Assessing and monitoring the impact on the agri-environment of genetically modified potatoes with resistance to *Phytophthora infestans*, causative organism of late blight disease (2012 – 2016)

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This document contains information required in notification concerning the release of genetically modified higher plants (angiospermae) as per Part II of the Third Schedule of S.I. No. 500 of 2003[1].

The GM line to be studied under this notification is part of a larger cohort of GM potato lines currently being researched in the Netherlands under Permits B/NL/10/06[2]

[1] http://www.irishstatutebook.ie/2003/en/si/0500.html#sched3
[2] http://gmoinfo.jrc.ec.europa.eu/gmp_report.aspx?CurNot=B/NL/10/06

A. GENERAL INFORMATION

1. Name and address of the notifier (company or institute)

Teagasc,
Oak Park,
Carlow,
Ireland

2. Name, qualifications and experience of the responsible scientist(s)

Dr. Ewen Mullins (PhD, UCC, 1996; B.Sc, NUI Maynooth, 1992)
Dept. Crop Science,
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Ireland

A Senior Research Officer in the Teagasc Dept, Crop Science Dr. Mullins is responsible for the GM crop risk assessment research programme at Oak Park, Carlow (http://www.agresearch.teagasc.ie/oakpark/people/emullins.asp)

3. Title of the project

Assessing and monitoring the impact on the agri-environment of genetically modified potatoes with resistance to *Phytophthora infestans*, causative organism of late blight disease (2012 – 2016).

B. INFORMATION RELATING TO (A) THE RECIPIENT OR (B) (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

(a) family name = *Solanaceae*
(b) genus = *Solanum*
(c) species = *tuberosum*
(d) subspecies = *tuberosum*
(e) cultivar/breeding line = cv. *Desiree*
(f) common name = potato

Grown widely on the European continent, a detailed description of the cultivar's properties is available on the European Cultivated Potato Database[3]. In summary cv. Desiree is late to intermediate in its maturity. Its tuber shape is oval to round with a red skin. *S. tuberosum* cv. Desiree is male fertile and its flower frequency is high, leading to the production of berries containing true potato seed. This has been confirmed in a Teagasc study that focussed on tracing gene dispersal (via pollen and seed) from *S. tuberosum* cv. Desiree using microsatellite markers[4]. As a result the management and flowering characteristics of cv. Desiree under Irish conditions have been thoroughly examined.

2 (a) Information concerning reproduction

(i) mode(s) of reproduction

Potato is a clonally propagated annual crop, with the primary mode of reproduction vegetative via tuber production. Consequently, the transfer of pollen from a donor potato plant to neighbouring recipient potato plants has no impact on the genetic constitution of the tubers harvested from these neighbouring recipient potato plants.

Sexual reproduction via true potato seeds, which are derived from berries, is also possible under field conditions but is dependent on the cultivar in question. Under Irish field conditions *S. tuberosum* cv. Desiree will produce berries with viable true potato seed but the plants that arise from true potato seed are agronomically weak and are not capable of competing against weeds and grasses, as observed during Teagasc gene flow studies. In addition, complete control of volunteers arising from true potato seed during the same studies was achieved by ploughing, harrowing or employing a broad spectrum herbicide (e.g. glyphosate).

(ii) specific factors affecting reproduction, if any

Tubers are frost sensitive and their reproducibility is dependent upon temperature, with tubers typically being destroyed at temperatures below -3^{o}C. It is reported that tubers will be destroyed by a continuous 25 hour period of < -2^{o}C or up to 5 hours at -10^{o}C[5]. If tubers are buried as a result of tillage operations post-harvest, the survivability of the tubers will increase.

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[3] http://www.europotato.org/display_description.php?variety_name=Desiree
[4] Petti, C. Meade, C and Mullins, E. (2007). Facilitating co-existence by tracking gene dispersal in conventional potato systems with microsatellite markers. Environmental Biosafety Research 6(4), 223-231.
[5] OECD (1997). Consensus document on the biology of Solanum tuberosum subsp. Tuberosum (Potato) – Series on the harmonization of regulatory oversight in biotechnology, No. 8.

(iii) generation time

Under Irish conditions, potato tubers are typically planted from March to May. Harvesting typically takes place from June to October, dependent on weather conditions. The start and length of the growing season is determined by the suitability of the soil temperature and cultivar used.

2 (b) Sexual compatibility with other cultivated or wild plant species, including the distribution in Europe of the compatible species.

Derived from South America, the potato is characterised by a reduced number of wild relatives in Europe; for Ireland, only by *Solanum nigrum* ('black nightshade') and by *Solanum dulcamara* ('bittersweet nightshade').

Previous studies[6] included *S. nigrum* in field trials with transgenic potato. Although seeds were recovered from these wild individuals, upon screening for the physiological traits conferred by the transgene, no hybrids were found; indicating the absence of pollen-mediated gene flow. Similarly, in the case of *S. dulcamara*, and irrespective of whether the wild relative was being used as a pollen donor or pollen receptor, no berry formation was observed. An additional study also concluded that while the two wild relatives of commercial potato were commonly found throughout Europe, they are not sexually compatible with commercial potato and the generation of hybrids and the potential for transgene escape into either *S. nigrum* and/or *S. dulcamara* was negligible[7].

As there was no Irish-specific data available on this subject, Teagasc re-examined this issue in 2006 by completing 1,514 controlled pollination crosses between *S. nigrum* and *S. dulcamara* with GM *S. tuberosum* cv. Desiree[8]. Crosses between *S. dulcamara* and *S. tuberosum* cv. Désireé did not lead to the formation of any berries. For crosses with *S. nigrum*, formed berries matured prematurely and bore only discoidal seeds with no evidence of embryo development. This phenomenon was equivalent to that described by Eijlander and Stiekema (1996) and confirmed that *S. tuberosum* is genetically incompatible with Irish ecotypes of *S. nigrum* and *S. dulcamara*.

3 Survivability

(a) Ability to form structures for survival or dormancy

Potato survives through tuber production and for some cultivars via the production of true potato seed. True potato seed forms in berries after successful pollination of flowering inflorescences.

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[6] Conner, A. J. (1993). Monitoring "escapes" from field trials of transgenic potatoes: A basis for assessing environmental risks. Seminar on Scientific Approaches for the Assessment of Research Trials with Genetically Modified Plants, Jouy-en-Josas, France, OECD.
Conner, A. J. (1994). Analysis of containment and food safety issues associated with the release of transgenic potatoes. In the Molecular and Cellular Biology of the Potato. W. R. Belknap, M. E. Vayda and W. D. Park. Wallingford, CAB international: 245-264.
McPartlan, H. and P. J. Dale (1994). "An assessment of gene transfer from transgenic potatoes to non-transgenic potatoes and related species." Transgenic Research 3: 216- 225.

[7] Eijlander, R. and W. J. Stiekema (1994). Biological containment of potato (Solanum tuberosum) - Outcrossing to the related wild-species *S.nigrum* and *S. dulcamara*. Sexual Plant Reproduction 7(1): 29- 40.

[8] Petti, C. (2007). Elucidating the propensity to genetically transform *Solanum tuberosum* L. and investigating the consequences for subsequent risk assessment studies. PhD Thesis, NUI Maynooth.

(b) Specific factors affecting survivability, if any

Tubers are frost sensitive and will be destroyed if they remain on the soil surface during periods of -3°C or lower[9]. Tuber survivability increases when tubers become buried during post-harvest tillage operations and this will lead to the emergence of volunteers in the subsequent rotational crop.

This was confirmed by Teagasc research conducted in 2010 and 2011, which surveyed 34 commercial fields across Wexford, Cork, Louth, Meath and Dublin for the emergence of potato volunteers, after potatoes had been cultivated in 2009[10]. As expected, the application of herbicides during the cereal crops significantly reduced the number of recorded volunteers observed in the rotation.

True potato seed (Figure 1a) are contained within berries (Figure 1b), which drop off before harvesting and lie on the soil surface as the crop senesces. Mixed with the soil during harvest and subsequent tillage operations, plants derived from true potato seed are agronomically disadvantaged and can be controlled without difficulty by mechanical cultivation or with an herbicide application.

Figure 1: Images of true potato seed (A) and maturing berries on *S. tuberosum* cv. Desiree at Oak Park in 2010.

A.

B.

4 Dissemination

(a) ways and extent (for example, an estimation of how viable pollen and/or seeds declines with distance) of dissemination

Potato can be spread as true potato seed, tubers and pollen. Tuber dispersal will occur pre-sowing and post-harvest and is primarily operator related. Poor storage of seed tubers and harvested tubers during transport can lead to tuber loss within the confines of the field and along the routes from field to warehouse. Tuber loss during harvest operations can lead to tubers lying on the soil surface and animal-mediated dispersion can cause a limited amount of tuber loss from the field.

