Til Landbrugsstyrelsen

Vedr. bestillingen "Risiko for GMO forekomst i prydplanter".

DCA - Nationalt Center for Fødevarer og Jordbrug

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Specialkonsulent

Dato 8. december 2017

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Afs. CVR-nr.: 31119103
Journal 2017-760-000511

Landbrugsstyrelsen har i bestilling dateret d. 9. november 2017 bedt DCA – Nationalt Center for Fødevarer og Jordbrug – om at udarbejde et notat med en vurdering af hvilke prydplantearter der kan være i risikogruppen for utilsigtet at indeholde transgener, med fokus på de arter hvor der foregår kommerciel forædling.

Notatet er udarbejdet af lektor Søren Borg fra Institut for Molekylærbiologi og Genetik ved Aarhus Universitet og seniorforsker Karen Koefoed Petersen fra Institut for Fødevarer ved Aarhus Universitet. Fagfællebedømmelsen er foretaget af seniorforsker Annie Enkegaard fra Institut for Agroøkologi ved Aarhus Universitet.

Besvarelsen er udarbejdet som led i "Rammeaftale om forskningsbaseret myndighedsbetjening mellem Miljø- og Fødevareministeriet og Aarhus Universitet" under ID 1.12 i "Ydelsesaftale Planteproduktion 2017-2020".

Venlig hilsen

Lene Hegelund

Risk of unintended presence of transgenes in ornamental plants

Request

The Danish Agricultural Agency has requested a short note in English stating:

1. Which ornamental species might be at risk in relation to unintended introduction of transgenes. These might include species where transgenes of ornamental or production value have been introduced within research projects.
2. The note should focus on ornamental plants that are commercially bred in Denmark, secondly in the remaining EU.
3. The note should evaluate potential transgenes involved and suggest how their presence may be checked.

Deliverable

By Associate Professor Søren Borg, Department of Molecular Biology and Genetics, Aarhus University, and Senior Scientist Karen Koefoed Petersen, Department of Food Science, Aarhus University.

INTRODUCTION

Transformation systems have currently been developed for more than 50 species of herbaceous ornamentals and several woody species, and efforts continue in many laboratories around the world (Chandler and Sanches, 2012). Several groups and companies are working on various ornamentals altering flower colour, plant architecture, floral longevity, fragrance, resistance to environmental stresses including abiotic stress, resistance to drought and cold, as well as biotic resistance to pests, fungal, viral and bacterial diseases. Only few genetically modified ornamental crops have been commercialized, and apart from a few major crops such as chrysanthemum, carnations, roses, and petunia, relatively few professional plant breeders work with each of the very large number of crops that make up the ornamentals industry. For a more comprehensive review, see Chandler and Sanches (2012) and Azadi *et al*. (2016).

In Denmark, breeding of potted plants mainly takes place as an R&D activity performed by individual pot plant producers. Therefore, there are many smaller players in the market and their breeding activities have resulted in many applications for plant variety rights (PVR) to the Community Plant Variety Office CPVO. During the last 22 years, applications have been submitted for more than 90 different genera (> 2500 varieties) of ornamental plants, herbaceous as well as woody, where breeding takes place in DK. Apart from the R&D activities taking place at growers, a few companies focus on breeding. Sakata Seeds and Ex-Plant (Pan American Seeds, Ball Horticultural Company) are breeders as well as seed producers. Nissen Consult, Vitroform and other small companies assist with breeding. In addition, the Danish universities are involved in breeding of ornamentals in collaboration with individual growers or groups of pot plant producers.

In ISAAA pocket folder no. 47, lawn and turf grasses are listed and may include “creeping legumes”. However, in a European context, turf grasses and legumes are not considered ornamentals but a natural base for sports and for leisure activities. Therefore, lawn and turf grasses including creeping legumes are not described here – even though appendix 1 lists *Trifolium repens* L.

Grasses and legumes produced in the European Union are not transgenic, but bred through essential biological processes. In USA, transgenic bentgrass was approved in 2017. Denmark has a world leading position in grass and clover seed production, and according to Tolstrup et al., 2003, there are no suggestions for control measures to maintain coexistence in forage grasses and clover.

Denmark also has a considerable production of chrysanthemum seed for the Asian food markets.

