# Part B: Information about the release application to be included on the public register

## B1 The name and address of the applicant

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## B2 A general description of the genetically modified organisms in relation to which the application is being made

The organisms to be released are genetically modified potato plants that have been modified to improve different traits: resistance to *Phytophthora infestans* (the organism responsible for the devastating late blight of potatoes), resistance to potato cyst nematodes (PCN) and improved tuber quality. These traits have been introduced in different combinations. To generate the genetically modified plants, *Agrobacterium*-mediated transformation was used, which utilises the natural ability of the soil bacterium *Agrobacterium tumefaciens* to stably incorporate foreign DNA into the genotype of plants.

Most of the transgenic lines contain plant resistance (*R*) genes (*Rpi-amr3i*, *Rpi-amr1e* and/or *Rpi-vnt1.1*), introduced as single *R* genes or combined as a 3-*R*-gene stack. Deployment of *R*-gene stacks has the potential to confer a more efficient and durable resistance by combining different recognition specificities. These genes were isolated from the wild potato relatives *Solanum americanum* and *Solanum venturii* and they confer useful resistance against different isolates of the late blight pathogen. *R* genes enable plants to recognise certain isolates of the pathogen, which possess a specific corresponding avirulent effector gene. The recognition event triggers a signalling cascade culminating in expression of the plant defense response, which acts to prevent further pathogen growth within the host plant. All of these *R* genes are regulated by their endogenous promoters and terminators. These terminator sequences regulate the expression of the genes and are **not related** to any technology that prevents seed propagation of plants.

Some of the transgenic lines contain a stack of two genes that confer resistance against PCN, which are an important problem in British agricultural potato fields. The expression of these genes is targeted to the plant root system, which is the organ invaded and affected by PCN. The first gene encodes a variant of a rice cysteine proteinase inhibitor (‘cystatin’). When this cystatin is expressed in potato roots it confers resistance against PCN thanks to its antifeedant activity. The second gene codes for a repellent peptide that is not derived from the gene of an organism. It has no known lethal effects as used. It merely prevents PCN from invading roots from soil. As consequence,

the nematodes deplete their lipid reserves and die. This is also the normal fate of PCN that fail to locate and invade wild type roots after hatching from dormant eggs. Deployment of a gene-stack conferring resistance by two different mechanisms has the potential to be a more efficient and durable strategy against PCN, compared to deployment of individual genes.

The stack conferring resistance against PCN has been introduced on its own, or combined with the 3-*R*-gene stack against late blight, with or without gene-silencing modules that improve tuber quality. These silencing modules are designed to silence the polyphenol oxidase gene *Ppo*, the asparagine synthetase-1 gene *Ast1* and the vacuolar acid invertase gene *Vlnv* in a tuber-specific manner. Silencing is triggered by the expression of sense and antisense sequences of the above-mentioned genes, and occurs via the endogenous gene-silencing mechanisms of plants.

The enzyme Ppo plays a major role in tuber discolouration after impact-induced bruising. Upon mechanical damage of the tuber, Ppo-mediated oxidation of polyphenols leads to the precipitation of black or brown pigment deposits. This phenomenon has a negative impact on tuber quality. One of the gene-silencing modules contains sense and antisense sequences derived from the gene encoding the predominant Ppo variant in tubers and its silencing significantly decreases enzymatic browning upon bruising.

Cold storage of tubers triggers the accumulation of reducing sugars (i.e., glucose and fructose). This process, known as cold-induced potato sweetening, is responsible for the potato blackening upon cooking at temperatures above 120 ºC in low-moisture environments. Blackening is the result of the accumulation of dark (and bitter-tasting) compounds, which are products of the non-enzymatic Maillard reaction between reducing sugars and amino acids. The enzyme VINV hydrolyses sucrose to glucose and fructose in the vacuole and its activity correlates with potato sweetening during cold storage. Silencing of the *Vlnv* gene decreases potato blackening upon cooking. To silence this gene in tubers, a second gene-silencing module contains sense and antisense sequences derived from the potato *Vlnv* gene flanked by convergent tuber-specific promoters.

The Maillard reaction also leads to the formation of acrylamide from reducing sugars and asparagine, which is the predominant free amino acid in potato tubers. Acrylamide is a neurotoxic compound and potential carcinogen. The Food Standards Agency (FSA) has recently released a report on foods with high potential for acrylamide formation, advising on actions to reduce dietary intake of this compound. Silencing of the *Vlnv* gene in tubers contributes to a reduction in the acrylamide-forming potential, since it decreases the availability of reducing sugars.