Pollen dispersal is only possible from male fertile potato cultivars. If successful pollination does occur between two adjacent potato crops, it will have no impact on

[9] Van Swaaij et al. (1987). Increased frost tolerance and amino acid content in leaves, tubers and leaf callus or rain rated hydroxyproline resistant potato clones. Euphytica 36:369-380

[10] Phelan, S., Bryne, S., Fitzgerald, T., Meade, C. and Mullins, E. Potential for seed-mediated gene flow from commercial potato crops and possible consequences for the coexistence of GM and non-GM potato systems. (in preparation for publication).

the formation or the genetic constitution of tubers on the recipient plant. Instead, pollination will lead to the formation of berries (Figure 1b), with the number of seeds within that berry dependent on berry morphology and viability. True potato seeds are not spread by birds[11]. Previous studies that examined the dispersal of pollen from field-grown transgenic potatoes concluded that transgene dispersal is limited (99.98%) to within 10m of the transgenic population[12,13,14]. A separate study reported the potential dispersal of transgenes up to 1000 m from the donor potato population with an inference that the pollen beetle (*Meligethes aeneus*) was instrumental in facilitating gene transmission[15]. A critical assessment of this result disputed the efficacy of the described methodology and concluded that an isolation distance of 20 m was adequate to mitigate pollen-mediated transgene escape from GM potato[14]. This conclusion was supported upon review[16] and was subsequently adopted as a guideline to facilitate the cultivation of GM potato in Denmark[17].

To quantify the potential and consequence of pollen-mediated gene flow under Irish conditions, Teagasc completed separate studies in 2005 and again in 2010. We utilised two conventional potato varieties and microsatellite markers to score for successful gene flow events between the pollen donor and receptor sub-plots. To maximise the potential for hybridization between the male-sterile receptor (cv. British Queen) and pollen donor (cv. Desiree) plots, we staggered the sowing of both populations to ensure a lengthened period of synchronous flowering was achieved between both cultivars. As cv. British Queen does not produce fertile pollen, the presence or absence of a berry on British Queen plants indicated the occurrence or non-occurrence respectively of successful pollen-mediated gene flow.

The 2005 study was designed with the donor plot of *S. tuberosum* cv. Desiree in the centre of the field and single drills of receptor cv. British Queen plants cultivated at set distances from the edge of the donor plot in a north, south, east and west orientation. By adopting this 'worst case scenario' system, results from 2005 indicated that pollen-mediated gene flow in potato can extend out to 21 m from the pollen donor population[18]. A total of 140 berries were counted at 21m from the pollen donor, yet only 2.8% (n = 4) of these berries contained seed and only 36% (n = 23) of this seed was able to germinate under the controlled conditions of a glasshouse.

In contrast to 2005, the 2010 study was completed across two sites with pollen donor and receptor plots running parallel to each other; thereby reflecting the real scenario of two coexisting commercial potato crops. Berry distribution across both sites

[11] Hawkes (1988). The evolution of cultivated potatoes and their wild tuber-bearing relatives. Kulturpflanze 36:189-208.
[12] Conner AJ, Dale PJ (1996) Reconsideration of pollen dispersal data form field trials of transgenic potatoes. Theor. Appl. Genet. 92: 505–508.
[13] McPartlan HC, Dale PJ (1994) An assessment of gene transfer by pollen from field grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Res. 3: 216–225
[14] Tynan JL, Williams MK, Conner AJ (1990) Low frequency of pollen dispersal from a field trial of transgenic potatoes. J. Genet. Breed. 44: 303–306
[15] Skogsmyr I (1994) Gene dispersal from transgenic potatoes to conspecifics: A field trial. Theor. Appl. Genet. 88: 770–774
[16] Eastham K, Sweet J (2002) Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer, European Environment Agency, Copenhagen, pp. 1–75.
[17] Tolstrup K et al. (2003) Report from the Danish Working Group on the co-existence of genetically modified crops with conventional and organic crops. DIAS Report Plant Production 94, p. 275
[18] Petti, C. Meade, C and Mullins, E. (2007). Facilitating co-existence by tracking gene dispersal in conventional potato systems with microsatellite markers. Environmental Biosafety Research 6(4), 223-231.

indicated the maximum distance for pollen-mediated gene flow was 10 - 11m (Figure 2). A total of 34 berries were collected from the British Queen plots within 11m of the cv. Desiree plot. A total of 1,765 true potato seed were rescued from these berries and from this population, 1,219 seed germinated under controlled glasshouse conditions.

(b) Specific factors affecting dissemination, if any
See previous section

5. Geographical distribution of the plant
The centre of origin for the potato is the Andes region of South America. The potato is the world's fourth largest food crop and is sown across Europe, where almost 6 million hectares are grown per annum representing a value close to €600,000,000[19]. The potato remains the most important field grown horticultural crop in Ireland supporting an industry worth an estimated €74m. As is the case in nearly all potato growing regions of the world, the most significant challenge to potato yields remains late blight disease, caused by the oomycete pathogen *Phytophthora infestans*.

6. In the case of plant species not normally grown in Ireland, description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts
Not applicable.

7. Other potential interactions, relevant to the genetically modified organism, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.
Potato is susceptible to a large range of fungal, bacterial and viral diseases. It is also prone to insect damage and vulnerable to nematodes. Insects like aphids (*Myzus persicea, Aphis nasturtii, A. frangulae* and others), leaf hoppers (*Empoasca* spp,) and the Colorado beetle (*Leptinotarsa decemlineata*) are well known parasites in European potato cultivation, as are nematodes (*Globodera* spp., *Ditylencus* spp., *Paraditylencus* spp., *Tricodorus* spp. and *Paratricodorus* spp).

Late blight disease (causative organism *Phytophthora infestans*) remains the single greatest challenge to potato cultivation. Damage can also be incurred by black scurf (*Rhizoctonia solanii*), potato wart disease (*Synchythrium endobioticum*), early blight (*Alternaria solani*), powdery scab (*Spongospora subterranea*), skin spot (*Polyscytalum pustulans*), silver scurf (*Helminthosporium solani*), grey mold (*Botrytis cinerea*), watering wound rot (*Pythium ultimum*), wilt (*Verticillium* spp) and storage rots (*Phoma foveata* and *Fusarium* spp.).

The baterial-based diseases include the quarantine diseases brown rot (*Pseudomonas solanaceareum*) and ring rot (*Corynebacterium sepedonicum*), along with the more regularly occurring common scab (*Streptomyces scabies*) and black leg (*Erwinia*

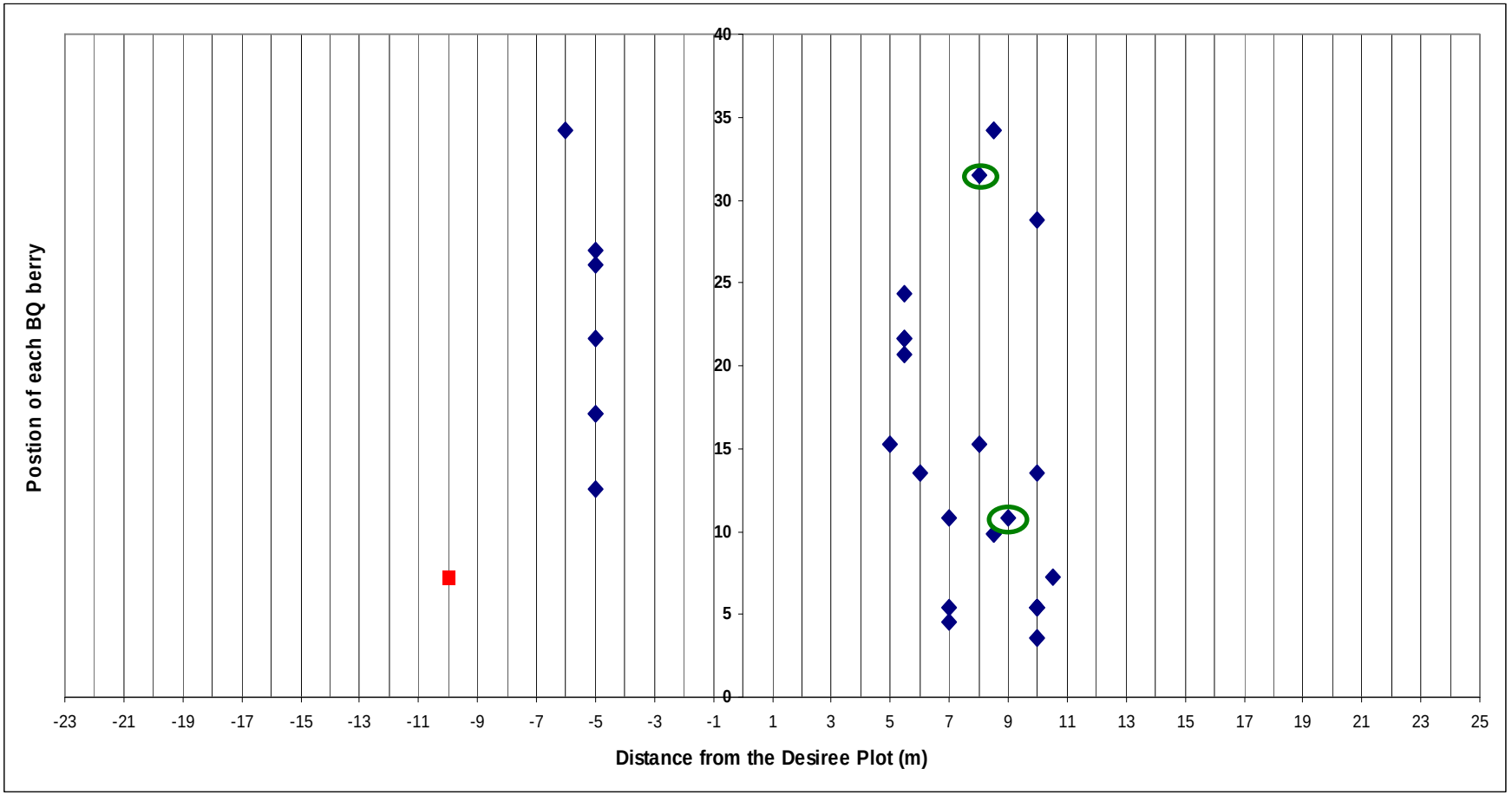
[19] Haverkort AJ, Boonekamp PM, Hutten R, Jacobsen E, Lotz LAP, Kessel GJT, Visser R, van Der Vossen E (2008) Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Research 51:47-57