COMMERCIAL PRODUCTION OF TRANSGENIC ORNAMENTALS

The low rate and extent of commercialization of genetically modified ornamental crops is not due to the lack of useful traits that may be engineered into ornamentals, nor is it due to market potential or a lack of research and development activities. Importantly, all GM ornamental varieties released commercially have been accepted in the market. The high costs of securing regulatory approval constitutes a challenge for the development of genetically modified ornamentals, making them unattractive from a business perspective.

The only GM ornamental products released to the world market so far are flower colour modified varieties of carnation (*Dianthus caryophyllus*) and rose (*Rosa hybrida*) developed by one company (Florigene Flowers/Suntory, Japan), and a Petunia GM variety introduced through a chimeric petunia CHS gene by manipulating the anthocyanin biosynthesis with altered flower colour developed by Beijing University. No other approved transgenic varieties are on the commercial world market.

Colour modification dominates the GM research that has been carried out in ornamentals so far. The key genes of the anthocyanin, flavonoid and carotenoid biosynthesis and metabolism pathways are identified, allowing modification of flower colour in many ways. GM of flower colour was first demonstrated more than 20 years ago in Petunia (Meyer *et al*., 1987). The same plant material was unintentionally used in crossings to produce new varieties introduced at the market. In 1993, the gene encoding flavonoid 3'5'-hydroxylase was isolated (Holton *et al*., 1993), providing the tool to allow the development of the colour-modified *D. caryophyllus* and *R. hybrida* now in the market.

TRANSFORMATION SYSTEMS FOR ORNAMENTALS

Transgenic ornamentals of various species have been produced by different systems, including *Agrobacterium tumefaciens*-mediated transformation, biolistic or particle bombardment, direct electrophoresis of DNA into meristems and, recently, new breeding techniques like CRISPR/Cas9 allowing precise single nucleotide manipulation in order to create gene knockouts and eventually a precise alteration of codons coding for specific amino acids. *A. tumefaciens* or biolistic bombardment are most commonly used. In theory, almost any plant species can be transformed, and transformation of more than 50 ornamental species has been reported (Chandler and Sanchez, 2012).

In collaborative research projects between Danish pot plant producers and Danish/German universities, *A. tumefaciens* mediated transformation protocols have been developed for a number of plants (Campanula (Sriskandarajah et al. 2008), Exacum (unpublished data), Symphyotrichum (Mørk et al. 2012), Kalanchoë (Gargul et al. 2013), Rosa (Zakizadeh et al. 2)) and CRISPR/Cas mediated transformation in Campanula is currently being developed.

In Denmark, the ornamental industry has also invested in research on transfer of genes from naturally occurring plasmids harboured in *Agrobacterium rhizogenes* (Christensen *et al*., 2008; Christensen and Müller, 2009). Plants resulting from *A. rhizogenes* transformation are not considered as GM according to European and Danish legislation (EU-direktivet 2001/18/EF; Bekendtgørelse af lov nr. 840 af 3. maj 2015).

TESTING FOR TRANSGENES IN PLANTS

Various techniques can be used to test whether seed or plant material is genetically modified. The most common methods used are different standard polymerase chain reaction (PCR) techniques. A technique using TaqMan probes releasing specific fluorophores for each successful PCR reaction cycle is the most sensitive. The use of different primers, probes and fluorophores, allows for testing of several genes in the same reaction. Testing of seeds or plant material should: 1) be carried out on a representative sample of material, 2) include appropriate positive and negative controls, 3) address the potential of false-positive results and 4) be conducted at a high level of sensitivity.

Novel molecular transformation techniques such as mutagenesis with oligonucleotides or site-directed nucleases (e.g., zinc-finger endonucleases, CRISPR-Cas9, or transcription activator–like effector nucleases (TALENs)) will henceforward facilitate targeted genetic modification that will be difficult to distinguish from natural mutations. The most recent example being the CRISPR gene-editing tool used to turn the flower colour of Japanese morning glory (the *Ipomoea nil* or *Pharbitis nil*) from blue to white by targeting the colour gene dihydroflavonol-4-reductase-B (DFR-B) (Watanabe *et al*. 2017). Whether this technique will be considered a genetic transformation or a targeted mutation in the future is currently being evaluated. New varieties produced by this technique will not be distinguishable from naturally occurring mutants.

