-pressure watering wands especially designed for insect control, release of natural predators and parasites, and/or a variety of organic and synthetic pesticides.
Japanese bettle, Rose chafers	Hand pick if infestation is light. The insecticides carbaryl (Sevin), acephate (Orthene), diazinon, and chlorpyrifos (Dursban) control these beetles.
Viruses such as Rose Mosaic Virus (RMV)	Ensure certified virus free rootstocks (if grafting). Destroy plants when infected. If available, heat treatment and meristem tip culture may be used to obtain clean material.

The following paragraphs are excerpts from an email from Jackson and Perkins noting their specific experience with pest and diseases when dealing with the transgenic rose lines IFD-52401-4 and IFD-52901-9.

Jackson and Perkins Wholesale, Inc. has grown the Florigene transgenic lines at the Somis, California facility for the past 4 years. These cultivars have grown normally in all respects. When grown under cover they suffer from 3 different pests, as do all commercially grown cut roses in California. These roses can become infected with the two spotted spider mite (Tetranychus urticae), with powder mildew (Podosphaera pannosa) and with downy mildew (Peronospora sparsa). These are ubiquitous pests throughout all of the United States and infect roses anywhere roses are grown commercially. All three pests can be controlled using common treatments and conventional protocols. These transgenic lines are completely normal with regard to susceptibility and control.

These three pests are most common when the roses are most actively growing. Mites prefer the warmth of long summer days. At the Somis location we treat for this pest usually 3-5 times a year. Powdery mildew is common when the seasons change from winter to spring and from summer to fall. The large temperature change from day to night encourages the conditions necessary for disease development. By properly controlling the greenhouse environment you can eliminate or nearly eliminate this disease. We spray for it 2 or 3 times a year. Downy mildew is mostly a fall to winter disease that likes cool moist conditions. It is a rare event in a greenhouse. We only spray for this occasionally - perhaps once a year.

In Colombia, transgenic rose lines IFD-52401-4 and IFD-52901-9 have been grown within a very large rose growing facility, adjacent to both the parental control and other commercial rose varieties. Under these conditions, pest and disease control measures are more intense as commercial flower must be completely free of disease and insect damage, as flowers are intended for export. The crop, including the transgenic lines, was chemically treated on a weekly basis, with treatments including Vertimec, Teldor and Previcur. The grower noted that the transgenic and parental controls were susceptible to Mildew, but that there was no difference between the transgenic lines and parental control.

References

Horst, R.K. 1995. Compendium of rose diseases. The American Phytopathological Society Press (APS press), Minnesota, USA.

Roberts, A.V., Debener, T. and Gudin, S. 2003. Encyclopedia of rose science. Volume 1. Elsevier Academic Press, San Diego, USA.

Stroom, K., Fetzer, J. and Krischik, V. 2008. Insect pests of roses. University of Minnesota. http://www.extension.umn.edu/distribution/horticulture/DG6953.html

FLORIGENE
FLOWERS

FLORIGENE PTY LTD
1 PARK DRIVE
BUNDOORA
VICTORIA 3083
AUSTRALIA
TEL +61 3 9243 3800
FAX +61 3 9243 3888

John Cordts
USDA
Unit 147 4700 River Rd
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USA

DATE: 23/9/09

Subject: Petition number 08-315-01p for a determination of non-regulated status for Rosa X hybrida varieties IFD-52401-4 and IFD-52901-9.

Dear John,

Please find enclosed the final completed petition, number 08-315-01p, for the determination of non-regulated status for the cultivation of two genetically-modified rose varieties. The points listed in the letter of completeness (dated April 10th, 2009) have all been addressed. The enclosed petition contains no CBI information.

Kind Regards,

Katherine Terdich
Regulatory Affairs

Petition for the determination of non-regulated status for *Rosa* X *hybrida* (rose) varieties IFD-524Ø1-4 and IFD-529Ø1-9

Submitted by
International Flower Developments Pty Ltd
1 Park Drive
Bundoora 3083
Victoria
Australia

Submitted September 2009

CERTIFICATION

The undersigned submits this petition under 7 CFR 340.6 to request that the Administrator make a determination that the article(s) should not be regulated under 7 CFR part 340. 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 all relevant data and information known to the petitioner that are unfavourable to the petition.

Signature _____ Date 21 Sep '09

Mr, Taizo Chinju
Director, International Flower development Pty. Ltd.

Address
1 Park Drive
Bundoora
VIC 3083
Australia

Tel +61 3 9243 3801
Fax +61 3 9243 3888
Email chinju@florigene.com.au

CONTACTS

Author and primary contact regarding this petition

Dr. Katherine Terdich
Florigene Pty. Ltd.
1 Park Drive
Bundoora
VIC 3083
Australia
Tel +61 3 9243 3826
Fax +61 3 9243 3888
Email kterdich@florigene.com.au

Signature Katherine Terdich Date 23/9/09

US-based contact

Mr. Cory Sanchez
Florigene Flowers
Suite 110
3350 SW 148thAve
Miramar, Florida 33027
Phone 954-874-1647
Fax 954-974-1699
Mobile 305-333-4466
csanchez@florigene.com

SUMMARY (STATEMENT OF GROUNDS)

International Flower developments Pty. Ltd. is petitioning for de-regulation of two transgenic varieties of rose;

Family	Rosaceae
Genus	*Rosa*
Species	*hybrida*
Common name	Rose (English), Rosas (Spanish)
OECD unique identifiers	IFD-524Ø1 -4; IFD-529Ø1-9
Transformation vector	pSPB130

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 cannot persist in the environment.
2. The genetic modification does not present a risk to plant, animal or human health nor to the environment.

1. The regulated articles cannot persist in the environment

The potential for gene dispersal from rose is nil because of the biology of the crop;

- The transgenic lines are genetic chimeras, and the pollen is non-transgenic
- Rose does not spread vegetatively.

In major flower production areas throughout the world, including the USA, no cultivated rose population has ever become established in the wild. Rose varieties have been grown in the USA and imported into the USA, for decades, but rose has not escaped from cultivation.

2. The genetic modification does not present a risk to plant, animal or human health nor to the environment

Rose has a long history of use as a safe crop in the USA. In previous decades the plant was grown in several states and in recent years billions of flowers have been imported into the USA, largely from South America. The crop is not known to be toxic, or to be a common cause of allergies.

Flowers of IFD-524Ø1-4 and IFD-529Ø1-9 have introduced genes from pansy and torenia and produce the anthocyanin delphinidin. These modifications do not introduce changes that increase the toxicity or allergenicity of the flowers;

- Delphinidin is a common anthocyanin, present in many other flower species, and at high concentrations in commonly consumed foods.
- The introduced, flavonoid 3' 5' hydroxylase occur in all plants foods containing delphinidin.
- Genetically-modified carnation flowers containing the same pansy flavonoid 3' 5' hydroxylase inserted in IFD-524Ø1-4 and IFD-529Ø1-9 have been traded in the USA for several years, with no reports of adverse effects.

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ABBREVIATIONS[1] AND SCIENTIFIC TERMS

Agrobacterium (AGL0):	*Agrobacterium tumefaciens*, strain AGL0
CaMV35S or 35S:	Cauliflower mosaic virus 35S promoter fragment
CHI:	Chalcone isomerase
CHS:	Chalcone synthase
DFR:	Dihydroflavonol reductase
DHK:	Dihydrokaempferol
DHM:	Dihydromyricetin
DHQ:	Dihydroquercitin
DNA:	Deoxyribonucleic acid
EFSA	European Food Safety Authority
F3H:	Flavanone 3-hydroxylase
F3'H:	Flavonoid 3' hydroxylase
F3'5'H:	Flavonoid 3' 5' hydroxylase
FLS:	Flavonol synthase
g:	gram
GMO:	Genetically Modified Organism
HPLC	High Pressure Liquid Chromatography
LB:	Left Border of T-DNA
mg:	Milligrams
mg/g:	Milligrams per gram
MG/L:	Milligram per liter
mm:	Millimeter
OGTR:	Office of the Gene Technology Regulator
p:	Promoter
PCR:	Polymerase Chain Reaction
RB:	Right Border of T-DNA
RHS:	Standard color reference from the Royal Horticultural Society, London
t:	Terminator
T-DNA:	Transferred-deoxyribonucleic acid
™:	Trade Mark
UK:	United Kingdom
US or USA:	United States of America
USDA – APHIS:	United States Department of Agriculture – Animal and Plant Health Inspection Service
WHO:	World Health Organisation

[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 Development of Color Modified Rose

Florigene Pty. Ltd., an Australian based biotech company, has marketed genetically modified, color-modified carnation flowers for ten years. Flowers are grown in Australia, Ecuador and Colombia and are sold in Australia, Japan, USA, Europe and Canada. The flowers are a novel flower color and have become well established in the US marketplace (www.florigene.com). The company has also developed genetically modified rose, also modified for novel flower color, in the same shades of color as that developed for carnation.

The two rose lines which are the subject of this application are grown in Colombia and one intention is to import cut flowers of these two varieties into the USA. However, commercial rose flowers growers based in the USA have also expressed an interest in producing the flowers for the domestic market. The purpose of this petition is to seek de-regulation to allow trials, propagation and commercial production in the USA, most probably in California.

II. The Rosaceae Family

A. *Rosa* Cultivation

Origin of cut flower varieties

The origin of the cultivated roses popular as cut flowers today, which are all complex hybrids, is very complicated. Rose breeding has a very long history and over 15,000 cultivars have appeared over the centuries. These have largely arisen through hybridization of less than 10 wild *Rosa* species centuries ago (Hurst, 1941). 'Pure' *Rosa* species are often termed species roses (Le Rougetel, 1983) and that terminology is adopted in this document. The origin of the cut flower type roses is summarized in Figure 1, using information described in Hurst (1941), Mastalerz and Langhans (1969) and Phillips and Rix (1988). Hybrid Tea roses contain *R. damascena*, *R. moschata*, *R. chinensis*, *R. gigantea* and *R. gallica* genes in their parentage (Figure 1). Cultivated roses are categorized according to breeding heritage and form, and a category may include lines that have arisen by mutation to give sports. Categories (e.g. hybrid tea, autumn damask, portland etc.) are shown in non-italic letters in Figure 1. The first hybrid tea rose was produced in France in 1867 by a Monseiur Guillot (Beales, 1985). Since then there have been hundreds of new hybrid tea cultivars introduced, and this type is the most common background in the varieties grown commercially for cut flower production. In addition, hybridization of hybrid tea roses to *Rosa* species and other cultivated rose types has given rise to the polyantha, pernetian, floribunda and grandiflora types (Figure 1). Hybrid tea cultivars are sometimes triploid (and thereby usually sterile) but generally tetraploid.

