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 Brassicaceae family holds many agronomically important crop plants, including oilseed rape and cruciferous vegetables. Cruciferous vegetables can be found in a wide variety morphotypes and have been consumed the world over for many years, having been bred and selected for their distinct flavor properties. The distinct flavor of Brassica vegetables is due to elevated levels of sulphur-containing metabolites, including the secondary metabolites known as glucosinolates. Glucosinolates are produced exclusively by plants of the order Brassicales (also referred to as Capparales) in order to deter herbivory (Mithen et al., 2010). The production of these sulphur-containing secondary metabolites is of economic significance due to their putative health-promoting abilities upon human consumption.

Myb28 has been repeatedly and independently well characterized as a vital regulator of aliphatic glucosinolate biosynthesis across the *Brassica* genus, including homologues in *Brassica napus* (Li et al., 2014), *Brassica juncea* (Augustine et al., 2013) and *Brassica rapa* (Kim et al., 2013; Seo et al., 2016). It would be useful to understand the consequences of a knock out of this gene via CRISPR on sulphur metabolism and the production of glucosinolates. These plants have modified Myb28 sequence in order to remove gene function so that we may characterize the effect this genetic disruption has on the production of these sulphur compounds in a commercial environment, similar to that of the broccoli lines.

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 179 075). The land to be used is the area that was previously sown with GM potatoes under consent 10/R29/01 from 2010 – 2012. The area planted with the gene edited plants will be approximately 100 metres squared. We estimate that the release will not exceed 50 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).

This is a research trial to determine the role of a gene, known as Myb28, in regulating sulphur metabolism, specifically the accumulation of aliphatic glucosinolates, in field-grown *Brassica oleracea*. Brassica plants of this type, when grown under glasshouse conditions, produce almost undetectable levels of these compounds, therefore this trial is required in order to better imitate the commercial interaction between these compounds and their environment and ultimately how this transcription factor Myb28 may mediate this interaction. Field evaluation of these traits allows for a better understanding on improvement of these crops in the future. This trial is being proposed purely for exploratory purposes and no future commercial use or feeding trials are intended at this time.

B5 The intended dates of the release.

If consent is granted, this year's field trial will start in April and will continue until August, from 2019 to 2021. The exact timing of sowing of the trial will depend upon weather conditions at the time. Harvesting of material will take place during June or July, depending on weather conditions.

B6 The environmental risk assessment.

The probability of *B. oleracea* seeds or pollen escaping from the trial site or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. Plants grown in the trial will have their inflorescences removed at the early stages, prior to pollen exposure and therefore seed set. Primary inflorescences produced will be harvested for analysis, along with leaf material. Following this, all plants will be uprooted and destroyed in their entirety by autoclaving at the John Innes Centre to prevent further flowering. Less than ten individuals will be allowed to flower

with their inflorescences contained within a pollen proof bag, preventing pollen or subsequent seed release. *As B. oleracea* is unable to clonally propagate and reproduces exclusively through sexual reproduction, it is unlikely any residual plant material will lead to further emergence of plants. Moreover, no *Brassica* plants will be grown within 20 metres of the trial site and surrounding areas will be monitored for the presence of species capable of crossing with *B. oleracea*. If any species are found which may cross pollinate within 20m of the *B.oleracea* plants in this study, they will be treated with a herbicide (glyphosate). The potential removal of defence compounds, glucosinolates, in trial plants suggests that they would not possess a selective advantage over any existing Brassica plants and will be very unlikely to outcompete any wild or ruderal plants.

The risk of non-sexual, horizontal gene transfer to other species is extremely low. Current data suggests an absence of the transgene used to generate the mutation in this line and therefore an absence of the plasmid (further analysis of components of the vector backbone will be conducted to confirm this), further reducing the risk of any potential gene transfer. In the event of horizontal gene transfer to bacteria, neither the trait genes nor the marker genes would be expected to confer a selective advantage in the field environment under consideration. The genes introduced in *B. oleracea* have been inserted *via Agrobacterium tumefaciens*-mediated gene transfer. We estimate the likelihood of horizontal gene transfer as low and the consequences were it to occur, as negligible.

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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 April-August. Visits will usually occur more frequently. The release site will be fenced to protect against animal damage and entry by unauthorized persons. The site will also be monitored by remote security cameras visible from the John Innes Centre (JIC) reception which is manned throughout the day by JIC reception staff and by security guards out of normal working hours. 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.

All harvested material (plant tops) will be placed in sealed bags or containers and removed from site to an authorised waste disposal facility. Disposal will be carried out by incineration through our contractor SRCL.

Post-trial the release site will remain fallow to enable easy identification of volunteers. The site will be inspected monthly and any volunteers identified

will be immediately destroyed either by application of a systemic broadleaf herbicide or by hand pulling plants and digging out of the root systems. These will then be autoclaved within the John Innes Centre.

Emergency procedures:

In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants, (including gene-edited, control and barrier Brassica plants) will be destroyed using a suitable herbicide or harvesting as deemed appropriate. All harvested material will be removed from the site and disposed of by incineration or deep burial at a local authority approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. The phone numbers of all key staff will be available to site security.

References

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