On the other hand, the enzyme Ast1 is the main responsible for asparagine formation in tubers. Therefore, silencing of the *Ast1* gene can further diminish the production of acrylamide by reducing the levels of asparagine in tubers. To

silence *Ast1*, the *Ppo*-gene-silencing module also contains sense and antisense sequences from the potato *Ast1* gene, all flanked by convergent tuber-specific promoters.

In addition to the previous traits, all the transgenic plants proposed for release in this application will also contain the *CSR* gene which confers resistance to some herbicides (sulfonylureas and imidazolinones). This trait will be used **only** for the ***in vitro*** selection of transgenic lines during tissue culture and these plants remain sensitive to other herbicides.

Even though the plasmids described above are designed to modify diverse traits, the goal of the proposed trial is to evaluate resistance to circulating *P. infestans* isolates in field conditions. Plants that only carry the PCN resistance trait will be used as negative controls.

## B3 The location at which the genetically modified organisms are proposed to be released

The plants will be released on an area of arable land no larger than 1000 metres squared located at the John Innes Centre (JIC, Ordnance Survey map grid reference TG 1707). Each year the area planted with the genetically modified plants will be approximately 100 metres squared. In accordance with potato planting practice, the plot will rotate within the release site each year of the trial. For each year of the field trial we estimate that the release will not exceed 250 transgenic plants.

## B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

Since 2001, we have been working towards identifying, mapping and isolating resistance (*R*) genes from potato that confer resistance against potato late blight (*Phytophthora infestans*). This research has been publicly funded. The genes identified are potentially valuable weapons in the fight against potato late blight as they confer resistance against many different isolates of this pathogen, including the strains which are currently responsible for major potato losses in the UK and Europe. Thus, there is a need to test these genes in a ‘real’ environment.

Previously, the gene *Rpi-vnt1.1* was isolated from the wild potato relative *Solanum venturii*. This gene was successfully tested in the field after being introduced into Désirée potato plants (Consent 10/R29/01). Recently, two other genes were isolated from the wild potato relative *Solanum americanum*: *Rpi-amr3i* and *Rpi-amr1e*. These three genes have now been transformed into Maris Piper potato, both as single genes or as a three-gene stack. Some of the plants proposed for release have the three-*R*-gene stack combined with genes

conferring resistance against potato cyst nematodes (PCN), with or without gene-silencing modules conferring increased tuber quality. Plants proposed as negative controls in the field trial will only carry the PCN resistance trait.

The aims of the trial are:
1) to demonstrate that the transferred late blight resistance genes offer a valuable method for controlling late blight of potatoes which does not rely on agricultural inputs (pesticides);
2) to confirm that the transferred resistance genes still function in a 'real life' situation (i.e. in a field as opposed to a lab/greenhouse);
3) to evaluate the performance of the three-*R*-gene stack in comparison to the *R* genes deployed individually;
4) to expose plants containing the newly identified genes to the local populations of late blight to confirm that they are indeed useful;
5) if infection does result in disease, to isolate the corresponding pathogen race.

Even though some of the plants will also carry genes related to nematode resistance and improvement of tuber quality, none of these traits are within the scope of the proposed trial. Those characteristics will be evaluated independently by our collaborators in the project.

Recently, the British Biotechnology and Biological Sciences Research Council (BBSRC) put in place the Horticulture and Potato Initiative (HAPI). This program is part of a BBSRC's strategy to support innovative developments in bioscience. The goal of the HAPI is to address challenges faced by the horticulture and potato industries in the UK, and funding has been granted for collaborative works between research institutions and industrial partners. One of those BBSRC's research grants supports this work. This means that if the project yields good results, the industrial collaborators within the partnership will support the steps towards commercialization, to make the benefits of the programme available to farmers, processors and consumers. Please see http://www.bbsrc.ac.uk/innovation/collaboration/collaborative-programmes/hapi/ for more details on the HAPI.

## B5 The intended dates of the release.

If consent is granted, this year's field trial will start in late May 2017 and will continue until 30th November 2017. The trial will then proceed for 3 more years (2018-2020), from 1st May until 30th November in each year. The exact timing of sowing of the trial will depend upon weather conditions at the time. Harvesting of tubers will take place during September or October of each year of the field trials.

## B6 The environmental risk assessment.

Four hundred years of cultivation have established that the potato has limited ability to survive in UK environments except when cultivated. Plants generated from tubers are readily eliminated and potato plants are not invasive of natural habitats. We expect no difference with respect to persistence in agricultural habitats or invasiveness into natural habitats as compared to conventional potato varieties under normal agricultural practice. The pollen of potato normally disperses less than 10 metres and cannot cross with other crop plants to produce hybrids. Through the precautionary measures undertaken for the duration of the release and the maintained distance from, or absence of, conventionally cultivated potatoes or wild species, the possibility of any gene transfer is effectively zero. Even in the very improbable event that pollen was to be transferred to non-genetically modified potato plants, no consequences are to be expected, since potato propagation conventionally takes place via tubers and not via seeds.

