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RESEARCH INSTITUTE

10.1.c Wob
juncto 63.2.d
Vo 1107/2009

PMV-01: Storage stability study 2

[redacted] PepMV, CH2 strain, isolate 1906

Content

Scientia Terrae *performs research based on the on this moment leading scientific views and knowledge. Since the PPP analysed in this study is a plant virus to be used for crossprotection or vaccination purposes, and no such products have previously been registered as PPP in Europe, no specific guidelines or reference protocols could be followed. Scientia Terrae used all its knowledge and expertise in the domain of Plant Virology to perform this study. The study was conducted with uttermost care, following internal quality standards. Scientia Terrae will not accept any responsibility for possible damage which is directly or indirectly the consequence of analyses, judgments or recommendations made in this report.*

1. STUDY DETAILS

PRODUCT:

[redacted]epMV, CH2 STRAIN, ISOLATE 1906

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GOAL OF THE STUDY:

The goal of the presented study is to determine the storage stability of the plant protection product (PPP) [redacted]CH2 STRAIN, ISOLATE 1906' (PMV-01) a biological product containing a mild variant of *Pepino mosaic virus* (PepMV) to be used as a plant vaccine to protect tomato plants from infection with other variants of PepMV, CH2 strain. The PPP is sprayed at high pressure to infect tomato plants with this mild PepMV isolate. As the efficiency of the PPP depends largely on the infectivity and thus on the concentration of virus particles and the viability/infectivity thereof, both parameters were analysed upon storage at different storage conditions.

BATCH SPECIFICATIONS:

Production date: [redacted]

Batch number: [redacted]

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STORAGE CONDITIONS:

The PPP was stored in the official product packaging material (sealed brown PET bottles). For each time point, two separate containers with 100ml (one for determination of viral concentration and one for bioassays on test plants) were kept at three different temperatures:

- Freezer: -18°C
- Fridge: +4°C (2-8°C)
- Room temperature: +20°C

TIME POINTS

[redacted]

**DAP: days after production*

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2. ANALYSES

- **Determination of PepMV (CH2 strain) concentration using a TaqMan RT-qPCR assay (Gutierrez-Aguilar et al., 2009)**
- **Bioassays (inoculation of test plants) to check the infectivity of the product and the viability of the virus particles**
  - **Using the concentrated product**
  - **Using a 10^{-2} dilution from the product:**

[redacted]

A TaqMan RT-qPCR assay results in Ct values (threshold detection cycles). Note that the Ct value is inversely correlated with the virus concentration: the higher the Ct-value, the lower the virus concentration. [redacted]

Reference

Gutiérrez-Aguirre I. et al., 2009. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162 : 46-55.

3. RESULTS

3.1. Determination of PepMV (CH2 strain) concentration in the concentrated product using the TaqMan RT-qPCR assay.

The PepMV concentration in the concentrated product was verified during storage using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). The Ct value of the product has to be lower than [redacted] (approx. $5*10^5$ viral genome copies per µl product) in order to guarantee a good efficiency of the

PepMV CH2 vaccine. The results are presented graphically in Figure 1.

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The Ct values remain lower than [redacted] over the entire [redacted] days period, for all three storage conditions, implying that the virus concentration remains in line with the defined criteria, at least up till [redacted] days after production. These results are more or less in line with the first storage stability study, although in this first study a slight decrease in virus concentration was seen from [redacted] days after production onwards. Due to the biological nature of this product, some variation in storage stability can be expected.

3.2. Bioassays to check the infectivity of the product and the viability of the virus particles

(a) Using the concentrated product

[redacted]

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When sampled and analysed at 7 days post inoculation all test plants from all storage time points (up till [redacted] were positive for PepMV ([redacted] implying that the concentrated product remains viable and infectious during storage [redacted]

(b) <u>Using a 10^{-2} dilution of the concentrated product</u>

[redacted]

All samples were analysed for presence and concentration of virus particles using the above mentioned TaqMan RT-qPCR assay. The resulting Ct-values are presented graphically in Figure 3.

[redacted]

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All test plants inoculated at [redacted] with 100x diluted product showed high virus concentrations 7 days after inoculation, regardless of the storage conditions (Figure 3). However, after storage [redacted] the number of viable virus particles has drastically decreased, as test plants were negative for PepMV from 7 DAP onwards. This clearly shows that cold storage is required. [redacted]

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2.3. CONCLUSION:

The virus concentration determined by RT-qPCR directly on the concentrated product remains sufficiently high [redacted] during the entire storage period of [redacted] regardless of the storage temperature (-18°C, 4°C or 20°C). The bioassays with the concentrated product show that the infectivity remains high until [redacted]

Study director: [redacted]

Study technician [redacted]

10.2.e

Report finalized on 1/06/2012

Signature of study director:

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[redacted]

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