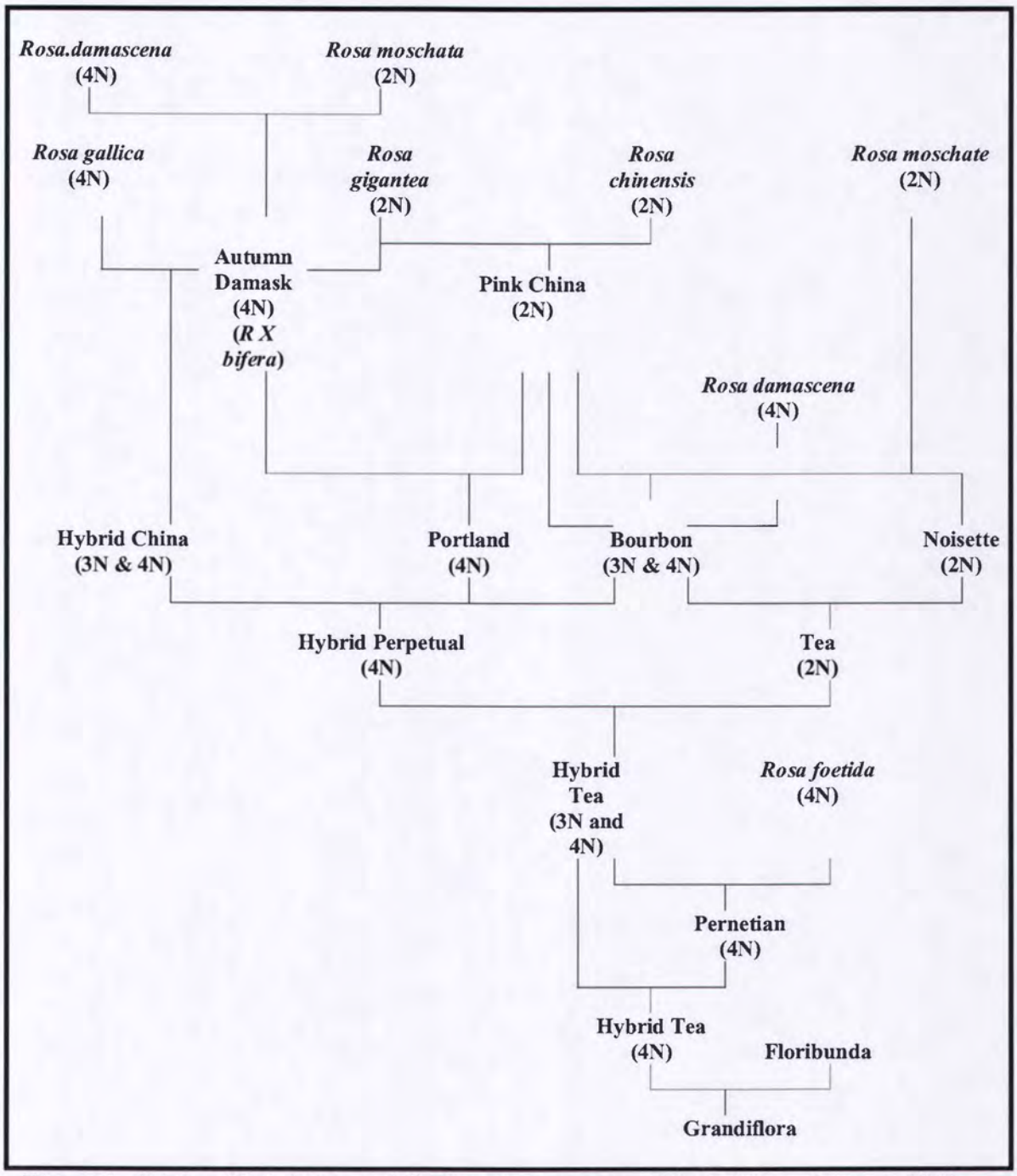


Figure 1: Origin of hybrid tea and floribunda type roses. Adapted from Stewart, 1969.

Cultivated rose

Many species of *Rosa* have been modified through selection and hybridisation to give rise to some 20,000 cultivars (OGTR, 2005). The flowers of cut-flower rose varieties are now quite different to a flower from a wild rose species, such as *Rosa multiflora*. This is shown in Figure 2.

Figure 2: Comparison of a commercial cut flower (left hand side) to a flower from a wild rose species Rosa multiflora (right hand side).

Cut-flower varieties of rose grow to 160 – 250 cm high, depending on variety. Rose flowers are typically sold dependant on stem length with the most common lengths being 45 – 75cm, although some European markets are now favouring even longer stems, up to 120cm. Cut flower varieties of rose produce flowers with a diameter up to 100 mm, containing 25 to 35 petals, but up to 80 petals in some hybrids. The ovary is inferior, developing below the petals and sepals. In contrast, wild *Rosa* species have small, open, 5 petalled flowers, with the stigma and style easily accessible. The flowers are typically in large clusters of 10 or more per stem (Figure 2).

Rose cultivation in North America

The cultivated rose is found in all temperate and subtropical parts of the globe. It is the most widely produced cut flower crop in the world (over six billion stems sold per annum in recent years) and varieties have also been established as perennial plants in private gardens, parks, public facilities and botanical gardens. Globally, 60-80 million new rose plants are planted per annum just to meet the demand for cut flower production.

Commercial cut flower varieties of rose have been bred for color and form of the flowers. In the case of cut flower varieties further selection has been imposed for characters such as

increased productivity, fewer prickles and improved vase-life. This generally makes these varieties unsuitable for establishment in gardens. Regardless of whether varieties are destined for cut flower production or establishment as garden roses hybrid tea roses are commonly propagated by budding onto rootstocks, to provide improved pathogen resistance and improved vigour. Commonly used rootstocks are *R. multiflora*, *R. fortuneana* and *R. canina*.

In North America the main growing area for cut flowers is in California, 73% of US production is based there. Roses are the most popular cut flower in the US with over 1.3 billion stems sold each year, for Valentine Day alone 189 million stems were purchased by consumers. In 2006 approximately 173 million rose stems were produced in California. This accounts for only 10% of the roses sold in the US, with the bulk of roses being supplied by Colombia, Ecuador, Europe and Africa (USDA Floriculture & Nursery Crop Outlook 2005; California Cut Flower Production and Industry Trends 2006; American Floral Endowment Consumer Tracking Study; Society of American Florists).

Texas also has a history as a rose grower however this is mainly for rose bushes not cut flowers. In the early 1970s more than fifteen million rosebushes were shipped annually from Texas throughout the United States but by the 1990s out-of-state competition, along with unpredictable weather and some lost crops during the 1980s, contributed to a decrease in production. In 2006 fewer than fifty rose growers produced approximately eight to ten million rosebushes annually on approximately 800 to 1,000 acres. This accounts for 16-20 percent of the United States rosebush crop (The handbook of Texas online, 2008).

B. Taxonomy of *Rosa*

The *Rosa* genus belongs to the Rosaceae family (Klastersky, 1968; Hickey and King, 1981) which contains many important fruit trees (e.g. apple, pear, plum, cherry) and berry plants (e.g. strawberry, blackberry). There are over 200 *Rosa* species (Hickey and King, 1981; Phillips and Rix, 1988) and all are native of temperate regions or tropical mountains of the northern hemisphere including North America, Europe, Asia and the Middle East. The greatest diversity of species is found in Western China.

The haploid *Rosa* genome contains 7 chromosomes and the genus contains species with diploid, triploid, tetraploid, pentaploid and, less commonly, hexaploid species (Ratsek *et al.*, 1941). There are two octoploid species (Stewart, 1969). Interspecific hybridization is common among wild *Rosa* species (Melville, 1975), and is described in more detail below under the title "Interspecific hybridization is common in the *Rosa* genus".

Rosa species in the North American flora

There are 105 species of *Rosa* listed on the USDA website. The distribution of the most widely dispersed *Rosa* species is shown in Table 1 and Figure 3. Only three species, *R. arkansana*, *R. multiflora* and *R. rugosa* are commonly established as weeds in the US (USDA, 2008).

Table 1: Most widely spread Rosa species in North America and Canada (35 species out of 105 total). USDA plant database www.plants.usda.gov 2008. .

***Rosa* Species**	**Common Name**	**Status**	**Number of states/ provinces**	
			US	**Canada**
Rosa acicularis	Prickly rose	Native	22	10
Rosa arkansana	Prairie rose	Native	22	5
Rosa blanda	Smooth rose	Native	25	8
Rosa bracteata	Macartney rose	Introduced	13	0
Rosa canina	Dog rose	Introduced	28	5
Rosa carolina	Carolina rose	Native	36	5
Rosa centifolia	Cabbage rose	Introduced	8	3
Rosa cinnamomea	Cinnamon rose	Introduced	12	5
Rosa ×damascena	Damask rose	Introduced	4	1
Rosa eglanteria	Sweetbriar rose	Introduced	42	6
Rosa ferruiginea	Redleaf rose	Introduced	6	2
Rosa foliolosa	White prairie rose	Native	5	0
Rosa gallica	French rose	Introduced	24	2
Rosa gymnocarpa	Dwarf rose	Native	5	1
Rosa laevigata	Cherokee rose	Introduced	10	0
Rosa majalis	Double cinnamon rose	Introduced	6	0
Rosa manca	Mancos rose	Native	3	0
Rosa micrantha	Smallflower sweetbrier	Introduced	22	4
Rosa multiflora	Multiflora rose	Introduced	40	5
Rosa nitida	Shining rose	Native	8	6
Rosa nutkana	Nootka rose	Native	11	1
Rosa ×odorata	Tea rose	Introduced	4	1
Rosa palustris	Swamp rose	Native	31	4
Rosa pisocarpa	Cluster rose	Native	4	1
Rosa rugosa	Rugosa rose	Introduced	21	6
Rosa setigera	Climbing rose	Native	32	1
Rosa spinosissima	Scotch rose	Introduced	16	5
Rosa spithamea	Ground rose	Native	2	0
Rosa stellata	Desert rose	Native	3	0
Rosa tomentosa	Whitewoolly rose	Introduced	5	1
Rosa villosa	Apple rose	Introduced	2	0
Rosa virginiana	Virginia rose	Native	20	6
Rosa wichuraiana	Memorial rose	Introduced	17	0
Rosa woodsii	Woods' rose	Native	21	8
Rosa yainacensis	Cascade rose	Native	2	0

Rosa acicularis

Rosa arkansana

Rosa blanda

Rosa canina

Rosa carolina

Rosa eglanteria

Rosa gallica

Rosa micrantha

Rosa multiflora

Rosa palustris

Rosa rugosa

Rosa setigera

Rosa woodsii

Figure 3: Distribution maps of the most widely spread Rosa species across the USA (including territories and commonwealth) and Canada. Source: USDA plant database www.plants.usda.gov 2008

Native ***Introduced***

Table 2 shows the distribution of *Rosa* species in California. Species are found in many general locations but also in forests, shrublands, woodlands, disturbed open sites, canyons and in dry or moist locations.

Table 2: Distribution of naturalized Rosa species in California. Taken from: Hickman, (1993) and http://www.calflora.org/species/sci-R.html.

Species	Distribution
R. californica	Yellow Pine Forest, Foothill Woodland, Chaparral, Valley Grassland, many plant communities, wetland-riparian. equally likely to occur in wetlands or non wetlands
R. canina	General, dry open areas
R. eglanteria	General, dry often disturbed open sites
R. gymnocarpa	General in forests, shrublands Yellow Pine Forest, Foothill Woodland, Chaparral, Valley Grassland, many plant communities usually occurs in non wetlands, but occasionally found on wetlands
R. minutifolia	Rare. It is listed by the state of California as endangered
R. multiflora	Weed, species characteristic of disturbed places occurs in wetlands in another region, but occurs almost always under natural conditions in non wetlands in California
R. nutkana	General moist flats Redwood Forest, Mixed Evergreen Forest, wetland-riparian equally likely to occur in wetlands or non wetlands
R.pinetorum	Pine woodlands, canyons, general Yellow Pine Forest, Red Fir Forest, limited to California alone
R. pisocarpa	General, moist or shady areas Yellow Pine Forest, slopes, usually occurs in non wetlands, but occasionally found on wetlands
R.spithamea	Open forest, chaparral especially after fire, general Mixed Evergreen Forest, Douglas-Fir Forest, Yellow Pine Forest, Chaparral
R. woodsii	General moist areas Yellow Pine Forest, Red Fir Forest, Lodgepole Forest, Subalpine Forest, Pinyon-Juniper Woodland, Joshua Tree Woodland, wetland-riparian equally likely to occur in wetlands or non wetlands

C. Pollination of *Rosa*

Interspecific hybridization

The concerted efforts of breeders over the years have resulted in successful interspecific hybridization between different *Rosa* species and between species and more complex hybrids (Figure 1). Comprehensive studies have shown that hybridizations can lead to fertile hybrids where parents have the same or different ploidy level (Blackhurst, 1948; Gustafsson, 1942; Ratsek *et al.*, 1941). The modern cultivars of *Rosa* X *hybrida* tend to be triploid or tetraploid (Rout *et al.* 1999), so crossing among them may not generate viable seed.

It is now clear that many earlier designated taxonomic *Rosa* species are in fact hybrids (Melville, 1975). One section of the *Rosa* genus is particularly prone to natural hybridization (Klastersky, 1968; Melville, 1975). This is section Caninae, which includes the two fully naturalized species occurring in Australia, *R. canina* and *R. rubiginosa*. Species in section Caninae are predominantly pentaploid (2n = 35) and aneuploid roses are also most commonly found in this section (Rowley, 1960).

Ploidy levels in *Rosa* spp significant to the USA is summarised in Table 3 below.

Table 3: Ploidy levels of significant Rosa species found in the USA (Neumeyer, 2003; Graham and Primavesi, 1993)

Species	Ploidy
Rosa X *hybrida*	Triploid / Tetraploid
Rosa acicularis	Diploid / Tetraploid / Hexaploid / Oktoploid
Rosa arkansana	Tetraploid
Rosa blanda	Diploid
Rosa canina	Pentaploid / Hexaploid
Rosa carolina	Tetraploid
Rosa eglanteria	Pentaploid / Hexaploid
Rosa gallica	Tetraploid
Rosa micrantha	Pentaploid / Hexaploid
Rosa multiflora	Diploid
Rosa palustris	Diploid
Rosa rugosa	Diploid
Rosa setigera	Diploid
Rosa woodsii	Diploid

Conventional breeding programmes of the rose focus on improvements of various characteristics to enhance ornamental value including color, size, form and keeping quality of the bloom and plant response to the environment. Rose breeding is mainly carried out by amateurs or commercially by highly competitive companies, thus the genetic knowledge is often proprietary and unpublished (Gudin 2001).