ORNAMENTALS THAT REQUIRE SPECIAL ATTENTION

Appendix 1 lists the approximately 90 genera for which Danish breeders have applied for PVR at the CPVO office. For app. 35 of these genera, transformation protocols are developed but genes of interest to the horticultural industry have not necessarily been inserted. It is most likely that transgenes will appear in crops like *Rosa hybrid*, *Kalanchoë* sp., *Campanula* sp., *Euphorbia pulcherrima*, *Hibiscus rosa-sinensis*, *Hydrangea macrophylla*, *Osteospermum, Dianthus* sp. and *Rhipsalidopsis*. For most of these crops, breeding efforts take place at growers with considerable R&D activities, and these activities have involved different types of transformation in collaboration with Danish and European universities. Transgenes may unintentionally be introduced via breeding material collected from other breeders, but the risk is not considered very high as breeders usually ask for background material (pedigree charts/family trees). The genes listed in the ISAAA pocket folder no. 47 are most likely to be introduced as well as genes from non-ornamentals resulting in enhanced cold and drought tolerance and the previously mentioned targeted mutations introduced by techniques like CRISPR/Cas9 which cannot be identified.

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Pocket K no. 47, Biotechnology in Ornamental Plants by International Service for the Acquisition of Agri-Biotech Applications

https://www.retsinformation.dk/Forms/R0710.aspx?id=132155

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0018:DA:HTML

Plant species (Latin name)	Plant species (Common name)	Wild relatives in Denmark	Commercial GMO varieties available	Transgenic plants or transformation protocol available	PVR applications 1995-2017 (no.)	PVR applications 2013-2017 (no.)
Adenium Roem. et Schult	Desert rose	no	no	no	12	1
Aeschynanthus sp.		no	no	no	10	12
Aloe vera (L.) Burm. f.		no	no	yes	1	10
Alstroemeria L.	Peruvian lilie	no	no	yes	1	0
Anemone hupehensis	Chinese or Japanese anemone	yes	no	no	1	0
Argyranthemum frutescens (L.) Sch. Bip.	Paris Daisy	no	no	no	13	1
Asplenium nidus L.	Bird's-nest fern	yes	no	no	1	0
Aubrieta Adans.		no	no	no	1	1
Begonia sp.		no	no	yes	10	0
Bougainvillea Comm. ex Juss.		no	no	no	1	0
Brachyscome Cass.		no	no	no	1	0
Buxus sempervirens L.	Common box	no	no	no	1	0
Campanula sp.		yes	no	yes	78	18
Capsicum annuum L.	Chili	no	no	yes (many)	7	0
Carex brunnea Thunb.		yes	no	no	1	0
Clematis L.		yes	no	no	96	36
Columnea L.	Flying goldfish plant	no	no	no	1	0
Colysis pteropus (Blume) Bosman (syn. Microsorum pteropus (Blume) Copel.; Polypodium pteropus Blume)		no	no	no	2	0
Cotoneaster Medik.		no	no	no	1	0
Crassula schmidtii Regel	Fairy tongue	yes	no	no	2	0
Crassula swaziensis Schönland		yes	no	no	2	1
Crossandra infundibuliformis (L.) Nees			no		1	0
Dahlia Cav.	Dahlia	no	no	yes	59	12
Dianthus sp.	Carnations	yes	yes	yes (many)	3	0
Dischidia ruscifolia Warb ex K.Schum. & Lauterb.		no	no	no	1	0
Echeveria DC.	Succulent	no	no	no	1	1
Echinodorus cordifolius (L.) Griseb.	akvarieplante - spade-leaf sword or creeping burhead	yes	no	no	1	0
Episcia cupreata (Hook.) Hanst.	Flame violet	no	no	no	1	0
Euphorbia sp.	Poinsettia,	yes	no	yes	123	7
Exacum sp.	Indian violet	no	no	yes	11	1
Fargesia murieliae (Gamble) T. P. Yi	Bamboo	no	no	no	3	1
Ficus sp. (benjamina)	Weeping fig	no	no	yes	10	0
Gardenia jasminoides J. Ellis	Gardenia	no	no	no	1	0
Gentiana scabra Bunge	Korean gentian	yes	no	(yes)	8	4
Graptopetalum bellum (Moran & J. Meyran) D. R. Hunt (syn. Tacitus bellus Moran & J. Meyran)	Cactus	no	no	no	2	0
Hebe Comm. ex. Juss.	Hebe	no	no	no	19	9
Hedera helix ssp	Ivy	yes	no	no	3	0
Hibiscus rosa-sinensis L.	Cineese hibiscus	no	no	yes	152	10
Holarrhena pubescens Wall. ex G.Don	Tellicherry-bark	no	no	no	1	0
Hydrangea macrophylla (Thunb.) Ser.	Hortensia, Big leaf hydrangea	no	no	yes	12	10
Hylotelephium telephium (L.) H. Ohba (syn. Sedum telephium L.)	Orpine, longlive many common names (rød sankthansurt)	yes	no	no	1	1
Ipomoea batatas (L.) Lam.	Sweet potato	no	no	yes	1	0
Ixora L.	West indian jasmin	no	no	no	4	0
Jasminum sp.	Jasmine	no	no	no	3	0
Juncus L.	rushes	yes	no	yes	2	0
Justicia L.	Water willow	no	no	no	1	0
Kalanchoe sp.	Flaming Katy	no	no/?	yes - many	281	50
Lavandula sp.	Lavender	no	no	yes	16	3
Lewisia cotyledon (S. Watson) B. L. Rob.	Siskiyou lewisia	no	no	no	4	4
Lupinus angustifolius L.	Blue lupin	yes	no	yes	2	1
Lycianthes rantonnetii (Carrière) Bitter	Blue potato bush		no		1	0
Malus sieboldii (Regel) Rehd.	Wild apple	yes	no	Within other Malus species	2	1
Mandevilla sanderi (Hemsl.) Woodson	Brazilian jasmine	no	no	no	4	0
Miscanthus sinensis (Thunb.) Andersson		no	no	yes	2	0
Muehlenbeckia complexa (A. Cunn.) Meisn.	Maidenhair wine	no	no	no	1	0
Nerium oleander L.	Oleander	no	no	yes	1	0
Osteospermum sp.	Cape daisy	no	no	yes	267	39
Otacanthus sp.	new York aster	no	no	no	1	0

