Grower Summary

FV 448

Carrot: An early warning system for risk of cavity spot in crops

Final 2016

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GROWER SUMMARY

Headline

A set of potential indicator genes for cavity spot has been identified. The aim is to prevent losses caused by cavity spot, by using a practical molecular test.

Background

Cavity spot is a major disease of carrots in the UK and is mainly caused by *Pythium violae*. Cavity spot reduces harvest quality. Visibly infected carrots are not acceptable for the fresh produce market or processing.

An early indication for cavity spot would be of great value, as it can be used as a decision support system. The test has to assess risk of cavity spot at two cost adding moments: before distribution of straw and before fields are covered. Selecting low risk fields will reduce losses and lead to reduced costs for labour and straw.

The aim of this project is to identify cavity spot specific indicator genes from the most prominent carrot cultivar in the UK 'Nairobi'. These genes will be used to develop a practical test that quantifies the expression of those genes, to determine the risk of cavity spot at an early stage. An early indication for cavity spot would be of great value, because it can be used as a decision support system. To find such indicator genes, NSure uses RNA sequencing (RNA-Seq). By using RNA-Seq, NSure can examine the expression of all genes present in any crop of interest and select those genes in which the expression is linked to a particular trait (Stattin et al., 2012; Kromwijk et.al, 2013).

The time projected to develop such a test requires two seasons of research (FV 448 and FV 448a). In the first season, samples were collected at different calendar dates from various fields in the area of Yorkshire and Nottinghamshire. In parallel, the fields were visually evaluated on the occurrence of cavity spot. Gene expression profiles on a selection of the collected samples were obtained using RNA-Seq and subsequently validated. A similar trial will be conducted in a second season to validate the functionality of the indicators selected in the first season. After defining the final set of indicators, NSure will define the decision criteria that determine whether a certain field shows a certain risk at developing cavity spot.

The project will result in a practical test to determine the risk on cavity spot. The test will consist of a simple sampling kit that enables the grower to collect a sample. The samples have to be sent to a lab either in the UK or to NSure's facilities in Wageningen. Within 48 hours after the samples have arrived, the grower will receive an indication of the risk of cavity spot. Samples

can be taken throughout the season in order to support decision taking at several crucial time points during the season.

Summary

Collection of carrot samples with a varying occurrence of cavity spot

In collaboration with Strawsons Ltd, M H Poskitt Ltd and Elsoms Seeds, NSure received carrot samples from fields in Nottinghamshire and Yorkshire, in September and October 2015. All fields were evaluated for the incidence of cavity spot. The incidence of cavity spot ranged from 0% to 65%, the range required to start the search for cavity spot related genes, as both high and low risk samples were needed.

Processing of the RNA-Seq data

Based on the quality evaluation results, NSure selected two fields that showed a low occurrence of cavity spot and two fields with a high incidence (Table 1). Frozen samples collected from those fields in September and October were studied in detail by RNA-Seq. By using RNA-Seq, NSure is able to examine the expression of all genes present in a sample. RNA-Seq was carried without any technical problems and reliable gene expression values were obtained.

Table 1. Sample selection for RNA-Seq and field evaluation of the selected fields

Field	**Quality evaluation 1**		**Quality evaluation 2**	
	%	Date	%	Date
1	2	17-11-2015	7	1-2-2016
2	3	20-11-2015	Harvested	-
3	47	18-11-2015	Harvested	-
4	64	18-11-2015	Harvested	-

The identification and validation of potential cavity spot indicators

To get an impression about the global differences between the samples that were sequenced, a Principal Component Analysis (PCA) was performed. PCA is a statistical method in which a set of data points are re-expressed in terms of basic components that explain the most variance in the data. As a consequence, PCA allows you to separate different samples in a graph according to the global differences between the samples. Samples that are similar in overall gene expression will cluster together, whereas samples that differ appear separated in the PCA plot. The results from the PCA are displayed in figure 1. Looking at the second component, which is represented on the y-axis, a segregation of the samples with high and low incidence on cavity spot can be observed. This suggest the presence of cavity spot related

genes which are differentially expressed in samples with a low or high incidence on cavity spot.