Pollination of *Rosa*

Wild *Rosa* species are insect pollinated (the pollen is too large and heavy to be dispersed by wind) and are capable of cross- and self- pollination. Examples of insects capable of pollinating *Rosa* species are given in Table 4. From this and anecdotal evidence it would appear there are numerous potential pollinating insects of *Rosa* plants in the North American fauna. The common pollinators of *Rosa multiflora* in the US were Syrphidae (hover flies) and *A. mellifera*, followed by other species of flies, *Bombus* spp., solitary bees, and Coleoptera (Jesse *et al.*, 2006).

Table 4: Examples of insects pollinating Rosa species

***Rosa* species/citation**	**Pollinating insects/location**
Rosa spp. (Fussell and Corbet, 1992)	Bumble bees (*Bombus* and *Psithyrus*). United Kingdom.
Rosa multiflora (Jesse *et al.*, 2006)	Syrphid flies, *A. mellifera*, and *Bombus* spp. Iowa USA.
Rosa setigera (Kevan *et al.*, 1990)	*Apis mellifera* and other bees; *Eristalis tenax* and other flies. Central North America.
Rosa canina, R. pimpinellifolia, R. rubiginosa, R. centifolia (Knuth, 1908)	Hoverflies, bumblebees, honey bees, wasps. Europe.

Unlike species roses such as *R. canina* and *R. multiflora* insect pollination of varieties such as hybrid tea roses is severely constrained in glasshouse conditions, and particularly where cut flower production takes place. This is because flowers are picked for sale well before petals are open (the so called 'tight bud' stage), and at this point the reproductive organs are inaccessible to insects. There are also physical constraints to insect movement into glasshouses (Hoekstra, 1989).

Fertility of Hybrid Rose

Unlike species roses, hybrid tea roses do not necessarily produce significant amounts of viable pollen and they may be infertile. A number of factors influence the viability of hybrid tea rose pollen in storage, including humidity and temperature (Gudin *et al.*, 1991; Pearson and Harney, 1984; Visser *et al.*, 1977). In general however pollen collected for breeding purposes remains viable for many weeks.

Though some hybrid tea varieties do not produce hips many do and though the amount of seed produced per plant is significantly lower than seed produced in wild roses (such as *Rosa rubiginosa*), seed formation does occur. Rose seed normally requires a significant cold period in order to germinate. Hips of wild rose species are much smaller and softer than those of hybrid tea varieties which may be extremely thick, and less palatable. Wild rose hips are distributed by animals, such as foxes (Saunders *et al.*, 2004; Coman, 1973) and birds (Banasiak and Meiners, 2008).

Seed set

Seeds (achenes) are hairy and produced in a fleshy pericarp called a rose hip. Mature rose hips are usually red, but a few (eg. *R. pimpinellifolia*) have dark purple to black hips. Hips contain from 5 to 25 seeds. Some rose hips (*R. canina and R. rugosa*) are very rich in vitamin C (OGTR, 2005).

Even if a successful cross-pollination of the cultivated rose did occur, seed set would be impossible during commercial production of cut-flowers. This is because flowers are harvested from rose before they are fully open and the process of hip formation takes 2 months and 3 months for the hip to reach maturity. Unsold flowers are removed from stock plants by the grower to maintain plant quality. Cut-flowers of rose, even if treated for increased vase-life cannot be kept for longer than 2 to 3 weeks. Separation from the plant would also deprive any developing embryos of essential nutrients, preventing maturation.

Rose does not spread vegetatively

Cultivated rose is vegetatively propagated by cuttings or grafting but the species does not spread vegetatively, i.e. the plant does not exhibit production of organs such as stolons, rhizomes, root-borne shoots, tubers, bulbs, corms or runners. Cuttings have to be struck in optimized conditions, and roots will not form on discarded materials, i.e. cut-flowers or old plants disposed of by growers or florists. The lack of any specialized vegetative propagules also means refuse is not an avenue for dispersal of rose.

D. Weediness of *Rosa*

Rosa X *hybrida* is not a weed, nor a federal noxious weed. Indeed there are no *Rosa* species listed as federal noxious weeds, however two *Rosa* species are listed as noxious weeds in some states. These species are *Rosa multiflora, and Rosa rugosa,* more information can be found at http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/index.shtml). *Rosa arkansana* is listed as a non-noxious weed of Nebraska and the Great Plains.

Despite decades of cultivation, and plantings in parks and gardens, *Rosa* X *hybrida* has not become a weed, anywhere in the world. Each year over 25 billion flowers are produced for the world's flower markets.

The cultivated rose has no capacity to escape from cultivation as the crop possesses no vegetative propagation mechanisms and there are no opportunities for seed-set.

Rosa multiflora and *Rosa rugosa* are classed as 'widespread' because of the number of states they are found in. *Rosa multiflora* is found in 40 states however within each state it is only 'widespread' (found in more than half of the counties) in one-quarter of these states; Arkansas, Connecticut, Ilinois, Louisiana, Massachusetts, New Jersery, Oklahoma, Pennsylvania, Rhode Island and Virginia. Similarly *Rosa rugosa* is found in 21 states however it is widespread in 3 states Connecticut, Massachusetts and Rhode Island (USDA, 2008).

E. Potential Modes of Gene Flow in *Rosa*

There are three theoretical mechanisms for gene dispersal from a cultivated rose plant:

1. Vegetative spread, leading to the formation of wild 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 rose.

The paragraphs below briefly outline why the probability of gene dispersal by any of these routes is unlikely.

1. Vegetative spread. Rose does not spread vegetatively, i.e. the plant does not produce organs such as stolons, rhizomes, root-borne shoots, tubers, bulbs, corms or runners. Roots will not form on discarded or old cut-flowers. Rose is not found growing wild, even in the immediate vicinity of rose growing farms where waste material has been discarded or has been left for composting.

2. Formation of seed. As flowers are removed from plants being grown for cut flower production, dispersal by seeds from cultivated rose plants is extremely unlikely. For gene dispersal by seed formation to occur from a cut rose flower, the following events would all need to occur successfully; arrival of viable pollen on the stigma of the rose, pollen germination, pollen tube growth to the ovule of the rose, fertilization, seed formation and seed dispersal. Notwithstanding the fact that successful pollination of a rose flower in a vase is highly unlikely, it would be even more unlikely for seed set to occur. This is because the process of seed development takes at least 2 months on the plant – where the growth of any developing embryo could be sustained. A cut-flower will remain in a consumers hand for three to four weeks at most before dying.

3. Pollen dispersal leading to a successful hybridisation event. The transgenic lines are genetic chimeras, and the pollen is non-transgenic. There is therefore no possibility of the dispersal of the transgenes by pollen-mediated gene flow.

III. Flower Color

A. Flower color

Flower color is one of the most important factors in the selection of cut-flower plant varieties and in the consumer's choice of flowers. Whilst there is a huge range of shades of color, some colors are not available for all flower crops. The most obvious "gap" in the selection choice is the range from mauve to violet to blue. These colors do not exist in the most widely grown cut-flowers; rose, carnation, chrysanthemum and gerbera. The absence of these colors is due to the absence of the biochemical pathway for production of specific colored compounds, delphinidin glucosides.

Lines IFD-524Ø1-4 and IFD-529Ø1-9 have been genetically modified to enable production of delphinidin. The color of the flowers from the transgenic plants is mauve. Photographs of the flowers can be found in section VII.

The anthocyanin biosynthesis pathway

Figure 4 overleaf shows a simplified version of the anthocyanin biosynthesis pathway. In roses 5,3GT (UDP-glucose: anthocyanidin 3-O-glucosyltransferase) catalyses 5,3 glucosylation instead of the 3GT enzyme shown in this pathway.

The genes responsible for the modified trait are the flavonoid 3'5'-hydroxylase (*F3'5'H*) gene from *Viola sp.* and the anthocyanin 5-acyltransferase (*5AT*) gene from *Torenia*. The flowers produce novel, delphinidin-based anthocyanins. A gene for kanamycin resistance (*npt*II) has been included in the transformation vector only for the selection of plants *in vitro*.

Flower color is generally the result of the relative concentration and type of two pigment classes - carotenoids and flavonoids. The key enzyme at the start of the flavonoid pathway is chalcone synthase, from which the dihydroflavonol dihydrokaempferol is produced after enzymatic reactions catalysed by chalcone isomerase (CHI) and flavanone3β-hydroxylase (F3H) (Figure 4). The dihydroflavonols are substrates for the biosynthesis of the colored anthocyanin pigments, which are produced in three steps; firstly a reduction, catalysed by the enzyme dihydroflavonol 4-reductase (DFR); secondly the production of colorless anthocyanidin through the action of the enzyme anthocyanidin synthase (ANS) and thirdly addition of a glucose residue to produce a colored anthocyanin molecule. Anthocyanins can be further modified in many ways, by addition of glucose, acyl and methyl molecules.

Anthocyanins are flavonoid based colored pigments. There are three groups of glycosylated anthocyanins. 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.

Non-genetically modified roses lack the part of the anthocyanin biosynthetic pathway that is responsible for the production of delphinidin (in petal tissue), as they lack a gene encoding the enzyme flavonoid 3' 5' hydroxylase (F3'5'H) that converts dihydrokaempferol (DHK) to dihydroquercetin (DHQ) and then to dihydromyricetin (DHM) (refer to Figure 4).

Delphinidin 3, 5-diglucoside is thus produced as a result of the expression of the introduced gene *F3'5'H* together with endogenous genes in the anthocyanin biosynthetic pathway. 5AT catalyses the transfer of coumaroyl or caffeoyl moieties to 5-glucose of anthocyanidin 3, 5-diglucosides. In the case of the GMO, 5AT modifies the delphinidin (delphinidin production is enabled by *F3'5'H*) generating delphinidin 3-glucoside-5-caffeoylglucoside (Katsumoto *et al.*, 2007). The production of delphinidin 3, 5-diglucoside and its acylated derivative, results in a change in flower color, from pink to the color shown in Section VII.

We demonstrated that the rose can synthesize aromatically acylated anthocyanins by the expression of torenia 5AT.

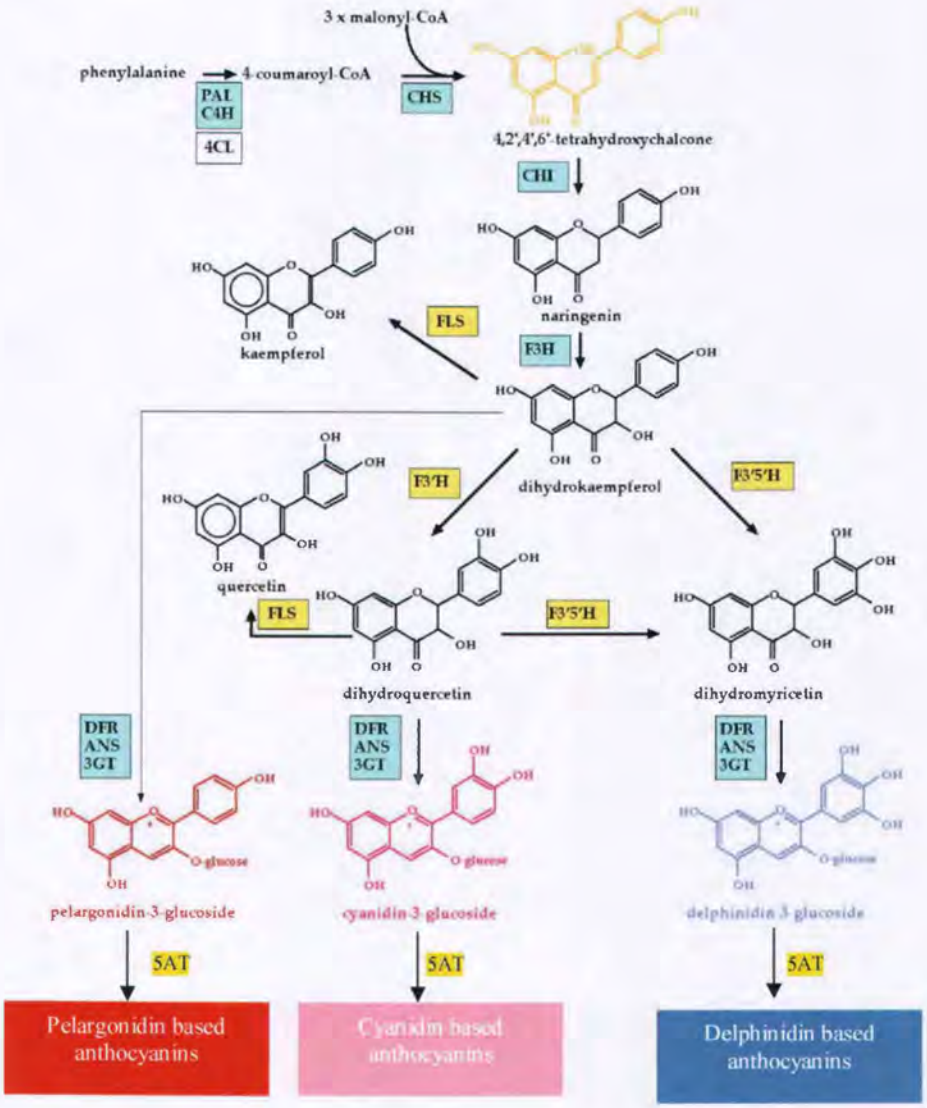


Figure 4. The anthocyanin biosynthesis pathway. *Abbreviations include; CHS – chalcone synthase; CHI – chalcone isomerase; F3H – flavanone 3β-hydroxylase; F3'H – flavonoid 3'-hydroxylase; F3'5'H – flavonoid 3', 5'-hydroxylase; DFR – dihydroflavonol 4-reductase;ANS – anthocyanidin synthase; FLS – flavonol synthase; 3GT – UDP-glucose: anthocyanidin 3-O-glucosyltransferase; PAL - phenylalanine ammonia-lyase; 4CL - 4-coumarate:CoA ligase; C4H - cinnamate 4-hydroxylase: 5AT –anthocyanin 5-acyltransferase.*

B. Genetic modification for flower color

The genetic modification carried out for IFD-524Ø1-4 and IFD-529Ø1-9 used transformation vector pSBP130. This transformation vector contains the *F3'5'H* gene and a torenia gene for anthocyanin 5- acyltransferase.