carotovora ssp *carotovora/atroseptica*, and *Erwinia chrysanthemi*). The verification of virus free stocks for seed potatoes is vital with attention focussed on generating stocks that are free of Potato virus Y (PVY), Potato virus A (PVA), Potato virus X (PVX), Potato leaf roll virus (PLRV), Potato virus S (PVS), Potato virus M (PVM), Tobacco rattle virus (TRV) and Potato mop-top virus (PMTV).

Potato's attributes make it a near ideal food source for both developed and developing countries. Potato yields more calories per acre than any grain. The potato tuber is composed mainly of carbohydrates but it also acts as a source of minerals, fibre and vitamins. Potato starch is approximately 25% amylase and 75% amylopectin. The high glycaemia index of potatoes is of concern for diabetics. The main toxic or anti-nutritional substances in potatoes are glycoalkaloids and nitrates. Glycoalkaloids which in high concentrations are toxic, are found in harmful amounts mainly in the above ground parts of the plant stems, leaves and fruits. In the tubers of cultivated potato varieties, the content is usually low, below 100 mg per kilogram fresh weight. A maximum glycoalkaloid content of 200 mg per kilogram fresh weight in table potatoes has been established.

Potatoes are also used as a source of animal feed throughout the world. In addition, wild animals, mammals and birds, occasionally feed on potatoes that are exposed after sowing or that remain in the field post-harvest. As is the case for humans, a high content of glycoalkaloids is toxic for animals.

Figure 2. Spatial berry production in each British Queen Plot of the 2010 gene flow trial sites (Walled and Quarry fields) relative to distance from the pollen donor Desiree central plot. The y-axis represents the pollen donating Desiree Plot. The vertical lines represents each of the drills (distance between each drill is 0.5m). The x-axis represents the distance of each of the drills from the Desiree plot. The negative values represent the left plot. (◆) = walled field, (■) = quarry field, (○) = ectopic berry.



C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification.

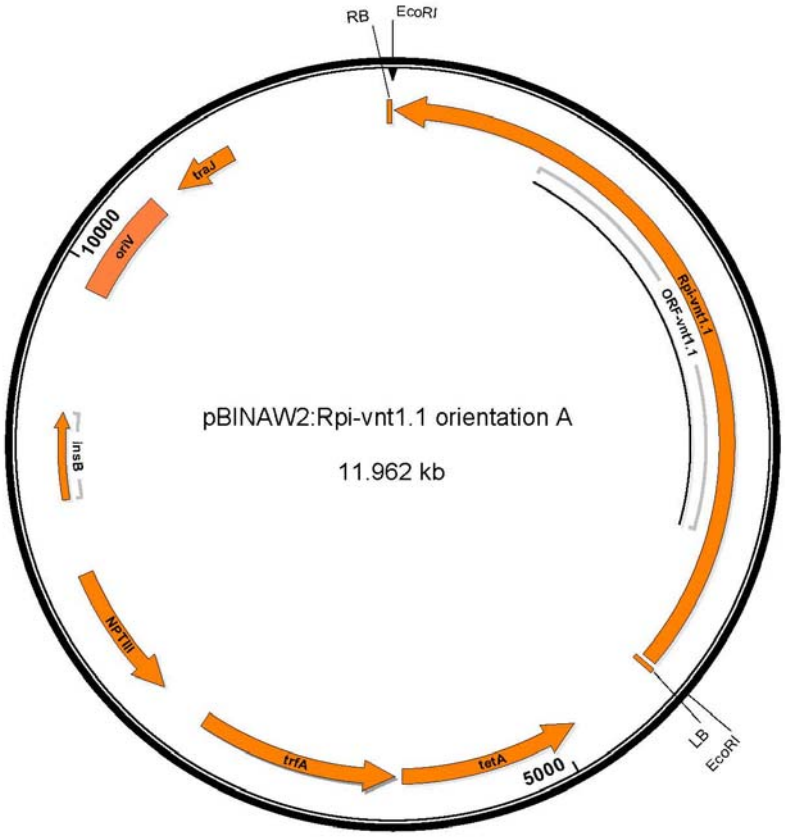
The cisgenic potato line A15-031 was generated via the insertion of the resistance to *P. infestans* (Rpi) Rpi-vnt1-1 gene into the genome of *S. tuberosum* cv. Desiree. The Rpi-vnt1-1 gene was originally taken from the wild potato species *S. venturii*. The genetic modification of the *S. tuberosum* cv. Desiree genome was mediated by *Agrobacterium tumefaciens*, in a process termed *Agrobacterium tumefaciens*-mediated transformation (ATMT).

2. Nature and source of the vector used.

Plasmid type pBINAW2:

The binary plasmid pBINAW2:Rpi-vnt1-1 (Figure 3) was employed to generate the A15-031 cisgenic potato line which is void of an antibiotic resistance, or similar type marker gene.

Figure 3: Graphical map of the pBINAW:Rpi-vnt1 plasmid.



The plasmid contains a number of open reading frames (ORFs) involved in the replication of the plasmid in bacteria. The expression of these ORFs is regulated by promoters of prokaryotic origin. In addition, the plasmid contains the NPT III gene on the vector backbone to facilitate the selection of transformed bacteria only. As such,

this ORF is not transferred into the potato genome during ATMT. The only sequences housed between the right and left border of the T-DNA are EcoRI restriction sites serving the sub-cloning procedures required to insert the Rpi gene, and a 4310 bp insert, containing the *Solanum venturii* late blight resistance gene Rpi-vnt1.1 and its native promoter and terminator.

3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion.
The T-DNA in pBINAW2:Rpi-vnt1-1 only contains the Rpi-vnt1-1 gene to confer resistance to *P. infestans*. The Rpi-vnt1-1 is a class of Rpi genes from tuber-bearing Solanum species that belong to the NB-LRR class of major resistance genes. The NB-LRR gene family is diverse and includes genes encoding a protein with a central NB domain (nucleotide binding domain) and a C-terminal LRR (Leucine Rich Repeats) domain. The N-terminal domain is more variable and has a coiled coil structure or a TIR domain (domain found in Toll receptor, Interleukin receptor and R-proteins)[20].

During the infection of potato by *P. infestans,* the pathogen's genes produce effector proteins, which are necessary for disease onset. A percentage of these elicitors are recognized by the proteins produced by the Solanum Rpi genes. It is this recognition which initiates a robust resistance response by the host against the pathogen. This 'hypersensitive' reaction leads to cell death only in the infected cells[21], which effectively forms a barrier, or blocks *P. infestans* from colonising the plant.

The gene used for the generation of the A15-031 line was Rpi-vnt1.1, which was inserted into the target potato cell's DNA with its promoter and terminator intact. Rpi genes vary in size from approximately 4kb to 11kb. The late blight resistance gene Rpi-vnt1.1 used for the generation of the A15-031 cisgenic line is 4310 bp.

D. INFORMATION RELATING TO THE GENETICALLY MODIFIED PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified
The Rpi-vnt1.1 gene that has been inserted into *S. tuberosum* cv. Desiree confers increased resistance to *P. infestans.* The respective gene encodes gene products that occur naturally in the wild potato species *S. venturii*.