Parthenocissus henryana (Hemsl.) Diels & Gilg	Chinese Virginia creeper	no	no	yes	1	0
Pelargonium sp.	Geraniums	no	no	yes	19	2
Pentas Benth.	Egyptian starcluster	no	no	no	10	0
Peperomia sp.		no	no	yes	2	2
Phalaenopsis Blume	Orchid	no	no	(yes)	2	0
Phlox drummondii Hook.	Drummond's flox	no	no	no	10	0
Platycodon grandiflorus (Jacq.) A. DC.	Balloon flower	no	no	no	5	0
Pogonatherum paniceum (P. Beauv.) Hack.	Baby panda grass	no	no	yes	1	0
Rhipsalidopsis	Easter cactus	no	no	yes	44	9
Rosa L.	Rose	yes	yes	yes	719	137
Saintpaulia ionantha H. Wendl.	African violet	no	no	yes	6	0
Salix sp.	Willow (ornamental?)	yes	no	yes	2	0
Sansevieria sp.	Mother in law's tongue	no	no	no	2	2
Saxifraga sp.	Rockfoils	yes	no	no	2	0
Scaevola L.	Fan-flower	no	no	no	1	0
Schlumbergera	Christmas cactus	no	no	no	52	5
Scutellaria sp.	Scullcaps	yes	no	yes	6	0
Siloxerus humifusus Labill.	Pussy's-toes tribe	no	no	no	1	1
Solanum jasminoides Paxt.	Potato wine	yes	no	no	1	0
Solanum pseudocapsicum L. (syn. Solanum diflorum Vell.)	Jerusalem cherry	(yes)	no	no	3	0
Solidago L.	Goldenrod	yes	no	no	1	0
Symphyotrichum novi-belgii (L.) G. L. Nesom var. novi-belgii (syn. Aster novi-belgii L.)	New York aster	yes	no	yes	69	19
Taxus baccata L.	yew	(yes)	no	no	1	0
Thuja L.		(yes)	no	no	1	0
Thunbergia Retz.	clockwine / Black-eyed Susan	no	no	no	1	0
Tilia cordata Mill.	Small-leaved lime	yes	no	no	1	0
Trachelium L.	Blue throatwort	no	no	no	3	0
Trifolium repens L.	White clover (different leaf colours)	yes	no	yes - but probably not regarding ornamental traits	13	5
Ulmus L.	Chineese elm	yes	no	yes	1	0
Wahlenbergia procumbens (Thunb.) A. DC.		no	no	no	1	0
x Doritaenopsis hort.	Orchid	no	no	yes	3	0
Zanthoxylum piperitum (L.) DC.	Japanese pepper	no	no	yes	1	0