In the genetically modified rose lines delphinidin-derivatives are produced, while the parent line produces cyanidin-derivatives only (Table 5). The production of the pigment delphinidin is unique to the transgenic rose lines. The pigment is not found in the parent line, whilst all three lines produce cyanidin-derivatives (Table 5).

Table 5: HPLC data showing amounts (mg/g fresh petal) and percentages of anthocyanidins

Variety	Anthocyanidin (mg/g fresh weight petal)		Delphinidin (%)
	Delphinidin	Cyanidin	
WKS82	0	0.0726	0
WKS82/130-4-1	0.0558	0.0059	90
WKS82/130-9-1	0.0960	0.0091	91

It is expected that the transgenic rose lines predominantly accumulate delphinidin in the form of delphinidin 3-glucoside-5-caffeoylglucoside ((Katsumoto *et al.*, 2007).

The genetic modification results in the biosynthesis of anthocyanin in the petals of the flower only. This is because the anthocyanin biosynthesis pathway is controlled in an organ specific way in plants, and the substrates on the pathway (required for delphinidin biosynthesis in the transgenic plants) are only produced in the flower.

IV. Description of Transformation System

The T-DNA within pSPB130 was transferred to rose by co-cultivation of leaf-derived callus with disarmed *Agrobacterium tumefaciens* strain AGL0. Co-cultivated explants were cultured on regeneration medium containing kanamycin (50 mg/L) as a selection agent over an eight week period. The antibiotic carbenicillin (200 mg/L) was included in all media to kill *Agrobacterium* after the transformation process.

V. Donor Genes

A. Transformation vector pSPB130

The transgenic roses IFD-524Ø1-4 and IFD-529Ø1-9 have been genetically engineered with the transformation vector pSPB130. The map of the transformation vector is shown in Figure 5.

The transformation vector was manufactured by Suntory Limited, Research centre, 1-1-1 Wakayamadai, Shimamoto-cho, Misima-gun, Osaka, Japan. The details of the elements used to make the vectors are summarised in Table 7.

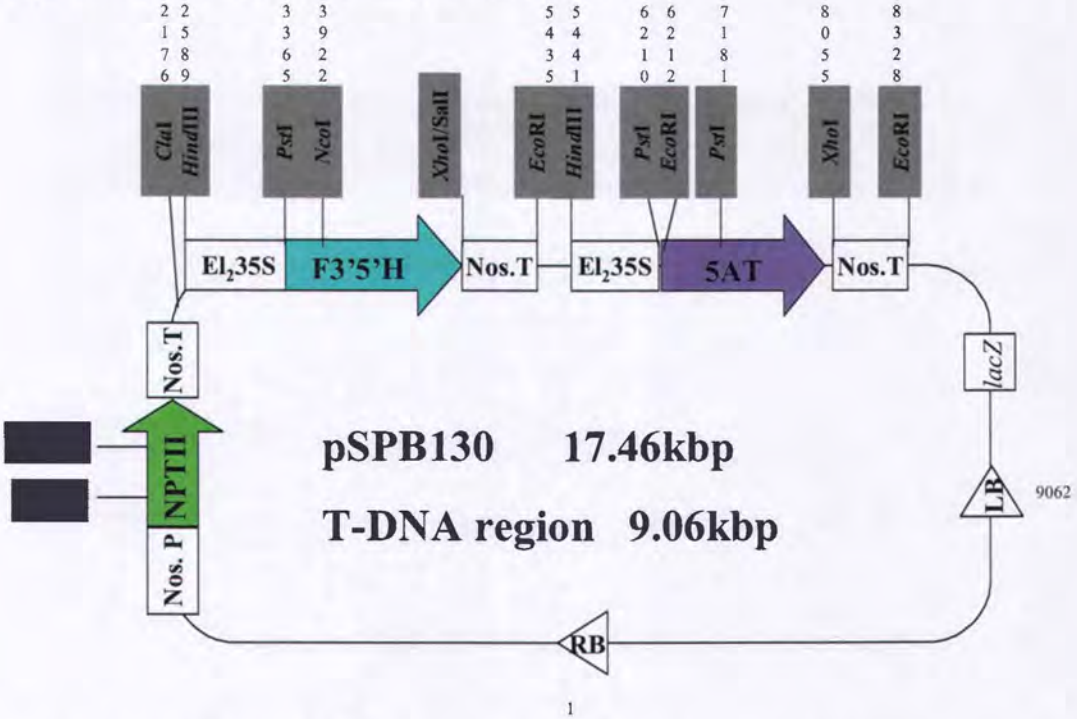


Figure 5: Map of pSPB130, showing T-DNA insert and key restriction enzyme cut sites.
LB: Left Border, El$_2$ 35S: 35S promoter, F3'5'H: Black Pansy flavonoid 3' 5' hydroxylase gene, T5AT: Torenia anthocyanin 5-acyltransferase, nos: terminator region of nopaline synthase, NPTII: Neomycin phosphotransferase, RB: Right Border.

Table 6: Origin of DNA within the borders of pSPB130

Designation on vector map	Genes and/or regulatory elements	Source organisms species name [common name]	Size of fragment (kb)
Nos. P	Promoter region of nopaline synthase	*Agrobacterium tumefaciens*	0.3
NPTII	Neomycin phosphotransferase (conferring resistance to Kanamycin)	*Escherichia coli*	1.0
Nos. T	Terminator region of nopaline synthase	*Agrobacterium tumefaciens*	0.3
$E1_2$ 35S (CaMV35S)	Enhanced cauliflower mosaic virus 35S promoter	Cauliflower mosaic virus (CaMV)	0.8
F3'5'H	Flavonoid 3', 5'-hydroxylase (referred to throughout the text as also as "Blue Gene" or "F3'5'H")	*Viola tricolor* [Black pansy]	1.8
Nos. T	Terminator region of nopaline synthase	*Agrobacterium tumefaciens*	0.3
$E1_2$35S (CaMV35S)	Enhanced cauliflower mosaic virus 35S promoter	Cauliflower mosaic virus (CaMV)	0.8
5AT	Anthocyanin 5-acyltransferase	*Torenia hybrida* [Torenia]	1.8
Nos. T	Terminator region of nopaline synthase	*Agrobacterium tumefaciens*	0.3
LB	Defines junction between T-DNA and plant genomic or vector DNA	*Agrobacterium tumefaciens*	0.15
RB	Defines junction between T-DNA and plant genomic or vector DNA	*Agrobacterium tumefaciens*	0.13

B. Function of the DNA contained in pSPB130

CaMV35S promoter

A constitutive promoter used to provide high levels of gene expression.

nptII conferring resistance to kanamycin

The *nptII* gene is the most commonly used antibiotic resistance marker gene for the production of genetically modified plants (Goldstein *et al.*, 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 gene functions as a selectable marker during the initial laboratory stages of development of the genetically modified rose.

Flavonoid 3' 5' hydroxylase cDNA

The enzyme flavonoid 3' 5' hydroxylase acts by converting the dihydroflavonols dihydrokaempferol (DHK) and/or dihydroquercetin (DHQ) into the dihydroflavonol dihydromyricetin (DHM) (Figure 4).

The cDNA for flavonoid 3' 5'-hydroxylase encodes the enzyme flavonoid 3' 5' hydroxylase allowing transgenic plants normally lacking this enzyme to produce violet or blue delphinidin derived pigments. The presence of this gene, a cytochrome P-450 (Holton *et al.*, 1993) is almost always essential for the production of blue to purple anthocyanins (Suzuki *et al.*, 2000).

5AT (anthocyanin 5-acyltransferase gene)

This gene encodes the anthocyanin 5-acyltransferase (5AT) enzyme. The function of this gene is acylation of anthocyanins. Acylation takes place on the glycosyl moieties of anthocyanidin 3-glucoside. Acylation makes anthocyanins more stable, more soluble in water (for uptake into the vacuole) and bluer (Nakayama *et al.*, 2003).

VI Genetic Analysis

A. Southern Blot Analysis

Experimental rationale and methods

Southern data for the transgenic line is shown in Table 7 and in Figures 6 – 10. Standard molecular analysis techniques were used to for the analysis.
DNA was extracted from petal tissue of WKS82 (hereinafter "the host") and IFD-524Ø1-4 and IFD-529Ø1-9 (hereinafter "the transgenics") using a DNeasy Plant Maxi kit (QIAGEN). 20μg of DNA was digested with restriction endonuclease *Eco*RI or *Pst*I (depending on probe – please refer to figure legends) and then electrophoresed through a 0.8% agarose gel. Following denaturing and neutralizing steps, DNA was transferred to a nylon membrane using 20 x SSC and the upward capillary transfer method. The membrane was hybridized at 42 °C for 16 hours with digoxigenin-labelled probes (either *F3'5'H*, *5AT*, *npt*II, LB or RB). The nylon membrane was then washed for 5 minutes twice at room temperature in 2 x SSC containing 1% (w/v) SDS and washed for 30 minutes twice at 65°C in 0.2 x SSC containing 1% (w/v) SDS. Hybridized signals were detected according to the method that was recommended by the manufacturer of the kit.

Several different methods are reported in the literature to estimate gene copy number and gene copy equivalents of T-DNA integrations in transgenic plants (Chee *et al.*, 1991; Croy, 1993). Here we have assumed our vector-derived DNA integrations are relatively simple based on the digestion pattern of the vector.

The calculation we have used for copy number is:
(Ref: http://www.med.umich.edu/tamc/spike.html)

Assumption 1: The tetraploid content of the rose genome is 500 x 10^6 bp
Assumption 2: There is 20 μg of genomic DNA per analysis

Therefore for pSPB130:
Mass of transgene =(17460 bp x20 μg)/(500 x 106 bp genomic DNA)
=0.698
Considering tetraploid nature =0.698/4
= 0.1745 ng
=174.5 pg

Thus, for the line IFD-524Ø1-4 and IFD-529Ø1-9, 174.5 pg of transformation plasmid pSPB130 is equivalent to a single copy.

Results

Results obtained following hybridization of the Southern blots with each of the five probes are shown in Figures 6 – 10 and are summarised in Table 7, 8 and 9.

Southern analysis showed hybridization with single or multiple bands for each probe (Table 7), showing genes contained within the T-DNA from the transformation vector pSPB130 are present in the transgenics. Each probe hybridized with appropriate bands in the *EcoR*I-digested or *Pst*I-digested pSPB130 plasmid DNA alone.

Table 7: Number of hybridizing bands found via Southern analysis of IFD-524Ø1-4 IFD-529Ø1-9 using various T-DNA probes

Probe	IFD-524Ø1-4	IFD-529Ø1-9	Figure
F3'5'H	1	1	6
5AT	2	1	7
npt II	1	3	8
LB	1	5	9
RB	2	2	10

Table 8 summarises the genetic fragments from *EcoR*I digested or *Pst*I digested pSPB130 DNA to which the probes hybridized. Figure 5 provides location of the *EcoR*I and *Pst*I sites in the transformation vector.