2. Information on the sequences actually inserted/deleted
(a) size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the genetically modified higher plant or any carrier or foreign DNA remaining in the genetically modified higher plant
The size of the T-DNA inserted into the A15-031 cisgenic line is 4310bp. The following sequences were integrated into the genome of *S. tuberosum* to generate the cisgenic A15-031 GM line:

[20] Branches & Tameling, 2009. To nibble at plant resistance proteins. Science 324:744-746
[21] Jones and Dangle, 2006. The plant immune system. Nature 444:323-329

- Rpi-vnt1.1 gene with its native promoter and terminator, originally derived from the tuber-bearing *S. venturii*

To test for the integration of the T-DNA into the cv. Desiree nuclear genome, Rpi-vnt1 specific primers (Appendix 1) were used for qualitative PCR analysis. No amplicons were produced using Desiree (untransformed) DNA, showing that only the T-DNA sequence was detected for A15-031 (as highlighted in Table 1).

Table 1. Summary of PCR tests for A15 cisgenic lines, including A15-031 containing the Rpi-vnt1.1 cisgene.

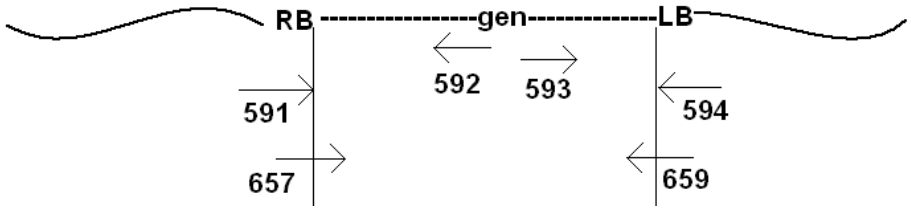
WUR code	T-DNA copynumber	Vector backbone		Border analysis		T-DNA analysis	DNA control	Relative expression
	Rpi-vnt1	*nptIII*	*tetA*	LB	RB	*Rpi-vnt1*	*EF1-alfa*	*Rpi-vnt1*
A15-007	3	-	-	-	-	+	+	1
A15-020	2	-	-	-	-	+	+	1.7
A15-028	2	-	-	-	-	+	+	3.0
A15-031	2	-	-	-	-	+	+	2.1
A15-045	2	-	-	-	-	+	+	1.3
A15-064	2	-	-	-	-	+	+	5.1
A15-070	4	-	-	-	-	+	+	8.6
A15-072	2	-	-	-	-	+	+	15.6
A15-044	2	+	+	+	+	+	+	4.5
Desiree	0	-	-	-	-	-	+	No expression

+: PCR band present, -: PCR band absent

To test for the presence of vector backbone DNA in the cisgenic A15-031 line, primers (Appendix 1) matching the TetA and NptIII genes outside the T-DNA borders (Figure 4) were used. No amplicons were produced using non-transgenic Desiree DNA (Table 1). Several plants were found to be positive for these PCR tests, showing that the experimental setup was suitable for detecting plants with vector backbone integration. One such plant is shown in Table 1 as a positive control (A15-44). A15 plants (including A15-031) that are negative for these PCR tests are therefore considered to not contain vector backbone integration (Table 1).

Specific primer pairs were designed to amplify within the left and right borders of the T-DNA and also overlapping the T-DNA borders. An overview of primer positions is given in Figure 4. If plants were negative for PCR with primers 591+592 and 657+592, it is concluded that the plant does not contain a viable right border after integration. If plants were negative for PCR with primers 594+593 and 659+593, it is concluded that the plant does not contain a viable left border after integration. Plant A15-44 was positive for the border PCRs and showed that the PCR approach was valid to prove presence or absence of border integrations. Cisgenic line A15-031 did not contain a left or right border sequence from the T-DNA.

Figure 4: Location of primers to detect left border (LB) and right border (RB) integration in A15 cisgenic lines



The integrity of the inserted Rpi-vnt1.1 gene was confirmed as intact based on Phytophthora leaf keys using specific *P. infestans* isolates and / or elicitors to control the expression of the respective Rpi genes[22], as per published protocols[23]

(b) in case of deletion, size and function of the deleted region(s)
No sequence has been deleted

(c) copy number of the insert
The number of T-DNA integrations was analysed using qPCR (Appendix 1). No amplicons were produced on non-transgenic Desiree DNA, showing that only the transgene copy number was measured. Ct values of Rpi-vnt1 amplicons derived from qPCR analysis were normalized by subtraction with the housekeeping EF1-α gene's C_t value. T-DNA copy numbers were calculated using single copy control plants as assessed by independent methods. *S. tuberosum* cv. This analysis confirmed that *S. tuberosum* cv. Desiree A15-031 possesses two T-DNA copies (Table 1).

(d) location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination
The cisgenic line A15-031 was developed via ATMT which delivers T-DNA into the nucleus of a targeted host genome[24]. The material has been propagated via shoot cuttings and the required phenotype is transferred in a stable manner.

3. Information on the expression of the insert:
(a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation
The level of cisgene mRNA expression was measured using qPCR (Appendix 1). Total RNA was isolated from non-inoculated leaf tissue. cDNA was synthesised and the Rpi-vnt1.1 transcript level was quantified using gene specific primers (Appendix

[22] Zhu et al. (2012). Functional stacking of three resistance genes against *Phytophthora infestans* in potato. Transgenic research. DOI 10.1007/s11248-011-9510-1
[23] Vleeshouwers et al. (2006). Agro Infection-based high throughput screening Reveals specific recognition of INF elicitins in Solanum. Molecular Plant Pathology 7:499 -510
[24] Zambryski, P. et al. (1980). Tumour DNA structure in plant cells transformed by *A. tumefaciens*. Science, 209: 1385-1391.

1) . No amplicons were produced on non-transgenic Desiree DNA, showing that only the cisgene expression level was measured. Ct values of Rpi-vnt1 amplicons derived from qPCR analysis were normalized by subtraction with the housekeeping EF1-α gene's C_t value. Relative constitutive expression levels were calculated by setting the lowest expression level (A15-007) to 1. The expression level of A15-031 was 2.1 fold higher than the control.

(b) parts of the plant where the insert is expressed (for example, roots, stems, pollen, etc.);

The cisgenic line A15-031 has been modified with the Rpi-vnt1.1 gene, which remains under the control of the native promoter and terminator sequence. As such, the expression of the insert is dependent on whether particular tissues of the plant are exposed to the pathogenic organism *P. infestans*. The Rpi gene in this notification belongs to the class of NB-LRR class of disease resistance genes. This class of genes are present in many cultivated plants and the model plant organism Arabidopsis. The interaction between the protein of the Rpi gene and the elicitor (effector) of the corresponding gene in the pathogen is responsible for the generation of a hypersensitive response at the cellular level of the plant against the pathogen. This results in localised cell death surrounding the point of initial infection. As a result, the development and advancement of the pathogen is blocked, leading to a resistant phenotype[25]. Depending on the resistance genes involved in the host-pathogen interaction, the expression of the NB-LRR genes can occur at the earlier or later stages of the infection process. The mechanism of resistance conferred by these plant genes indicates that the use of multiple Rpi genes can be seen as adding additional barriers that prevent the pathogen from colonizing host tissues and ultimately evolving to break down the expression of resistance by the Rpi genes[26].

4. Information on how the genetically modified plant differs from the recipient plant in:

(a) mode(s) and/or rate of reproduction;

The cisgenic GM line A15-031 intended for release was selected true to type from a larger population of A15 lines (Table 1). The intended cisgenic potato line has been grown under field conditions in the Netherlands and no changes were observed between the GM line and its equivalent comparator non-GM *S. tuberosum* cv. Desiree.

(b) dissemination;

The Rpi gene transformed into *S. tuberosum* cv. Desiree confers decreased susceptibility to *P. infestans.* The cisgenic material has gone through glasshouse and field selection in the Netherlands and the inclusion of Rpi-vnt1.1 gene in the genome of cv. Desiree has not affected the mode and/or pattern of gene dissemination by cv. Desiree: which has been previously studied at Oak Park.

(c) survivability

The survival of potato tubers is dependent upon the temperatures the tubers are exposed to. It has not been observed in the Netherlands that the inclusion of the Rpi – vnt1.1 gene has had a significant impact on the frost tolerance of generated tubers.

[25] Jones, J. and Dangl, J. (2006). The plant immune system. Nature, 444, 323-329.

[26] Dangl, J. and Jones, J. (2001). Plant pathogens and integrated defense responses to infection. Nature 411:826-833.

5. Genetic stability of the insert and phenotypic stability of the genetically modified higher plant

The cisgenic A15-031 line to be studied at Oak Park is phenotypically stable as per previous testing conducted in the Netherlands. As the desired trait of increased resistance to *P. infestans* has been consistently expressed in successive generations it has been concluded that the inserts are genetically stable.

6. Any change to the ability of the GMHP to transfer genetic material to other organisms

The cisgenic A15-031 material is not expected to interact any differently within the agri-environment (with the exception of how it resists *P. infestans* infection) than its equivalent comparator *S. tuberosum* cv. Desiree. Previous discussions[27] concerning the potential impact of horizontally transferred antibiotic resistance genes from GM potato are irrelevant for this notification as the intended cisgenic material does not contain an antibiotic resistance marker gene.