Most of the lines included in this application carry late blight resistance (*R*) genes. Non-transgenic potato plants also contain many *R* genes, which are active against a wide range of potential pathogens. Many conventional potato varieties also contain additional *R* genes against *P. infestans* that have been introgressed from wild *Solanum* species. An intended effect of the introduced trait is increased survivability of the genetically modified potatoes exposed to *P. infestans*. This possible selective advantage, however, is of importance only in the agricultural field, and will not otherwise improve the survivability in the surrounding environment.

Some of the transgenic lines included in this application will carry genes coding for a cystatin and for a repellent peptide. The expression of these genes is targeted to the plant root system and confers resistance against potato cyst nematodes (PCN). Cystatins are present in many foods, e.g. rice seeds, maize kernels and chicken egg white, and the repellent to be used is not lethal to animals, but merely prevents plant parasitic species from invading roots. Resistance to PCN is not a characteristic that would enhance the invasiveness of potatoes. Further, there's no evidence of PCN contamination in the soil of our experimental field as well as no evidence that PCN limit the distribution or abundance of wild Solanaceae in the UK.

The resistance traits to be expressed are predicted to affect only the target pathogens, *Phytophthora infestans* and PCN (if present). The expected environmental impact is negligible and will reduce the level of other agricultural inputs such as use of fungicides or nematicides to control late blight or potato cyst nematodes in potato crops.

The gene-silencing modules present in some of the plants are designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. They are not expected to affect the fitness of the plants in field conditions.

All the plasmids used to generate the plants included in this application carry

an allele of the tomato acetolactate synthase (*ALS*) gene encoding a variant of the ALS enzyme that is resistant to inhibition by sulfonylureas and imidazolinones. Resistance to ALS-inhibiting herbicides is present in several commercially-available crops. This trait will be used **only** for the ***in vitro*** selection of transgenic lines during tissue culture. The plants remain sensitive to other herbicides such as glyphosate or glufosinate, which could readily be used to eliminate them in the field. In addition, sulfonylureas and imidazolinones will not be used in the context of this trial, so no selective advantage will be conferred to this plants.

Finally, in the unlikely event of backbone integration, no detrimental effect is expected from any of the elements in the vector backbone and no other emergent advantages or disadvantages are expected from the proposed combinations of genes and traits.

The interactions of the genetically modified potato lines with non-target organisms and the effects resulting from this will be comparable to those of conventional potato varieties. Due to a reduced need for fungal treatments, an increase in the populations of those non-target organisms that respond to fungal treatments might be expected. Similarly, the nematode resistance trait has been previously tested in the field. Works performed with similar transgenic lines established considerable advantages of this approach to soil micro-organisms relative to nematicide use, with no detrimental effects on non-target organisms and soil health.

No toxic or allergenic effects are expected from any of the additional proteins expressed in the transgenic lines proposed for release. Measures which are taken under current release practice will both protect the trial against damage by wild animals, and also ensure that seed stock and plant material are harvested, transported and disposed in a way that minimises or prevents contact with people or animals. No effects on biogeochemical processes are expected, other than those that apply also to non-genetically modified potatoes.

## B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The release site will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals during May-November (the potato growing season) of each year of the trial. Visits will usually occur more frequently. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the national inspectorate immediately. Should the need arise to terminate the release at any point the emergency plans detailed below will be followed.

At the end of each season, the plot will be left fallow and monitored for groundkeepers during the remainder of the year. Any groundkeepers identified

will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed by heat treatment. The monitoring of the plot for groundkeepers will be continued at monthly intervals for the duration of the four-year trial by walking the trial site.

Following completion of the four-year trial the release site will remain fallow to enable easy identification of volunteers. The site will be inspected monthly between April and November and any volunteers identified will be immediately destroyed either by application of a systematic broadleaf herbicide or by hand pulling plants and digging out tubers/root systems. These will then be autoclaved within the Sainsbury Laboratory. If volunteers are found at the end of the two-year 'fallow' period, DEFRA recommendations will be followed for the management of the release site. Both raw data and reports of inspections of groundkeepers and volunteers will be maintained and provided to DEFRA. The cultivation of the release site after the monitoring programme has concluded will be according to local crop rotation practice for potatoes.

**Emergency procedures:** At any time point post planting, should the release need to be terminated, any plant material will be sprayed with an appropriate systemic broadleaf herbicide and tubers dug up by fork and hand and transferred to an authorised waste facility for disposal by deep burying or incineration. Should the release site be subject to vandalism, care will be taken to ensure that all uprooted plant material within and outside of the trial site is identified and destroyed accordingly as described above.