Table 8: Sizes of expected hybridizing bands with probes used on EcoRI-digested or PstI-digested pSPB130 plasmid DNA. LB: Left Border, CaMV35S: 35S promoter, nptII: Kanamycin resistance gene, nos t: nos terminator, F3'5'H: Pansy flavonoid 3'5' hydroxylase gene, nos p: nos promoter, 5AT: Torenia anthocyanin 5-acyltransferase gene, RB: Right Border

Probe	Approx. size *EcoR*I or *Pst*I fragment	Sequences contained within the fragment
*npt*II	2.7kb	*npt*II 3', nos t, CaMV35S
F3'5'H	2.8 kb	*F3'5'H*, nos t, CaMV35S
5AT	2.1 kb	CaMV35S, 5AT, nos t
LB	14.6kb	LB, RB, nos p, *npt*II, nos t, CaMV35S, *F3'5'H*, nos t
RB	14.6kb	LB, RB, nos p, *npt*II, nos t, CaMV35S, *F3'5'H*, nos t

Table 9: Hybridizing bands detected in lines IFD-524Ø1-4 and IFD-529Ø1-9

Probe	Approx. size (kb) of hybridising bands	
	IFD-524Ø1-4	IFD-529Ø1-9
*npt*II	2.7	2.7, 4.1, 10
F3'5'H	2.8	2.8
5AT	3.5, 2.1	2.1
LB	1.2	1.6, 2.0, 2.2, 5, 7
RB	3.5, 4.2	10, 12

Figure 6: Southern analysis of IFD-524Ø1-4 and IFD-529Ø1-9 probed with F3'5'H. 20µg of DNA digested with PstI was loaded onto the gel. Dilution series of PstI-digested pSPB130 plasmid DNA representing 0.15, 0.30, 0.74 and 1.5 gene copy equivalents for the transgenic rose lines IFD-524Ø1-4 and IFD-529Ø1-9 is also shown.

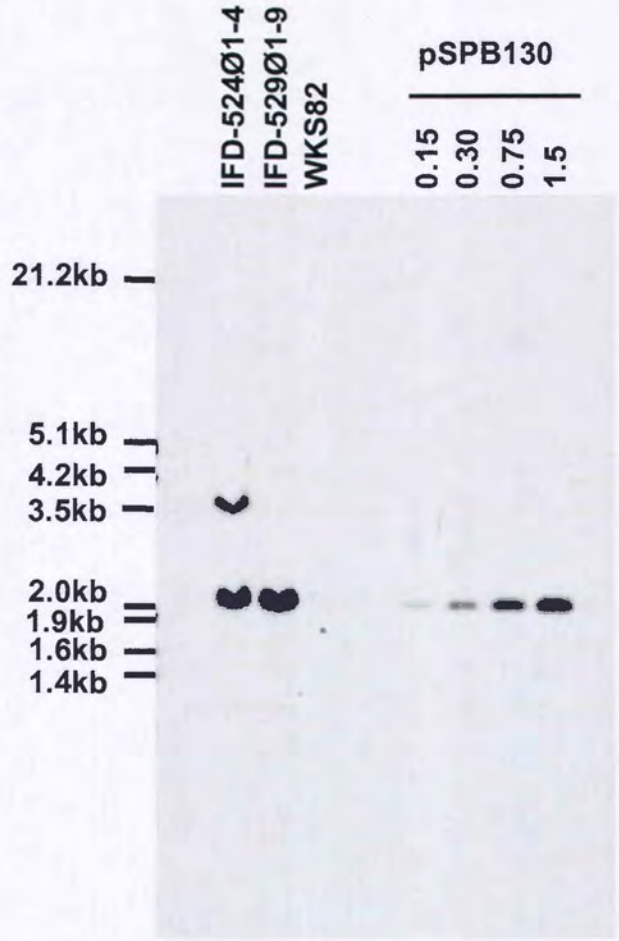


Figure 7: Southern analysis of IFD-524Ø1-4 and IFD-529Ø1-9 probed with 5AT. 20µg of DNA digested with EcoRI was loaded onto the gel. Dilution series of EcoRI-digested pSPB130 plasmid DNA representing 0.15, 0.30, 0.74 and 1.5 gene copy equivalents for the transgenic rose lines IFD-524Ø1-4 and IFD-529Ø1-9 is also shown.

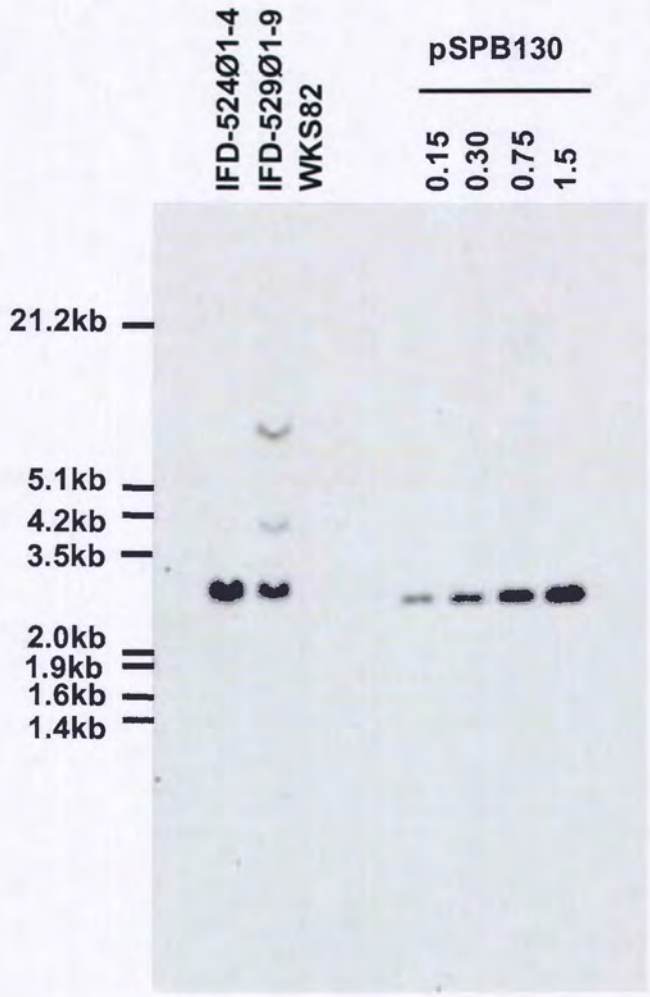


Figure 8: Southern analysis of IFD-524Ø1-4 and IFD-529Ø1-9 probed with nptII. 20µg of DNA digested with PstI was loaded onto the gel. Dilution series of PstI-digested pSPB130 plasmid DNA representing 0.15, 0.30, 0.74 and 1.5 gene copy equivalents for the transgenic rose lines IFD-524Ø1-4 and IFD-529Ø1-9 is also shown.

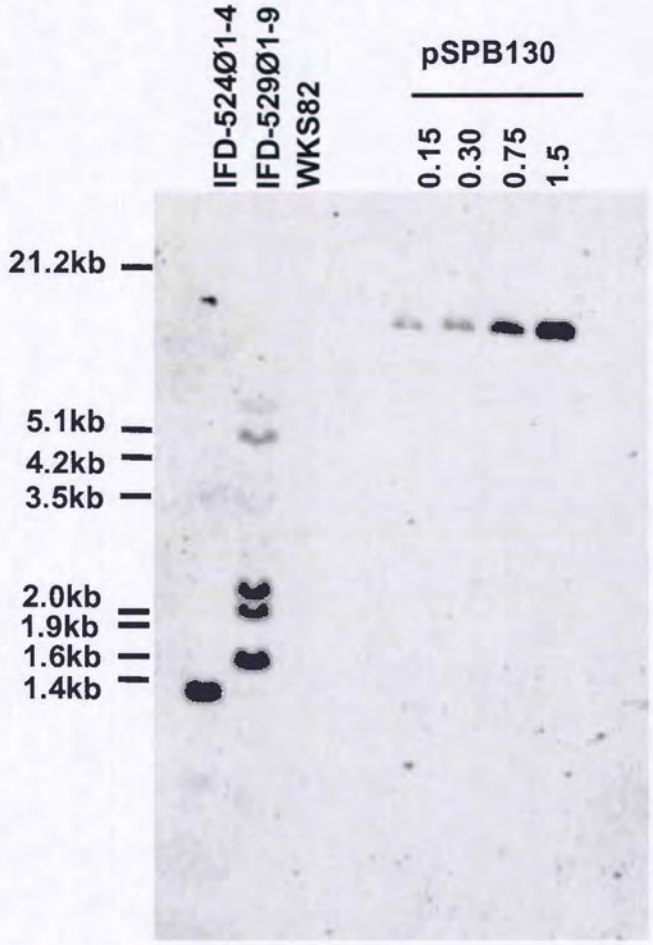


Figure 9: Southern analysis of IFD-524Ø1-4 and IFD-529Ø1-9 probed with LB. 20µg of DNA digested with EcoRI was loaded onto the gel. Dilution series of EcoRI-digested pSPB130 plasmid DNA representing 0.15, 0.30, 0.74 and 1.5 gene copy equivalents for the transgenic rose lines IFD-524Ø1-4 and IFD-529Ø1-9 is also shown.

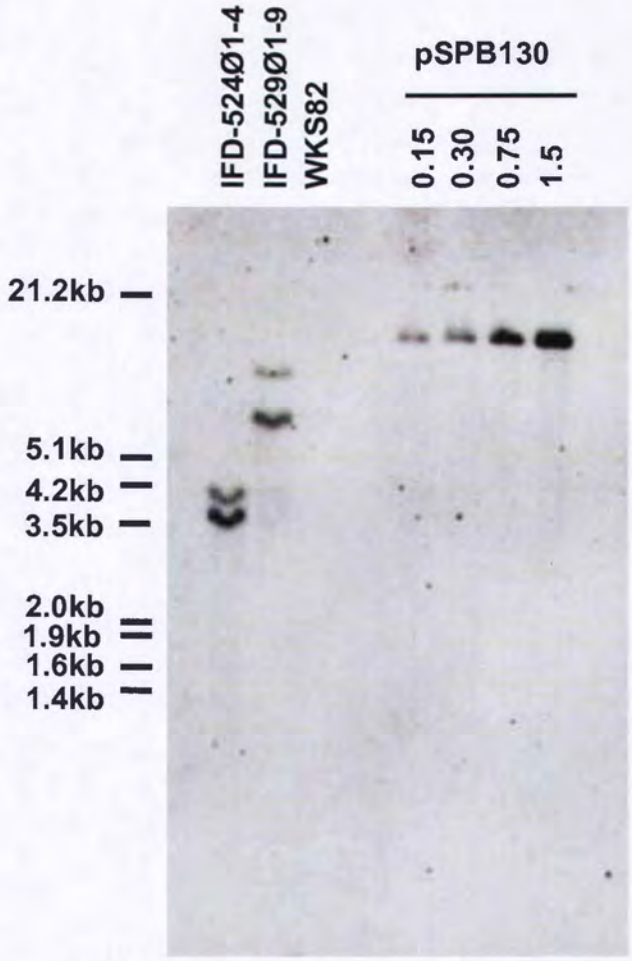


Figure 10: Southern analysis of IFD-524Ø1-4 and IFD-529Ø1-9 probed with RB. 20µg of DNA digested with EcoRI was loaded onto the gel. Dilution series of EcoRI-digested pSPB130 plasmid DNA representing 0.15, 0.30, 0.74 and 1.5 gene copy equivalents for the transgenic rose lines IFD-524Ø1-4 and IFD-529Ø1-9 is also shown.

B. Mendelian Inheritance

Crossing and *in situ* experiments, described below, have shown that the transgenic lines are L1 periclinal chimeras (the L1 layer is transgenic) and accordingly the pollen is non-transgenic (pollen is derived from the L2 layer; Howell, 1998). The L1 layer derived tissue does not give rise to generative cells this explains why the progeny from crosses using pollen from the transgenic rose line resulted in non-transgenic seed.

Crossing experiments

Crossing experiments were carried out in the greenhouse to avoid insect-pollinations. Pollen from the host or the transgenic was transferred to the female when the pistils were fully mature, after which pistils were covered with bags to avoid secondary fertilization. Fresh pollen that was collected from anthers and left at room temperature for a day in a desiccator with silica gel before it was used. Figure 11 illustrates the various steps in the pollination and hip formation process.

The grandiflora variety rose "Queen Elizabeth", the floribunda variety "Gold Bunny" and the wild rose *Rosa multiflora* were used as female parents in these experiments.