7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification

Commercial potato already contains several NB-LRR type genes, several of which are derived from the wild potato species *S. demissum*[28]. No member of the NB-LRR protein class has so far been identified as possessing toxic and/or allergenic properties. The Rpi-vnt1.1 gene transformed into *S. tuberosum* cv. Desiree is derived from the related species *S. venturii* and has evolved to solely prevent infection by *P. infestans*. This *Solanum* genetic sequence is not expected to exert any toxic, allergenic or harmful effects on animal/human health and/or the environment.

8. Information on the safety of the genetically modified higher plant to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the genetically modified higher plant is intended to be used in animal feedstuffs.

The cisgenic A15-031 line intended for this release will not be used as animal feedstuff. Potato tubers and material not required for experimentation post-harvest will be destroyed by burial or by steam sterilisation.

9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable).

The target organism is *P. infestans*, which causes late blight disease of potato. The introduction of the Rpi-vnt1.1 gene will minimise and/or prevent the ability of *P. infestans* to infect potato tissue by conferring broad spectrum *P. infestans* resistance on the transformed potato. These Rpi genes encode receptors that recognize specific effectors delivered by *P. infestans* into infected plant cells. This recognition triggers a cascade of signalling events in the plant which delivers resistance through the development of hypersensitive lesions that appear on infected leaves. Equivalent levels of NB-LRR induced hypersensitivity is also displayed in conventional potato

[27] http://www.efsa.europa.eu/en/efsajournal/pub/323.htm
[28] Wastie, R. L. (1991). Breeding for resistance. Advances in Plant Pathology, 7, 193-224.

cultivars such as cv. Sarpo Mira and cv. Bionica, which possess resistance to certain genotypes of *P. infestans*.

10. Potential changes in the interactions of the genetically modified higher plant with non-target organisms resulting from the genetic modification.

The reaction between the Rpi genes and the corresponding avirulence factors of *P. infestans* are highly specific[29]. Due to this level of specificity between host and pathogen no effects on other organisms other than *P. infestans* can be expected by the release of the determined cisgenic plant material. It is hypothesised that the reduced fungicide treatments, which are planned as part of the experimental research of the intended release, will impact on levels of biodiversity across the plot. This will be investigated over the course of the notification, with specific emphasis on soil microbial populations.

11. Potential interactions with the abiotic environment.

The Rpi-vnt1.1 gene introduced into *S. tuberosum* cv. Desiree is solely related to conferring broad spectrum resistance against multiple genotypes of *P. infestans*. It does not serve a function in abiotic stress. As such it is not expected that the expression of hypersensitivity by the cisgenic *S. tuberosum* cv. Desiree A15-031 line will alter the GM line's response to frost, drought or salt tolerance, relative to their non-GM comparators.

12. Descriptions of detection and identification techniques for the genetically modified plant.

A PCR-based assay has been developed to identify the presence of the Rpi-vnt1.1 gene and the protocol is detailed in Appendix 1.

13. Information about previous releases of the genetically modified plant, if applicable.

The cisgenic line described in this notification is currently being trialled in the Netherlands under Notification B/NL/10/06. No unanticipated effects have been recorded. During the course of this EU 7th Framework funded project, the same material will also be cultivated in Finland under license by the respective competent authority.

[29] Vleeshouwers, V. G. et al. (2008). Effector Genomics Accelerates Discovery and Functional Profiling of Potato Disease Resistance and Phytophthora Infestans Avirulence Genes PloS ONE 3, e2875).

E. INFORMATION RELATING TO THE SITE OF RELEASE (ONLY FOR NOTIFICATIONS SUBMITTED IN ACCORDANCE WITH ARTICLE 14)

1. Location and size of the release site(s).

The location of the proposed field study is the Teagasc Crops Research Centre, Oak Park, Carlow. In 2012, it is proposed that a single plot will be sown, of a size no greater than 1 acre. To ensure statistical validity of collated datasets, two sites will be sown in 2013, 2014 and 2015. Each site will not exceed 1ha in total and each site will be defined and measured by GPS, to facilitate site identification and monitoring in subsequent years.

2. Description of the release site ecosystem, including climate, flora and fauna.

Oak Park is home to the National Centre for Arable Crops Research. Situated on 225 hectares, the main objective of the work programme at Oak Park is to support the Irish arable crops sector. To ensure that the research and services at Oak Park are of the highest quality the Centre possesses laboratories and workshops equipped with state of the art technology for analytical and research purposes.

Situated 52 51' 12" N latitude and 6 55' 15" W longitude, Oak Park is 58m above sea level. Average rainfall (30 year mean) is 786mm, with yearly mean air temperatures recorded at 9.4^{o}C. The soils at Oak Park are derived from limestone drift material which overlies limestone bedrock. Two broad groups of soils occur on the farm – light textured gravelly soils derived from limestone gravels and heavy textured soils derived from limestone till commonly known as boulder clay. The light textured soils which are mainly derived from outwash gravels vary from very coarse textured (10-13% clay), shallow (30 cm deep) brown earths on eskery gravels to wet gravelly soils on the lower part of the farm near the lake. The major portion of the gravelly soils on the farm consist of moderately deep (50-70 cm deep) free draining brown earths (grey brown podzolics) sometimes with a textured B subsoil horizon overlying coarse gravels. The heavy textured soils on the farm derived from boulder clay consist mainly of deep (100 cm +) free-draining grey brown podzolics. The soil profile consists of a loamy surface horizon (18-22% clay) over a thick textured B horizon which contains up to 42% clay. The trial sites will be isolated from all conventional potato experimental plots and will have been free of potato cultivation for a minium of 5 years. Typical fauna within the estate of Oak Park include rabbits, hares, foxes, mice and rats. Bird species include pigeons, blackbirds, crows, ravens, swallows, swifts and pheasant. The lake is home to swans, ducks and migratory birds. During the growing season, observed fauna include cabbage butterfly, bumblebees and honey bees.

3. Presence of sexually compatible wild relatives or cultivated plant species.

Potato has no sexually compatible wild relatives in Ireland. Of the two wild relatives of commercial potato in Ireland, a previous survey by Teagasc identified populations of *S. nigrum* on the Oak Park estate. Hand-mediated crosses between *S. nigrum* and *S. tuberosum* cv. Désireé did not lead to the formation of viable progeny. This result confirmed that *S. tuberosum* is genetically incompatible with the Irish ecotypes of *S. nigrum* found in Oak Park. As part of other research programmes, conventional potatoes are cultivated on the Oak Park estate on an annual basis. No potatoes will be cultivated within 40m of the perimeter of the GM field study.

4. Proximity to officially recognised biotopes or protected areas which may be affected.

There is no protected biotype located in the vicinity of the trial site.

F. INFORMATION RELATING TO THE RELEASE (ONLY FOR NOTIFICATIONS SUBMITTED IN ACCORDANCE WITH ARTICLE 14)

1. Purpose of the release.

As part of a crop biotechnology initiative, Teagasc has been researching the impact of specific GM crops on the Irish agri-environment since 2002. Resulting studies have highlighted the relevance of GM late blight resistant potato in light of future environmental and legislative challenges[30] and the potential impact this crop could have on the Irish agri-environment[31].

In 2011, Teagasc secured funding as part of a pan-European research consortium through the EU's Seventh Framework Programme (FP7) for Research and Technological Development[32]. Entitled 'AMIGA' (*Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems*), the project has 22 partners from Research Centres, Universities, State Agencies across 15 EU countries[33]. AMIGA has 4 primary objectives:

- Provide baseline data on biodiversity in agro-eco-systems in the EU,
- Identify suitable bio-indicators that permit a better integration of GM field experimentation across specific agricultural ecosystems in the EU,
- Deliver an improvement of knowledge on potential long-term impacts of specific GM crops,
- Assess the economic effects of cultivation of GM crops in the EU.

The final outcome of the AMIGA project is to establish a database of pre-market risk assessment and long-term monitoring to evaluate impacts and ultimately assist policy-makers and regulatory agencies such as the EPA. As a partner in this consortium Teagasc has been tasked with three primary research goals that underpin the purpose of this release. Specifically, the purpose of this release is to:

- Quantify the impact of GM potato cultivation on bacterial, fungal, nematode and earthworm diversity in the soil, compared to a conventional potato system.
- Identify integrated pest management (IPM) strategies and components which could be positively or negatively affected by the adoption of GM late blight resistant potato.