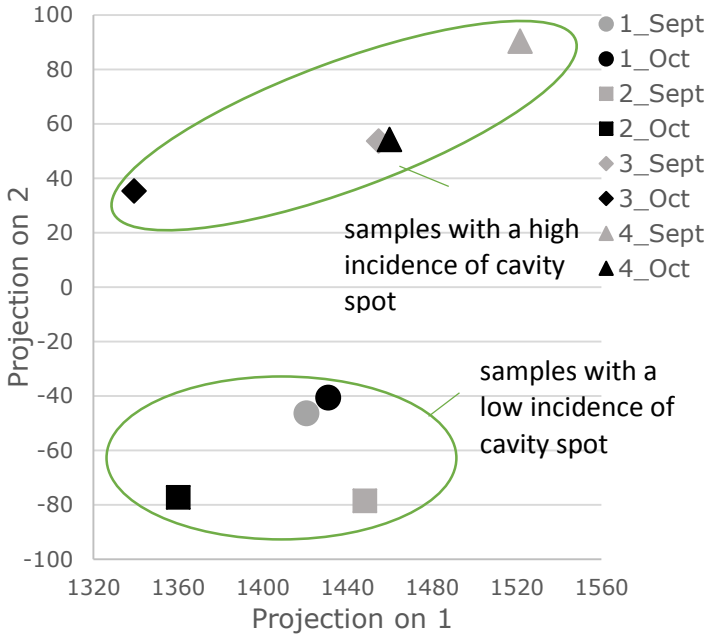

Figure 1. PCA plot using the observed expression values of all genes active in a certain carrot sample. The sequenced samples are indicated in different colours and shapes to indicate the field and timepoint. In total 2 samples per field (1-4) were sequenced, one sample collected in September and one collected in October. During the quality assessments performed at a later stage, fields 1 and 2 were characterized as fields with a low incidence on cavity spot, while fields 3 and 4 showed a high incidence. A separation of the low and high risk samples can be observed when looking at the second component which is projected on the y-axis. On the x-axis, three fields (2, 3 and 4) show a global difference in expression in time, meaning that other biological processes, which are specific for the carrots growing in that particular field, were triggered in time.

To obtain the best suitable cavity spot indicators, a stringent selection was performed based on pairwise comparisons between low and high risk samples. This resulted in approximately 70 candidate genes. As expected several of those genes are known to be involved in defence related processes. In figure 2, gene expression profiles are shown of 4 genes which are differentially regulated in samples collected from fields (3 and 4) characterized with a high incidence on cavity spot in comparison to fields (1 and 2) that showed a low incidence on cavity spot. Genes *30777* and *45235* are higher expressed in the high risk samples, while genes *6895* and *48839* are lower expressed. Further validation in samples collected from other fields led to a selection of potential carrot cavity spot indicator genes worthwhile to validate in a second season.

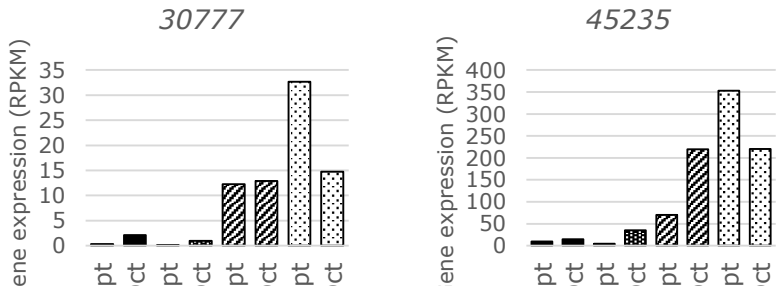

Figure 2. Gene expression profiles of 4 potential cavity spot related indicators. The RPKM value was used to indicate the level of gene expression, which is the standard quantification of gene expression when studying RNA-Seq data. Genes 30777 and 45235 are higher expressed in the high risk samples (3 & 4). The expression of genes *6895* and *48839* is down regulated in the high risk samples.

Next to frozen samples, which were used for the RNA-Seq study and indicator selection, juice samples were collected using the NSure sampling kit which is used for commercial tests (Figure 3). It is essential that similar results are obtained when analysing a frozen sample or a sample using the NSure sampling kit. For nearly all measured genes, similar results were obtained, showing that this sampling method can be used, once the putative indicator genes have been validated.

Financial Benefits

Carrot is one of the major crops in the UK. The total cultivated area exceeds 9,000 ha. 60% of the acreage, approx. 5,500 ha, is stored under straw. One hectare results on average in a gross income of £8,000. This means that the total turnover of covered carrots is approx. £44 million.

Losses due to cavity spot vary between years and geographical regions. Until recently, Scotland for example, had no occurrence of cavity spot. Other regions have more severe problems. In some fields the damage exceeds 40%. On average, cavity spot destroys 3% - 7% of the annual yield, resulting in a loss of between £1.25 and £3 million. However, this percentage seems to have increased over the years. Last season for example, the percentage was estimated to be between 5% and 10%, which almost doubled the losses.

The average cost of covering consist of straw (£3,000 per ha) and logistics (transport and covering). In total the costs for a carrot crop with straw covering are approx. £4,000 per ha.

It is clear that a high cavity spot occurrence means that a grower will not earn (instead: will lose) money on those batches. A predictive test that determines high risk fields, will support a grower to pick only low risk fields for covering. A reduction of cavity spot occurrence of only 1% on average, already makes a difference in turnover of £440,000 per year. This is exclusive of extra costs for covering, logistics and sorting. A predictive test would make UK carrot industry much more profitable.

Figure 3. Sampling procedure using the NSure sampling kit. 25 carrots are washed and from each carrot a vertical slice of 10 cm is collected with a potato peeler. The slices are transferred into the juice centrifuge and the extraction fluid is added. The juice is transferred in one half of the extraction bag which contains a sieve. The pipette is used to suck up some juice from the other half of the bag. Two droplets of juice are applied to the sampling card and the cards need to be dried for minimum of 2 hours.

Action Points

None for growers at this stage.

During this first season (FV 448), a set of potential indicator genes for cavity spot has been identified. In order to develop a reliable test, these genes need to be validated in a second season (FV 448a). Part of the work to be done involves the final description of the sampling protocol. This protocol will explain activities to be conducted by growers.