Seed was collected from hips and analysed by PCR for the presence of transgenes.
Genomic DNA was extracted from collected seeds using Nucleon Phytopure kit (Amersham Biosciences) for plant DNA extraction. PCR amplification of the transgene (pansy F3' 5'H gene) using the PEPLI-g Kit (QIAGEN) was attempted in order to confirm the transmissibility of progeny transgene.

Before opening Remove Anthers Pollination Bagging Seed set

Figure 11: Pollination and seed formation

Results are shown in Tables 10 and 11. It was found that the success of the crosses (measured by hip formation) was similar whether pollen was used from the transgenic line or the parent line it was derived from. This was the case whether the female was a cultivated garden rose (Table 10) or a wild rose (Table 11) though the percentage of hip formation was much lower when the female was the wild rose. 129 seeds from hips formed in crosses with the cultivated rose and 19 seeds from hips formed in crosses with the wild rose were analyzed by PCR. The introduced gene was not found in any seed, demonstrating that the introduced gene is not present in the reproductive cells of the transgenic line.

Table 10: Seed set in crosses between transgenic and garden rose

	WKS82 (male)		IFD-524Ø1-4 (male)			
	Crosses that produced hips	%	Crosses that produced hips	%	Number of seeds with introduced gene	%
Queen Elizabeth (female)	19/20	95	19/20	95	0/84	0
Golden bunny (female)	16/20	80	14/20	70	0/45	0

	WKS82 (male)		IFD-529Ø1-9 (male)			
	Crosses that produced hips	%	Crosses that produced hips	%	Number of seeds with introduced gene	%
Queen Elizabeth (female)	19/20	95	20/20	100	0/94	0
Golden Bunny (female)	16/20	80	14/20	70	0/94	0

Table 11: Seed set in crosses between transgenic and wild rose

	WKS82 (male)		IFD-524Ø1-4 (male)			
	Crosses that produced hips	%	Crosses that produced hips	%	Number of seeds with introduced gene	%
Wild rose (female)	6/152	3.9	12/159	7.5	0/19	0

	WKS82 (male)		IFD-529Ø1-9 (male)			
	Crosses that produced hips	%	Crosses that produced hips	%	Number of seeds with introduced gene	%
Wild rose (female)	6/152	3.9	9/134	6.7	0/11	0

In situ hybridization

In situ hybridization experiments confirmed IFD-524Ø1-4 and IFD-529Ø1-9 are L1 periclinal chimeras, this means that plants only carry the transgenes in the epidermal L1 layer, therefore the pollen is non-transgenic. The *in situ* hybridization experiments were carried out by the research group of Professor Masao Tasaka, the graduate school of biological sciences, Nara Institute of Science and Technology Ikoma, Nara (Japan). The methodology is described in; Aida, *et al.*, 1999. Essentially, digoxigenin-labeled RNA probes are used, which are deposited as a colored pigment at the sites of expression. Tissues are fixed before sectioning, removing any anthocyanin pigments. *In situ* images are shown in Figure 12 below.

Petal tissue from two rose lines was hybridized with a probe to flavonoid-3'5'-hydroxylase, one of the genes contained in the transformation vector. The presence of transcript, visualized by stained cells (arrows) was detected in the transgenic lines but only in the epidermal L1-derived cells of the petal.

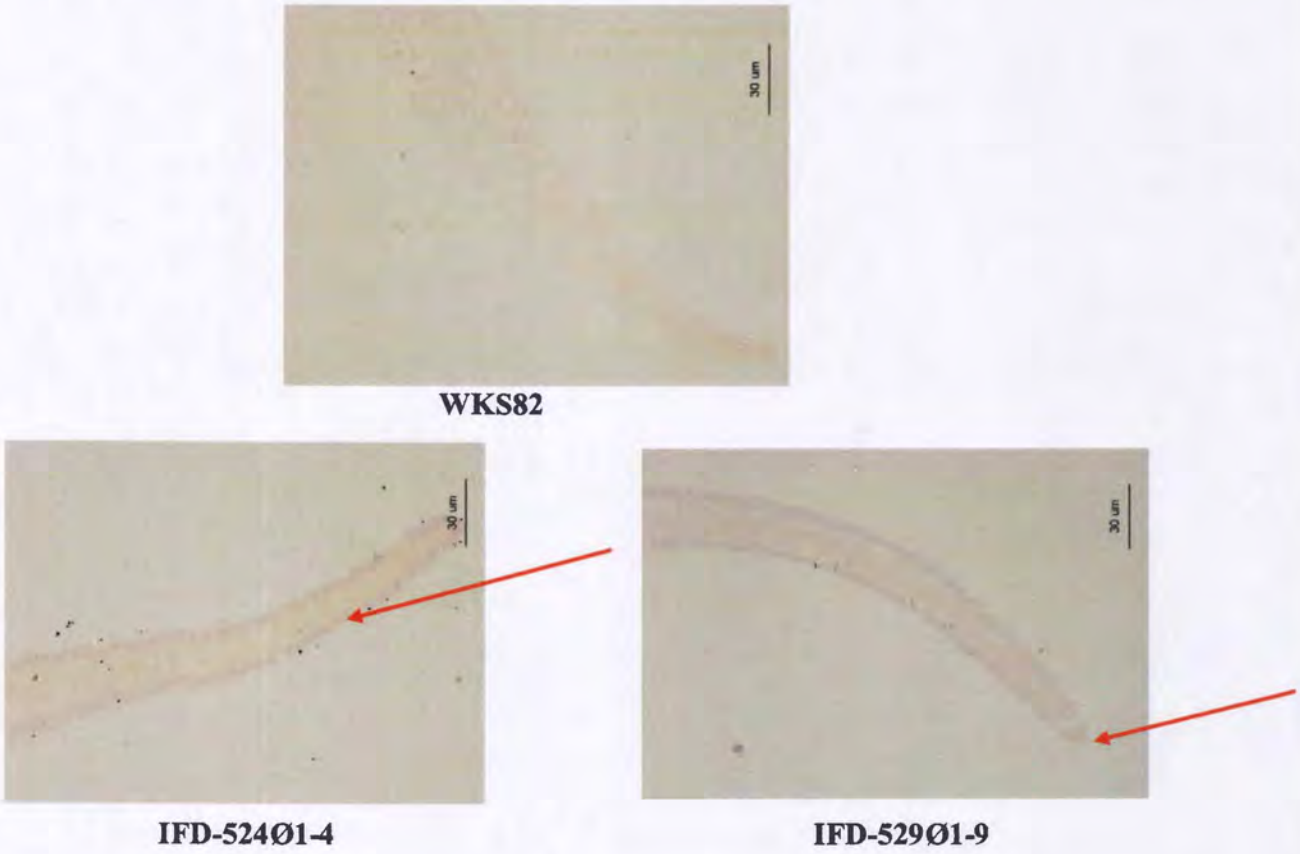


Figure 12: In situ hybridization images.

C. Expression of Inserted Genes

Introduction

All inserted genes encode proteins that are biologically active enzymes. Concentration, *Vmax* and *Km* of these proteins have not been measured. Detection of delphinidin-type anthocyanins using HPLC indicates expression of the introduced *F3'5'H* gene and enzymic activity of the enzymes encoded by this gene. Successful isolation of transgenic shoots on selection also indicates that the introduced *npt*II encoding neomycin phosphotransferase is active. Proteins were not detected by direct means such as via gel electrophoresis (they are present in minor amounts and thus not readily detectable by this means).

Northern analysis

The results of Northern analysis are shown in Figure 13. Total RNA was extracted from host and transgenic petals using an RNeasy Plant Mini Kit (Qiagen). 20μg of total RNA was electrophoresed through a 1.2% formamide-containing agarose gel, and then transferred to a nylon membrane. Following the DIG Northern Starter Kit (Roche) protocol, RNA probes of the transgenes *F3'5'H*, *5AT*, and *npt*II gene were created. The membrane was then hybridized at 68 °C for 16 hours with each probe separately. The membrane was washed for 5 minutes twice at room temperature in 2 x SSC containing 0.1% SDS and washed for 30 minutes twice at 68 °C in 0.1 x SSC containing 0.1% SDS. Hybridized signals were detected according to the method that was recommended by the manufacture of the kit.

The transcript for *F3'5'H, 5AT* and *npt*II were all detected in the transgenic, see Figure 13.

Figure 13: Northern analysis of IFD-524Ø1-4 and IFD-529Ø1-9. *20μg of total RNA for IFD-524Ø1-4, IFD-529Ø1-9 and the host WKS82. Northern was probed with F3'5'H, 5AT and nptII*

VII. Trial Analysis and Morphological Observations

A. Flower color

The phenotypic change due to the genetic modification is altered of flower color. The colors of flowers from the parent line and the two transgenic lines are shown in Figure 14, 15 and 16.

An industry standard for recording color is the Royal Horticultural Society (UK) color charts. Table 12 lists flower color using the codes provided in these charts.

Table 12: RHS color codes

Line	Main petal color
Parent	75B
IFD-524Ø1-4	84C
IFD-529Ø1-9	76A

Figure 14: Color and form of WKS82 parent

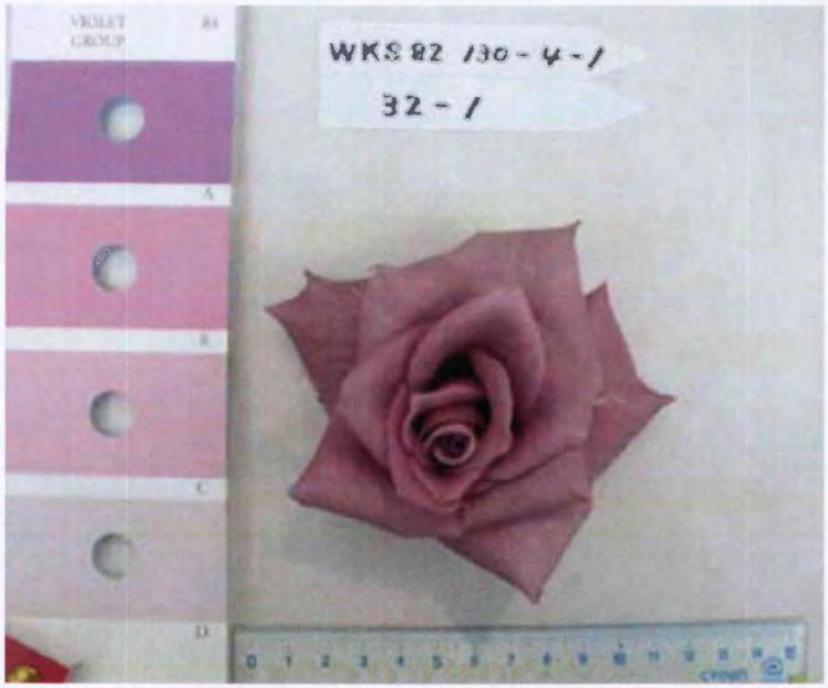


Figure 15: Color and form of IFD-524Ø1-4

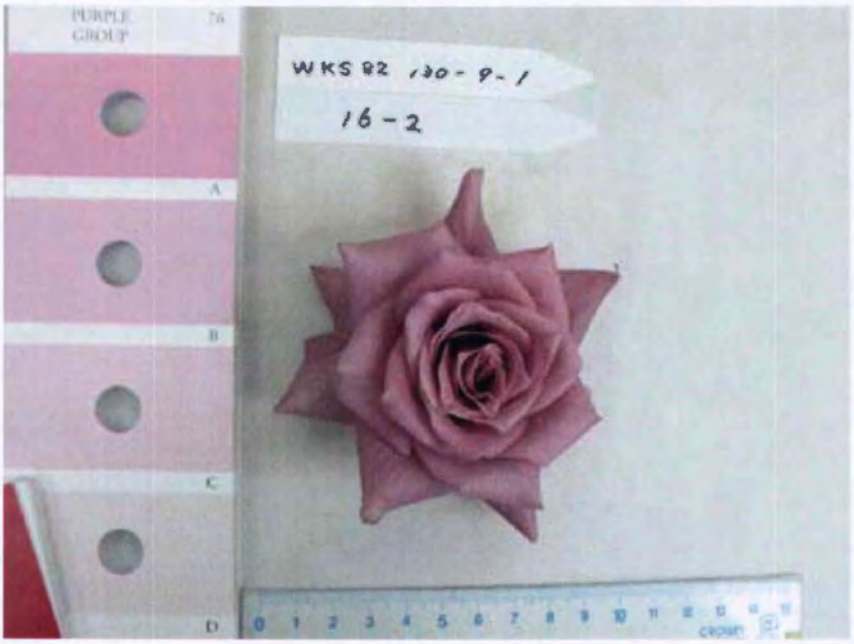


Figure 16: Color and form of IFD-529Ø1-9

B. Flower morphology

The collection of morphological and reproductive data from the plants, which began in May 2005, was continued under notification 05-318-08n in California USA. Fifteen plants of each line were used in the trial; all plants were maintained in five-gallon pots inside a glasshouse (Figure 17). Table 13 provides a summary of results. The data collected in the trials was analyzed using the student T-TEST. The following data is comparable to data collected in Australian and Japanese trials.