[30] O'Brien, M. and Mullins, E. (2009). Relevance of genetically modified crops in light of future environmental and legislative challenges to the agri-environment. *Annals of Applied Biology*, 154, 323-340.
[31] Collier, M. and Mullins, E. (2010). The CINMa Index: Assessing the potential impact of GM crop management across a heterogeneous landscape. *Environmental Biosafety Research*, 9, 135-145.
[32] 'FP7' refers to the Seventh Framework Programme for Research and Technological Development. This is the EU's main instrument for funding research in Europe and is also designed to respond to Europe's employment needs, competitiveness and quality of life (http://cordis.europa.eu/fp7/home_en.html)
[33] http://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&rcn=28673

- Employ the project's resources as a tool for education and demonstration in order to proactively engage and discuss the issues that most concern stakeholders and the public at large in regards to the cultivation of GM crops in Ireland.

To achieve the project's objectives, identical field experiments will also be conducted by partners in the Netherlands and in Finland. The GM potato seed to be sown in Oak Park has been developed as part of a publicly funded project in the Netherlands[34]. The GM line contains broad-spectrum blight resistance, conferred by the Rpi-vnt1.1 gene taken from the wild potato species *S. venturii*.

Critically, there is no involvement by the ag-biotech industry in the proposed field experimentation in Oak Park. As a public research body, Teagasc commit to making all datasets publicly available once the project's deliverables have passed the scientific standard of international peer-review. Upon completion of this notification, it is not the intention of Teagasc to seek consent for the placing on the market of the GM potato lines cultivated in this study.

2. Foreseen date(s) and duration of the release.
It is intended that the GM lines will be released from March to October in 2012, 2013, 2014 and 2015. Dependent on climatic conditions and seed availability, the GM lines will be sown during April – June. Data collection will continue through the growing season and the crop will be harvested by year's end.

3. Method by which the genetically modified plants will be released.
For 2012, the GM lines will be planted as mini-plants, having been propagated from sterile *in vitro* cultures and grown up under glasshouse conditions. For 2013-2015, the GM lines will be sown as tubers or mini-tubers. In 2012, sowing will be by hand, with standard mechanical machinery used for 2013-2015.

4. Method for preparing and managing the release site prior to, during and post-release, including cultivation practices and harvesting methods.
Stewardship of the site will be in accordance with standard conventional practises for the cultivation of commercial potato. This relates to the application of fungicides (when required as experimental controls), insecticides, herbicides and fertilisers. The GM potato lines will be planted to a replicated randomised block design in order to ensure statistically appropriate replication. While it is intended to capitalise on the high levels of *P. infestans* inoculum that is typical of Irish summers, in instances where disease pressure is low, artificial inoculations will be completed on bait plants (*S. tuberosum* cv. Bintje) within the plot. Once data collection is complete, the crop will be desiccated (using a regulated defoliating agent) and GM lines will be harvested by hand or machine.

5. Approximate number of plants (or plants per m^2).
In 2012, approximately 100 plants will be sown. For 2013-2015, the number of respective tubers will depend on the outcome of the experimental research in the previous year. Teagasc will ensure all information (including planting plans and detailed plot maps) in this regard is presented to the EPA prior to the respective

[34] http://www.durph.nl/UK/

sowing dates. GM tubers will be sown as per conventional crop densities of approximately 30 plants per m^2 and the sites for 2013, 2014 and 2015 will not exceed 1 ha in size.

G. INFORMATION ON CONTROL, MONITORING, POST-RELEASE AND WASTE TREATMENT PLANS (ONLY FOR NOTIFICATIONS SUBMITTED IN ACCORDANCE WITH ARTICLE 14)

1. Any precautions taken:

(a) distance(s) from sexually compatible plant species, both wild relatives and crops;

There are no sexually compatible wild species of commercial potato in Ireland. A minimum distance of 40m will be maintained between neighbouring conventional potato crops and the perimeter of the GM trial. This is equivalent to the distance consented to under Notification No. B/IE/06/01[35].

(b) any measures to minimise/prevent dispersal of any reproductive organ of the genetically modified higher plant (for example, pollen, seeds, tuber).

Pre-sowing and post-harvest, all GM tubers/plantlets will be stored in the contained facility at Oak Park, where GM potato research has been ongoing for several years under EPA license. GM material will be transported to the cultivation site in closed labelled containers and GM material that is not sown will be bagged before removal off site for appropriate storage or destruction. The latter will be achieved by validated steam sterilisation or burial in a designated pit (~6 feet in depth) for larger volumes. Accurate records will be kept of how many tuber/plantlet populations are stored before sowing, how many have been sown, how many destroyed/remain in storage, how many harvested.

The consequence of pollen dispersal outside the perimeter of the site will be negated by the implementation of a 40m isolation distance from any sexually compatible potato crop. Based on previous experience of potato gene flow trials conducted at Oak Park, the most effective method to minimise potential gene dispersal arising from formed berries is to contain material within the site of study. Therefore, formed berries will not be physically removed from the plants, but will be left to drop off. Animal and/or bird predation is not applicable, owing to the glycoalkaloid content of berries. Due to the poor agronomic competitiveness of potato volunteers the most effective way to eliminate the resulting volunteer population that will arise from the dropped berries is to apply a broad spectrum herbicide and then return the site into grass immediately after harvesting.

As the GM sown sites will be an active research experiment, project staff will visit sites several times weekly during the growing season for the purposes of data collection but also to minimise/prevent the movement of material from the site. Specifically, any tubers exposed above the soil surface will be covered or if this is not possible, they will be bagged before removal off site for appropriate destruction. This

[35] http://www.epa.ie/downloads/pubs/other/gmo/field/EPA_gm_potato_field_trial.pdf

will be achieved by steam sterilisation or burial in a specific pit (~6 feet in depth). The chopping of potato tubers will not be employed as a method of disposal.

An intensive harvesting approach will be adopted to ensure that as much as logistically possible all tubers are removed from the site. This will involve a minimum of two additional harvest cultivations after the initial harvesting, which will be completed mechanically or by hand. Once complete, the site will be walked a number of times by project staff to ensure that no tubers/tuber pieces remain on the surface. All tubers harvested will be collected in labelled containers, which will be sealed before being transported off site. All machinery used on the site will be cleaned and thoroughly inspected prior to, and after use on the site, with project staff signing hygiene declarations to confirm machinery is free of cisgenic material on departure from the site. All containers and vehicles used to transport material will be checked to ensure there is no risk of accidental material loss during transfer to and from the field site. A complete record will be maintained for each site; cataloguing all crop management operations and the date they started and finished.

2. Description of methods for post-release treatment of the site.
Based on previous Teagasc research the most appropriate cultivation to minimise groundkeepers and volunteers post-potato is to sow perennial ryegrass on the sites after GM potato. Although the grass will compete better with groundkeepers than a spring cereal, potato groundkeepers can still be expected to emerge through the grass canopy. These will be destroyed through the application of a commercially available herbicide. As part of a case-specific monitoring plan, the site will be monitored for at least 4 years post-harvest of the potato crop for the emergence of groundkeepers or volunteers that arise from dormant true potato seed and/or dormant groundkeepers.

3. Description of post-release treatment methods for the genetically modified plant material, including wastes.
Potato harvesting will be completed by hand or by mechanical means using conventional potato harvesters. These will be inspected and cleaned thoroughly before and after harvesting of the site. Harvested tubers will be removed off the field in closed, labelled containers and stored in a secure location within the biotechnology building. The plots will undergo repeat harvesting to minimise tuber loss post-harvest and the site will then be surveyed by project personnel who will collect mini tubers and tuber pieces in sealed bags for disposal by steam sterilisation or burial. Above green tissues from the GM plants will be destroyed prior to harvesting with a chemical application and will then remain on the release site for decomposition. It is intended that the GM tubers collected from the 2012 harvest will be used to supplement the number of tubers required for the following 2013 season and the same will occur from 2013-2014 and 2014-2015. Between growing seasons, all GM tubers will be stored within the confines of the EPA licensed laboratory-glasshouse facility in Oak Park. This will ensure their complete separation from any non-GM commercial potatoes. No tuber material will be supplied to animals for feed purposes during the course of the study.

4. Description of monitoring plans and techniques.
An on-going environmental risk assessment will be carried out in accordance with the principles and methodology outlined in Parts B and C of the Second Schedule of S.I. No 500 of 2003. The goal of the monitoring strategy for this release is to identify as

quickly as possible intended and unintended effects that may arise during the release of the GM potatoes. This is a primary research objective of the AMIGA project, which is tasked with quantifying the environmental impact of the cisgenic potato lines. Specifically, project participants will study indicators of biodiversity both above and below ground that might identify characteristics which may cause adverse effects. Teagasc will evaluate the potential consequences of each adverse effect, if it occurs.

In addition, a separate Teagasc project will conduct feeding studies on the GM potatoes, and a suitable non-GM comparator. It is intended that this will form the basis of a Teagasc PhD Walsh Fellowship. The studies will be based on EFSA guidelines and international best practise as per the recently completed GMSAFOOD project[36]. Teagasc was a main partner in this project and research staff based at Teagasc Moorepark will supervise the proposed feeding studies.