Figure 17. Location and appearance of established rose plants maintained during the trial 05-318-08n.

Table 13: Means of morphological data against WKS82 control (15 plants per line tested).

Measurement	WKS82	IFD-524Ø1-4	IFD-529Ø1-9
Plant height, cm, April	23.6	20.0	22.2
Plant height, cm, September	62.1	79.1	71.0
Stem length, April, cm	34.5	35.5	33.2
Stem length, June, cm	45.7	39.4	34.9
Stem length, August, cm	51.4	45.0	47.7
Flower height	46.8	42.1	50.3
Flower diameter	88.1	96.7	104.0
Petal number	23	25	24
Outer petal length, mm	44.5	49.1	44.7
Outer petal width, mm	49.8	54.3	54.5
Number of sepals	5	5	5
Leaflet length, cm	10.3	12.5	11.9
Leaflet width, cm	8.6	9.6	9.4
Number of pistils	50	46	41
Number of stamens	84	82	94
Style length, mm	5.0	5.1	4.9
Number of intact anthers	79	77	88
Anther length, mm	2.9	3.0	2.9
Anther width, mm	1	1	1

Data is color-coded; no statistical difference (brown), P=0.05 (green), P =0.01 – 0.05 (yellow) and P<0.01 (blue)

There were statistically significant differences between control and transgenic for some of the characters measured. However, in the majority of cases the difference in mean value was less than 15%, which is the approximate within group standard deviation (data not shown). The modified flower color phenotype is stable. Each plant maintained has produced 45 – 60 flowers, representing approximately 900 flowers per transgenic line. There has been no flower color variation, even though flowers have been produced in both summer and winter.

C. Pollen viability

Anthers of 10 flowers each from the host and the transgenic were chosen for testing. Pollen was observed through a stereoscopic microscope (Leica MzflIII) after being dyed with acetocarmine.

Table 14: Pollen viability

	WKS82	IFD-524Ø1-4	IFD-529Ø1-9
Pollen viability (% staining with acetocarmine)	81.3±4.9	83.3±3.8	83.9±3.9

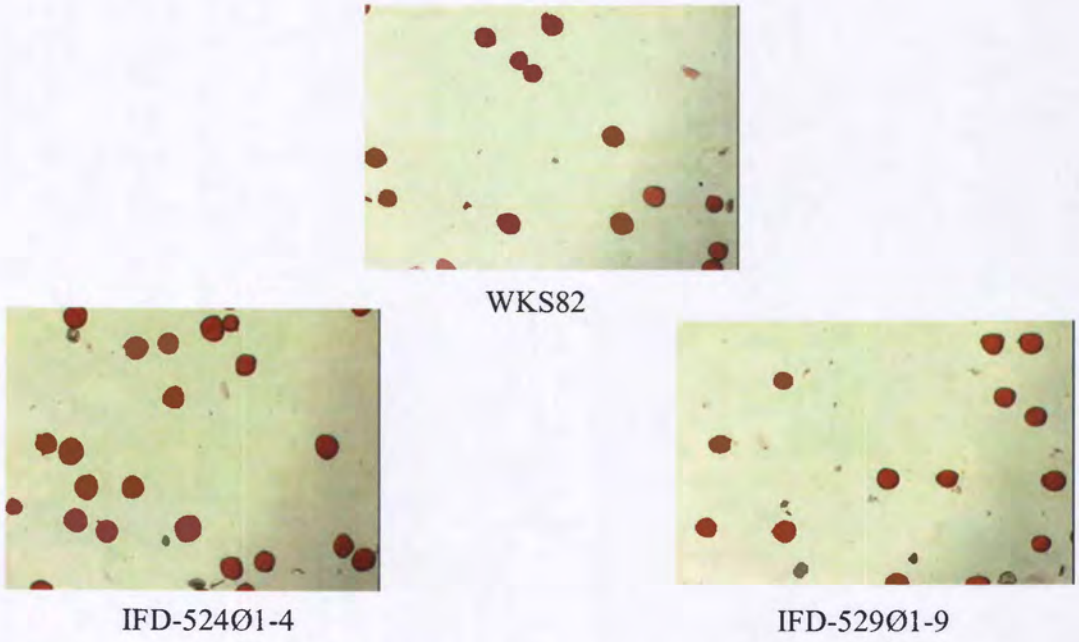


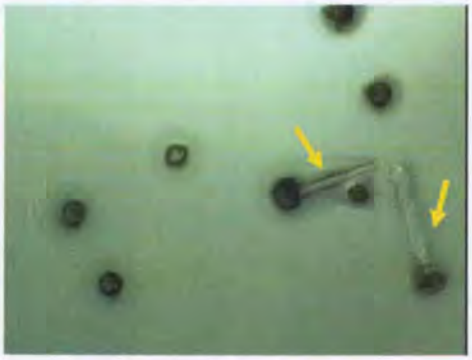
Figure 18: Pollen viability of WKS82 compared with IFD-524Ø1-4 and IFD-529Ø1-9 stained with acetocarmine. Pollen stained red is viable.

D. Pollen Germination

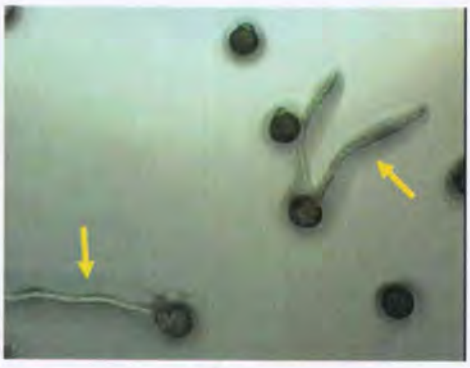
Boric acid media (sucrose concentration: 10%, boric acid concentration: 50ppm, agar concentration: 1%) was used to investigate gemination of pollen. Pollen from 10 flowers of each of the host and transgenic was used. After placing pollen in the media, they were cultivated at 25℃ for 2 hours and elongation of pollen tubes was observed through a stereoscopic microscope (LEICA MZFLIII). Pollen from both the host and the transgenic germinated and there was no significant difference in germination percentage.

Table 15: Pollen germination

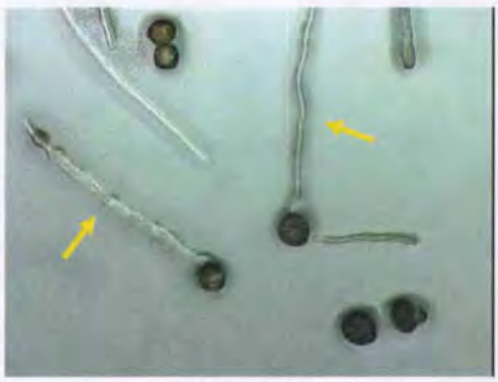
	WKS82	**IFD-524Ø1-4**	**IFD-529Ø1-9**
Pollen germination (%)	27.2±8.6	31.0±8.3	27.2±9.7



WKS82



IFD-524Ø1-4



IFD-529Ø1-9

Figure 19: Pollen germination. Arrows show pollen tubes formed.

E. Pollen Diameter

Pollen from 10 flowers each from the host and the transgenic were used. Pollen diameters were measured using a light microscope (Leica DM6000 B).
No significant difference in pollen diameter was observed between the host and the transgenic line.

Table 16: Size of pollen

	WKS82	**IFD-524Ø1-4**	**IFD-529Ø1-9**
diameter(μm)	47.3±2.0	47.8±1.1	48.2±0.7

WKS82

IFD-524Ø1-4

IFD-529Ø1-9

Figure 20: Morphology of pollen

VIII. Environmental Consequences of Introduction

There are negligible probable environmental consequences from the cultivation of rose lines IFD-524Ø1-4 and IFD-529Ø1-9. Rose has never become established in the US flora, despite years of cultivation. The biology of the rose plant precludes it from becoming feral and posing a risk of becoming a weed and there is no chance of genes from flowers of IFD-524Ø1-4 and IFD-529Ø1-9 becoming incorporated into the genome of any other plants of the North American flora via natural hybridization, due to the fact that the pollen is non-transgenic.

Details of the biology of rose and a discussion of possible routes of gene dispersal have been provided in Section II of this petition, and are summarised here;

A. Vegetative spread

Rose is vegetatively propagated for commercial production but the crop does not possess vegetative propagules such as stolons or rhizomes. Discarded material is unable to spread by vegetative means. Rose has never been found growing wild, even in the immediate vicinity of large rose growing facilities where waste material has been discarded or has been left for composting.

B. Gene dispersal by pollen dissemination

Many rose cultivars produce little or no pollen, and pollen dispersal is by insect-pollination, not wind. Though IFD-524Ø1-4 and IFD-529Ø1-9 flowers do have viable anthers and viable pollen, the transgenic lines are genetic chimeras and the pollen is non-transgenic. There is therefore no possibility of the dispersal of the transgenes by pollen-mediated gene flow.

C. Gene dispersal by seed formation

As all flowers are removed during cultivation and cut- flowers are unable to form seeds there is negligible realistic potential for gene flow by seed formation.

D. Related weeds

Rose is related to three major *Rosa* weeds in North America (see section II). There are many *Rosa* species naturalized in the United States flora and though some of these species do occur in states where rose is currently grown there is no evidence for hybridization to rose (section II).

E. Potential impact on threatened and endangered species

The transgenic rose will be grown in a limited number of locations, by experienced rose growers that specialise in cut flowers, inside a horticultural greenhouse setting (Figure 21). Therefore there is negligible opportunity for discarded material to become established in natural habitats. The relatively small number of plants to be cultivated, the way in which they will be cultivated (Figure 21) and shipped as well as the short longevity of the flowers

are all factors that preclude any direct or indirect interaction between IFD-524Ø1-4 and IFD-529Ø1-9 and non-target organisms.

Figure 21: Appearance of typical rose grower facilities in California USA; facility consists of several large secure greenhouses (left) with rose plants grown in raised beds (right).

IX. Theoretical Adverse Consequences of Introduction

A. Toxicity of non-GM rose

Roses are widely cultivated and have a long history of safe use. *Rosa* species have been used for centuries as a source of oils for the fragrance industry, hips for herbal teas, infusions, jams, preserves, and more recently, rose hips have been used in the floral industry.

Rose plants or products are not toxic or poisonous and are not listed on any of web-based toxic or poisonous plant websites.

B. Potential toxicity of lines IFD-524Ø1-4 and IFD-529Ø1-9

Delphinidin

Lines IFD-524Ø1-4 and IFD-529Ø1-9 accumulate delphinidin and delphinidin-derivatives, whilst the parent organism, non-genetically modified rose, does not.

Neither Delphinidin nor its derivatives are toxic compounds, when consumed or when handled. There is no toxicity data in the Merck Index for the aglycone, the mono -glucoside or the di-glucoside of delphinidin. Anthocyanins have a low acute toxicity of ca. 20,000 mg/kg BW in rodents, and a very low order of toxicity (WHO, 2001).

Delphinidin is found in many raw foods such as red grapes, black currants, egg plant (aubergine), blueberry, elderberry, bilberry and Jalapeno pepper. The daily estimated consumption of delphinidin as the anthocyanidin, from commonly consumed fruits and vegetables in the American diet, has been estimated at 2.5 mg per day (Wu *et al.*, 2006). Finns eat ca. 80 mg anthocyanin per day, with berries such as blackcurrant and bilberry a primary source of anthocyanin (Heinonen, 2007). In countries like Finland the per capita consumption of delphinidin is therefore much higher than the United States (for example, 100g of berries may have approximately 500 mg of anthocyanins). Single-dose intake of several hundred milligrams is quite reasonable (Manach *et al.*, 2005). Table 17 provides an estimation of the amount of delphinidin in common food plants.

Table 17. Estimation of delphinidin content in common delphinidin containing foods

Mean mg delphinidin per 100g edible portion					
Blackcurrant, raw	181.11	Black beans	11.98	Strawberry, fresh	0.32
Bilberry	161.93	Cranberry, raw	7.66	Raspberry	0.29
Cowpea	94.6	Bananas	7.39	Red cabbage	0.1
Blueberries, fresh	47.4	Pecan nuts	7.28	Green peas	0.03
Blackcurrant juice	27.8	Red grapes, raw	3.67	Red plum	0.02
Blueberries, frozen	21.59	Red onion, raw	2.28	Apples, raw	0.02
Eggplant, raw	13.76	Sweet potato, purple	0.90	Mango, raw	0.02

(USDA, 2007)

In the tissues of the foods where it is concentrated, the pigment is present at a higher concentration than in the flowers of IFD-524Ø1-4 and IFD-529Ø1-9. The concentration of delphinidin in line IFD-524Ø1-4 is approximately 0.0558 mg delphinidin per gram fresh weight petal. This 100 fold less than the amount found in blueberry, which may have up to 5 mg anthocyanin per gram fresh weight. (Table 17).