Observations during release

As part of the research work, the site(s) of cultivation will be monitored on a weekly basis by project staff during the growing season. Soil samples will be taken at regular intervals and in accordance with scientific standards, in order to quantify the impact of the GM lines on soil microbial populations (e.g. bacteria, fungi, nematodes and earthworms). Agronomic assessments will be made during the growing season to ensure that the performance of the GM lines is substantially equivalent to that of the respective comparator plants, which will be grown in parallel to the GM lines within each site. As Teagasc has extensively studied the degree of pollen flow from *S. tuberosum* cv. Desiree and the consequence of this gene flow across several sites in separate years at Oak Park [37], no additional pollen flow studies are planned during this study. Site visits will be recorded in field notebooks and all experimental data and observations will be freely available to the EPA as they require. Information regarding any unexpected occurrences of relevance regarding potential adverse effects on the environment and/or human and animal health directly related to the GM potato lines will be communicated to the EPA without delay and required measures will be implemented accordingly.

Observations after release

The results of the feeding studies will be forwarded to the EPA within one month of completion. After harvesting of the GM potato sites, a volunteer monitoring and management programme will commence. Each site will be sown with perennial ryegrass which is a strong competitor for nutrients over potato volunteers and to a lesser extent groundkeepers. Project staff will monitor the sites for groundkeeper proliferation and counts will be made (using a $1m^2$ quadrat) to gauge the degree of groundkeeper emergence on each site. At 3-4 leaf stage, groundkeepers will be destroyed by selective herbicides (e.g. glyphosate). The site will then be tilled and put back into grass. This process will be repeated each year, for 4 years after the initial GM plantings.

5. Description of any emergency plans.

While a primary goal of the FP7 project is to proactively interact and debate the issues with the general public, it is incumbent on Teagasc to prepare for the possibility that

[36] http://www.gmsafoodproject.eu/Default.aspx?section=376

[37] Petti, C. Meade, C and Mullins, E. (2007). Facilitating co-existence by tracking gene dispersal in conventional potato systems with microsatellite markers. Environmental Biosafety Research 6(4), 223-231.

the site could be vandalised. As part of the stakeholder consultation, An Garda Siochana will be informed of the planting plans and the risk of vandalism. However, should the trial become a target for vandalism, action will be taken to prevent the removal of potatoes from the site by persons not associated with the project proper. These measures will include the removal and destruction of uprooted plants and/or the termination of the trial via herbicide application. Teagasc will inform the EPA of such actions.

6. Methods and procedures to protect the site.
The trial sites will be situated on the Oak Park campus in Carlow, which is currently monitored by CCTV systems and an externally contracted security provider. In addition, the individual GM potato plots will be fenced off to prevent animals from entering.

H. RISK ASSESSMENT

1. Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats
The R gene that has been transformed into *S. tuberosum* cv. Desiree to produce the cisgenic line A15-031 will only confer a competitive advantage in the presence of *P. infestans* inoculum. In this scenario, A15-031 will display broad spectrum resistance to the oomycete pathogen, which its non-cisgenic comparator *S. tuberosum* cv. Desiree will not. Potatoes are not competitive outside the confines of the managed cropping system. The Rpi-vnt1.1 gene does not confer a competitive ability to A15-031 that would permit it to compete outside the managed confines of the agricultural system. Similarly, the Rpi-vnt1.1 does not alter the reproductive characteristics of the cisgenic line A15-031. Hence the pollen, tuber and true potato seed production of A15-031 will be equivalent to that of the comparative non-cisgenic cv. Desiree, in the absence of *P. infestans* disease pressure. Therefore, the introduced cisgene RPi-vnt1.1 is not anticipated to confer any difference compared to conventional potato varieties with respect to persistence in agriculturally managed habitats or invasiveness in non-manged ecosystems. Risk management of the field research will include ensuring that the cisgenic line A15-031 is sufficiently isolated with a 40m segregation distance from any other non-GM potato crops to prevent admixture events and that the occurrence and eradication of groundkeepers and volunteers post-harvest is carefully monitored for up to 4 years after the experimentation is completed. As a result, the impact of the proposed notification will be negligible on neighbouring habitats and there is no propensity for the cisgenic A15-031 from becoming any more persistent that its equivalent non-cisgenic comparator.

2. Any selective advantage or disadvantage conferred to the GMHP
The cisgenic line A15-031 only possesses a significant selective advantage over its non-cisgenic comparator within the managed environment of a cropping system and only in the presence of *P. infestans*. In this situation, A15-031 will exhibit broad spectrum resistance to the pathogen, in contrast to the non-cisgenic cv. Desiree which will display high susceptibility to the disease; in the absence of standard fungicide treatment. In the absence of *P. infestans*, A15-031 is substantially equivalent to its

non-cisgenic comparator, with the exception that A15-031 contains the Rpi-vnt1.1 gene, from the wild potato species *S. venturii*. Volunteer management practises will ensure effective control of groundkeeper populations. Coupled with the fact that *P. infestans* resistance is not a key determinant for inducting potential invasiveness, it can be concluded that A15-031 will not exhibit a fitness advantage that will permit it to establish outside of a managed tillage system.

3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage to those plant species.

There is no risk of successful gene transfer to wild *Solanum* species based on Irish-specific research conducted by Teagasc, which supports peer-reviewed literature on the subject. Pollen-mediated gene transfer is possible between conventional potato varieties but for this notification, the potential for pollen-mediated gene transfer is mitigated due to the imposition of a minimum 40m isolation distance around the perimeter of the GM trials. In the unlikely event that cisgenic pollen reaches conventional varieties, the consequences of such an event are negligible, as the transfer of the cisgene does not confer a selective advantage or disadvantage (see ii). The cisgenic line A15-031 will produce tubers and berries that bear true potato seed and hence a potential for seed-mediated gene flow does exist. However, a comprehensive risk management strategy has been designed to contain both berries and tubers on site, pending removal for storage or destruction. Critically, there is no risk of the cisgene being introduced into conventional potato crops as potato is propagated clonally.

4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and pathogens (if applicable)

The target organism of the Rpi-vnt1.1 cisgenic sequence is the oomycete pathogen *P. infestans*. The immediate effect of expressing this sequence in the presence of disease is that the GM potato lines will exhibit broad spectrum resistance to multiple genotypes of *P. infestans*. The cisgenic sequence has not evolved to target other organisms and is *P. infestans*-specific. By conferring resistance on the host to *P.* infestans, the inoculum pressure in the field will be reduced as *P. infestans* will not be able to sporulate to the degree that it would on conventional cultivars. This is equivalent to the scenario that occurs in conventional potato fields which receive fungicide control sprays every ~7 days to combat *P. infestans*, which in turn reduces the ability of the pathogen to sporulate. As conventional fungicide programmes impact significantly on a range of non-target organisms, it can be expected that this scenario will be reversed in the presence of the cisgenic A15-031 and that the environmental impact of the GM line will be minimal compared to conventional agricultural practise.

5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organism), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

The Rpi-vnt1.1 is specific to *P. infestans*, conferring broad spectrum resistance to multiple genotypes of *P. infestans*. Irish populations of *P. infestans* have not

previously been exposed to this R gene and the physical manifestation of resistance in the plant will be displayed by the presence of hypersensitive lesions appearing on the leaf surface, where *P. infestans* spores have attempted to gain entry to the host. Due to the specificity of the host's response, no effects on other organisms than the target pathogen are expected, other than those that apply to the interaction with conventional potatoes under standard management practises. It is expected that there will be an impact on non-target organisms that are typically targeted by standard fungicide programmes. Indeed, quantifying and investigating this will form part of the research targets with specific emphasis on monitoring any fluctuations in the soil ecology in response to the removal of fungicide treatments as per standard practise.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP releases

The cisgenic line A15-031 is equivalent to the conventional potato cultivar Desiree with the exception of the presence of the Rpi-vnt1.1 gene. Conventional potatoes already possess Rpi genes that have been overcome by the pathogen. The Rpi-vnt1.1 is derived from the wild potato species *S. venturii* and there is no evidence to suggest that this cisgene, or any other Rpi genes that exist in conventional potato varieties exert any toxic or allergenic effects to human health. The impact on human health is therefore negligible.

7. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.

Cisgenic potatoes produced during this notification will not be used for animal feed purposes. Measures are included in the risk management programme to mitigate the impact of wild animals feeding on the site. Hence the impact on animal health is negligible.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

No effects on biogeochemical processes are expected with the cultivation of the cisgenic line A15-031. This is because the Rpi-vnt1.1 gene has evolved to interact only with *P. infestans* and hence confer resistance upon the host against the pathogen. The protein produced as a result of the expression of the Rpi-vnt1.1 gene only interacts with *P. infestans* effector proteins. In contrast to standard potato cultivation regimes, the growing of A15-031 is likely to impact positively on soil organisms and this will be studied during the course of the notification by project staff.