Examples of widely grown ornamental plants that contain delphinidin-based pigments include *Agapanthus* (Bloor and Falshaw, 2000), cyclamen, *Hydrangea* (Takeda *et al.*, 1990), verbena, *Petunia* (Ando *et al.*, 1999), *Delphinium* (Kondo *et al.*, 1991), *Lobelia* (Yoshitama, 1977) freesia, pansy, and *Hyacinth* (Hosokawa *et al.,* 1995). These plant species are freely sold in the United States as both garden plants and/or cut-flowers. The concentration of delphinidin in some common delphinidin containing species is shown in Table 18, and compared to lines IFD-524Ø1-4 and IFD-529Ø1-9. The levels of delphinidin detected in the transgenic rose flowers are within the range seen in common, widely cultivated ornamental plants.

The concentration of delphinidin and other anthocyanidins was determined in flower samples by high pressure liquid chromatography (HPLC). Flavonoids from freeze-dried petals were extracted with 10 x volume (to wet weight of the petals) 50% acetonitrile containing 0.1 % (v/v) trifluoroacetic acid. Prior to HPLC analysis the anthocyanins present in the petal limb extracts were acid hydrolysed to remove glycosyl and acyl moieties from the anthocynanin core. HPLC analysis was essentially as described in Fukui *et al.* (2003). The delphinidin percentage was determined from a single assay of petal samples, and is expressed as mg/g fresh weight petal.

Table 18: Delphinidin concentration in some common delphinidin containing species. Data has been generated by Florigene's own analysis, based on methods found in Fukui et al., 2003.

	Delphinidin (mg/g FW)	**Delphinidin as % all anthocyanidins**
IFD-524Ø1-4	0.06	90
IFD-529Ø1-9	0.10	91
Agapanthus	0.12	82
Brachycome	0.75	83
Cineraria	0.96	71
Delphinium	0.52	98
Dampiera	1.64	100
Iris	1.26	100
Rhododendron	0.14	50
Lisianthus	2.80	90
Pansy	3.90	84
Wisteria	0.39	89

Proteins from introduced genes

The proteins encoded by the inserted genes are common proteins and are not toxic proteins. The NPTII enzyme is widespread in the environment as it is naturally produced by a common gut bacterium *Escherichia coli* (Jefferson *et al.,* 1986). It has been estimated that a human would ingest at least 1.2 x 10^6 kanamycin resistant bacteria daily (Flavell *et al.*,

1992). The F3'5'H protein are found in all plant foods producing delphinidin. This includes several raw foods containing high levels of delphinidin, normally consumed and handled by humans. The 5AT protein is also found in all plant foods containing the related anthocyanin pigments cyanidin and pelargonidin.

C. Allergenicity of non-GM rose

Flowers are not listed with, tree nuts, legumes, fruit, shellfish, eggs, cows milk etc as a common source of food allergy and rose pollen cannot be a source of hay fever type allergies, as the pollen is not wind-borne. Allergic reactions to rose are rare. Florigene has close relationships with rose growers in Australia, Colombia and Ecuador and none of these contacts have experienced allergy problems among their staff. We have found in the literature just three reports that suggest the possibility of occupational rose allergy developing in people working in rose cultivation (Unlu, *et al.*, 2001), rose oil extracting plants (Akkaya *et al.*, 2004) and processing powdered rose hips (Kwaselow, *et al.*, 1990).

D. Potential allergenicity of lines IFD-524Ø1-4 and IFD-529Ø1-9

The transgenic rose has no increased allergenicity potential when compared to any non-GM rose. As stated above flowers are not listed as a common source of allergy. Pollen from roses is not considered a cause of hay fever and in any case the transgenic lines have non-transgenic pollen.

From a risk assessment point of view we have assumed a high level of expression of all the genes, in order to determine if there is any potential increase in allergenicity. While there is no literature we are aware of reporting studies of the allergenicity of delphinidin or its derivatives, reactions to anthocyanin containing foods have been studied - most people are exposed to at least some anthocyanin in the course of their regular diet. A recent review states that there are no reports of allergic reaction to either grape skin extract or grape color extract - both of which are widely used food colorants (Lucas *et al.*, 2001), and contain delphinidin. The proteins (enzymes) encoded by the *F3'5'H* and *5AT* genes are not likely to be allergenic. The F3'5'H protein is found in all plant foods producing delphinidin and the 5AT protein is found in all plant foods containing anthocyanins.

The *npt*II gene is a commonly used selectable marker in the production of genetically modified plants (Miki and McHugh, 2004). It has been assessed by many regulatory agencies worldwide, including the Office of the Gene Technology Regulator (OGTR) in Australia and the Food Safety Authority in Europe (EFSA). It has been found to pose no risk to human or animal health or to the environment several times in the past but most recently in 2007 by EFSA and 2008 by OGTR (OGTR, 2008; EFSA, 2007).

In addition transgenic rose will not enter the food chain;

- It would make no economic sense to purchase flowers for large scale use as an animal feed
- The flowers are likely to be purchased for the use they are intended, not for consumption as a food

X. Information about current and previous assessments or approvals

A. Japan

An application to the Japan authorities for the commercial release of the two rose lines has been successful. The approval numbers are 08-46P-0001 and 08-46P-0002.

2008-02-01
Japan
Decision on LMO for FFP under Article 11
<http://bch.cbd.int/database/record.aspx?id=43762>
IFD-524Ø1-4 - WKS82/130-4-1
Suntory Ltd.
Rosa hybrida - Rose
Modified Flavonoid Biosynthesis Pathway

2008-02-01
Japan
Decision on LMO for FFP under Article 11
<http://bch.cbd.int/database/record.aspx?id=43763>
IFD-529Ø1-9 - WKS82/130-9-1
Suntory Ltd.
Rosa hybrida - Rose
Modified Flavonoid Biosynthesis Pathway

The letter of approval can be found on the next page along with the English translation.

１９消安第２６７０号
環自野発第080131001号
平成２０年１月３１日

サントリー株式会社
代表取締役社長　佐治　信忠　殿

農林水産大臣　若　林　正　俊

環境大臣　鴨　下　一　郎

遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律に基づく第一種使用規程の承認について

平成19年６月８日付けで承認申請のあった第一種使用規程（２件）について、遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律（平成15年法律第97号）第４条第１項の規定に基づき、別紙のとおり承認したので通知する。

（別紙）

承認番号　08－46P－0001

承認を受けた者の名称、代表者の氏名及び主たる事務所の所在地	サントリー株式会社 代表取締役社長　佐治　信忠 大阪府大阪市北区堂島浜二丁目１番40号
承認を受けた第一種使用規程	
遺伝子組換え生物等の種類の名称	フラボノイド生合成経路を改変したバラ（*F3'5'H, 5AT, Rosa hybrida*）（WKS82/130-4-1, OECD UI: IFD-52401-4）
遺伝子組換え生物等の第一種使用等の内容	切り花の用に供するための使用、栽培、保管、運搬及び廃棄並びにこれらに付随する行為
遺伝子組換え生物等の第一種使用等の方法	—

承認番号　08－46P－0002

承認を受けた者の名称、代表者の氏名及び主たる事務所の所在地	サントリー株式会社 代表取締役社長　佐治　信忠 大阪府大阪市北区堂島浜二丁目１番40号
承認を受けた第一種使用規程	
遺伝子組換え生物等の種類の名称	フラボノイド生合成経路を改変したバラ（*F3'5'H, 5AT, Rosa hybrida*）（WKS82/130-9-1, OECD UI: IFD-52901-9）
遺伝子組換え生物等の第一種使用等の内容	切り花の用に供するための使用、栽培、保管、運搬及び廃棄並びにこれらに付随する行為
遺伝子組換え生物等の第一種使用等の方法	—

English translation – Japanese approval

19, No. 2670, Food Safety and Consumer Affairs Bureau
No. 080131001, Nature Conservation Bureau

31 January 2008

Masatoshi Wakabayashi
Minister of Agriculture, Forestry and Fisheries

Ichiro Kamoshita
Minister of Environment

Mr Tadanobu Saji
CEO
Suntory Ltd.

Dear Mr Saji,

RE: Permission of Type 1 Use Regulation based on Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Genetically Modified Organisms

We hereby notify that permissions have been granted as per enclosure for the two applications dated on 8th June 2007 regarding Type 1 Use Regulation, based on Article 4, Clause 1, Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Genetically Modified Organisms (No. 97, 2003).

Attachment

Approval Number: 08-46P-0001

Applicant's Name and Address	Suntory Limited Nobutada Saji (CEO) 2-1-40 Dojimahama, Kita-ku, Osaka City, Osaka
Approved Type 1 Use Regulation	
Name of the Type of Living Modified Organism	Rose Variety with Modified Flavonoid Biosynthesis Pathway (F3'5H, 5AH, Rosa hybrida) (WKS82/130/4-1, OECD UI: IFD-52401-4)
Content of the Type 1 Use of Living Modified Organism	Appreciation, cultivation, storage, transportation and disposal of cut flowers, and other acts incidental to them
Method of the Type 1 Use of Living Modified Organism	

Approval Number: 08-46P-0002

Applicant's Name and Address	Suntory Limited Nobutada Saji (CEO) 2-1-40 Dojimahama, Kita-ku, Osaka City, Osaka
Approved Type 1 Use Regulation	
Name of the Type of Living Modified Organism	Rose Variety with Modified Flavonoid Biosynthesis Pathway (F3'5H, 5AT, Rosa hybrida) (WKS82/130/9-1, OECD UI: IFD-52901-9)
Content of the Type 1 Use of Living Modified Organism	Appreciation, cultivation, storage, transportation and disposal of cut flowers, and other acts incidental to them
Method of the Type 1 Use of Living Modified Organism	

B. Australia

A contained trial, DIR 060/2005, was approved by The Office of the Gene Technology Regulator (OGTR) in March 2006. The trial was completed and an application for commercial release of one line (IFD-524Ø1-4) was submitted in June 2008 (DIR 090/2008). As part of the assessment process a Risk Assessment and Risk Management Plan (RARMP) was prepared by the OGTR. The RARMP concluded that there are negligible risks associated with this commercial release of rose genetically modified for altered flower color, therefore approval for the commercial release of this line was granted on the 19th June 2009.

The RARMP as well as the licence is available online at http://ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir090

C. USA

In the USA the GMO has been notified in the state of California. The notifications (which give approval for field trials) extracted from the USDA-APHIS database are shown below;

07-184-101n Jackson & Perkins
Applicant #: JP 006
Reg article: Rose
OO-Modified Flower Color,
Release
Received: 03-JUL-2007
Status: Acknowledged 13-JUL-2007
Tech review: 09-JUL-2007
State notify: 09-JUL-2007
CA Release 12-JUL-2007

05-318-07n Jackson & Perkins
Applicant #: JP 004
Reg article: Rosa hybrida
OO-Flower Color Altered
Release
Received: 14-NOV-2005
Status: Acknowledged 12-DEC-2005
Tech review: 22-NOV-2005
State notify: 22-NOV-2005
CA Release 06-DEC-2005

05-318-08n Jackson & Perkins
Applicant #: JP 005
Reg article: Rosa hybrida
OO-Flower Color Altered
Release
Received: 14-NOV-2005
Status: Acknowledged 12-DEC-2005
Tech review: 22-NOV-2005
State notify: 22-NOV-2005
CA Release 06-DEC-2005

04-173-01n Jackson & Perkins
Applicant #: JP 001
Reg article: Rosa hybrida / OO / Flower color altered
Receive: 6/21/04
Tech review: 6/24/2004
State notify: 6/24/2004
Status: Acknowledged 7/06/04
State response:

CA Import 6/30/2004

04-173-02n Jackson & Perkins
Applicant #: JP 002
Reg article: Rosa hybrida / OO / Flower color altered
Receive: 6/21/04
Tech review: 6/24/2004
State notify: 6/24/2004
Status: Acknowledged 7/06/04
State response:
CA Import 6/30/2004

D Colombia

An application for field trial analysis has been approved in Colombia, Colombian Agricultural Institute ICA, Resolution No. 3857 (16 DIC. 2005). This trial is currently being undertaken with a view to commercial release.

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