9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs

The field sites to be researched during the notification will be managed according to standard conventional potato practises, with the exception that the cisgenic plots will receive a reduced or zero fungicide input. At the end of each year, plots will be mechanically harvested 3 times, to facilitate the mitigation of seed-mediated gene flow as per the risk management protocols. With the exception of a potentially

positive impact on soil-flora populations and dynamics, the impact of cultivating the cisgenic potatoes on the environment will be negligible.

Step 1 ***Potential adverse effect (hazard) which may be caused by the characteristics of the GM plant***	***Step 2*** ***Evaluation of the potential consequences of each adverse effect if it occurs***	***Step 3*** ***Evaluation of the likelihood of the occurrence of each identified potential adverse effect***	***Step 4*** ***Estimation of the risk posed by each identified characteristic of the GMO***	***Step 5*** ***Application of management strategies for risks from the deliberate release***	***Step 6*** ***Determination of the overall risk of the GMO***
Increased invasiveness in natural habitats or persistence in agricultural habitats.	The potential consequences are negligible because the Rpi-vnt1.1 gene is specifically targeted against *P. infestans.* In addition, potatoes are not an invasive crop species. Within the managed system physical and chemical methods are used to control volunteers.	Highly unlikely. The Rpi-vnt1.1 gene neither confers characteristics to the GM potato that add competitive abilities in unmanaged ecosystems or allow the cisgenic line to compete against plants of similar type for space. None of the characteristics transferred to the potato plants is anticipated to affect pollen production/fertility, seed dispersal or frost tolerance.	Negligible .	Groundkeepers and volunteers arising from true potato seed will be controlled by a robust management protocol which is based on experience of gene flow trials previously conducted by Teagasc at Oak Park.	Negligible.
Selective advantage – improved resistance to *P. infestans*	The consequence of the Rpi-vnt1.1 cisgene is to increase resistance to *P. infestans,* therefore a selective advantage is conferred in the cisgenic line in comparison to untreated non-resistant conventional potatoes.	Likely. The intended effect of the genetic modification is to improve the resistance to *P. infestans*. Thus under *P. infestans* pressure resistant potatoes are intended to have a selective advantage in comparison to untreated non-resistant conventional potatoes in the agricultural environment.	This will only be advantageous in the confines of a managed cropping system. Potato plants are rarely seen outside the field and there is no evidence to show that resistance to *P. infestans* is the key determinant for potential invasiveness of potatoes.	Robust management protocol for volunteer management and monitoring for potential escapes through surveys of surrounding area.	Negligible.

Selective advantage or disadvantage conferred to sexually compatible plant species	Negligible. Potato is clonally propagated so there is no potential of the cisgene introgressing into conventional potato systems. .	Very unlikely. There are no sexually compatible wild relatives present in Ireland. Pollen-mediated gene flow to cultivated potatoes is possible, but is mitigated by the imposition of a minimal isolation zone of 40m around the GM plots.	In the unlikely case that pollen is transferred to non-genetically modified potatoes, the consequences are negligible since potato is a vegetatively propagated crop.	Isolation distance to other potato crops.	Negligible.
Potential environmental impact due to interactions between the GM plant and target organism (*P. infestans*)	Minimal. The intended effect of the transferred resistance genes is to reduce infection by *P. infestans*, thereby this will reduce the sporulation potential of *P. infestans*.	Very likely if climatic conditions are suitable for *P. infestans* outbreaks during the growing season.	The risk of the intended effect is minimal to the environment and will only impact on *P. infestans* within the GM plots. The outcome desired with the introduction of the Rpi-vnt1.1 cisgene is what is typically achieved with standard *P. infestans* control measures in conventional potato systems.	None.	Negligible.
Potential environmental impact due to interactions between the GM plant and non-target organisms	The potential environmental impact with NTOs is negligible as the Rpi-vnt1.1 is targeted to *P. infestans*. Any other impact will be equivalent to that typically recorded with	Very unlikely due to specificity and mode of action of R-genes.	Any effect on non-target organism due to the introduced trait of *P. infestans* tolerance is anticipated to be comparable to that of non-genetically modified potatoes under conventional agricultural	Monitoring plan including observations on disease and pest susceptibility.	Negligible.

	the cultivation of conventional potatoes.		practice. Due to a reduced need for fungal treatments an increase in the populations of non-target organisms might be expected.		
Potential effect on human or animal health due to introduced R-genes	Negligible. Rpi genes are not known to confer toxic or allergenic properties.	Very unlikely. Rpi genes not known to confer toxic or allergenic properties.	Material from field trial not intended for human/animal consumption.	Measures with regard to planting, harvest, storage and transportation minimize the contact to humans and animals.	Negligible.
Potential effects on biogeochemical processes (changes in soil decomposition of organic material)	Negligible. None of the newly expressed proteins is expected to be exuded from the cisgenic plants to the soil.	Unlikely. Soil fertility is not expected to be affected differently due to the cultivation of the genetically modified potato plants as compared to conventional potatotes. None of the newly expressed proteins is expected to be exuded from the plants to the soil.	Negligible. Any effect is expected to be comparable to that of non-genetically modified potatoes under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in soil microflora is hypothesised and will be the focus of research.	None.	Negligible.
Possible environmental impact due to changes in cultivation practice	Minimal. Potential positive effects on the population of soil organisms, due to a reduction in fungal treatments.	Likely. Application of conventional agricultural practice, except for a reduction in fungal treatments against *P. infestans*.	Potential positive effects on the population of soil organisms.	None.	Negligible

APPENDIX 1.

DNA isolation and quality control

Trans and cis-genic plants were maintained in vitro culture. After transfer of cuttings to the greenhouse young leaf material was collected and genomic DNA was isolated according to van der Beek et al. (1992). This method was modified to a high-throughput procedure, using the Retsch machine (Retsch Inc., Haan, Germany) and 96-deep-well Costar microtiter plates (Corning Inc., Corning, NY, U.S.A.). DNA yield was confirmed by agarose gel electrophoresis, successively DNA yield was quantified by OD_{260} measurements. After dilution to 20ng/µl, DNA quality was confirmed by EF1α primers (Table 2) which target an endogenous housekeeping gene in the potato genome. All DNA samples were positive for this test (Table 1).

RNA isolation, quality control, and cDNA synthesis

Young leaf material was collected and genomic RNA was isolated using the Qiagen RNaesy kit. RNA quality was confirmed by agarose gel electrophoresis, and quantified by OD_{260} measurements. 1 ug of RNA was used for cDNA was synthesis using the iScript kit from Biorad**,** according to the manufacturer's instructions.

Regular PCR mix (Goldstar™):

10xPCR buffer	1.5	µl
$MgCl_2$ (25 mM)	1.2	µl
dNTPs (5 mM)	0.3	µl
primer 616 (10 µM)	0.3	µl
primer 617 (10 µM)	0.3	µl
Goldstar Taq (5 U/µl)	0.03	µl
MQ	7.37	µl
DNA (20 ng/µl)	2.00	µl
Total	15.00	µl

PCR-program:

94°C 5 min
94°C 30 s
Ta[1)] 30 s X 35 (Annealing temp as indicated in Table 2)
72°C 1 min
72°C 10 min

qPCR mix

Samples are split in 2 x 20µl reactions (technical replicates) and qPCR is performed in a CFX96 realtime system (Biorad) using the standard PCR program.

Green Supermix ™ (Biorad)	2.5	µl
primer 3µM	4.5	µl
primer 3µM	4.5	µl
MQ	11.5	µl
20ng/ul DNA	2	µl
Total	45	µl

Table 2. Primers used for backbone and T-DNA PCR

Primer name	sequence (5'---> 3')	Target gene	Annealing Temp (°C)	length of PCR product (bp)
	ATTGGAAACGGATATGCTCCA	*Ef1*-alfa	57	200
	TCCTTACCTGAACGCCTGTCA			
	CCTTCCTCATCCTCACATTTAG	*Rpi-vnt1*	60	302
	CTCATCTAATAGATCCTCCAC			
	CTGCTAGGTAGCCCGATACG	TetA	61	296
	CCGAGAACATTGGTTCCTGT			
	GAAAGCTGCCTGTTCCAAAG	NptIII	61	162
	GAAAGAGCCTGATGCACTCC			
MN591	cccgccaatatatcctgtca	pBINAW2	61	435
MN592	gaagcttcgtgcaacctctc	*Rpi-vnt1*	60	
MN593	acaccgttcgtcccaattta	*Rpi-vnt1*	60	513
MN594	tggcaggatatattgtggtgt	pBINAW2	58	
MN657	TATCCTGTCAggtacgaattc	RB	54	425
MN659	tggtgtaaacTCTAGAGGATC	LB	51	498

Table 3. Primers used for Q-PCR

sequence (5'---> 3')	Target gene
ATTGGAAACGGATATGCTCCA	*Ef1*-alfa
TCCTTACCTGAACGCCTGTCA	
ATGAATTATTGTGTTTACAAGACTTG	*Rpi-vnt1*
CAGCCATCTCCTTTAATTTTTC	