Report on the findings of genetically modified organisms of Finnish Food Safety Authority Evira and the measures to be taken

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Background

The Board for Gene Technology reported on March 15th 2017 to Evira a suspicion that unauthorized orange coloured petunias might appear on the Finnish market. This information was given by Professor Teemu Teeri at the University of Helsinki. The report mentioned two petunia varieties 'Bonnie Orange' and 'African Sunrise'. On March 23rd 2017 Evira made a proposition to the Board for Gene Technology of how to investigate whether the suspicion was accurate. No GMO petunia varieties have been cleared for deliberate release, i.e. growing or marketing within the EU. Nor is the importation of these varieties permitted.

Evira's seed inspection unit investigated the situation with seed varieties in Finland and found out that seeds of one variety, 'African Sunset', was for sale in one seed company. The company handed the entire seed batch over to Evira.

Producers of ornamental plants for greenhouse growers were asked for information about orange coloured petunia varieties they have either in their production or for sale by Evira's Plant Health Unit. Phytosanitary inpectors took samples from all the varieties that were at hand at that moment. All samples were delivered for testing to Evira's laboratory.

The investigation by Evira was performed as follows

Evira's plant health unit asked for information on the origins, consignors and delivered numbers of plants of these varieties. The investigation was limited to only include orange flowered varieties of *Petunia* and *Surfinia* (*Surfinia* = *Petunia × atkinsiana* Surfinia Group). The genus *Calibrachoa* was excluded from this investigation.

Inspection and sampling

Whether these varieties contained genetically modified elements needed to be verified with reliable testing. Phytosanitary inspectors were instructed to take sufficient samples – 4-5 plants with roots – from each variety. Disposable gloves were used to avoid contamination and gloves were changed between sampling of each variety. The plants were packaged in sufficiently sized plastic bags, each variety in its own bag.

The information of the samples was recorded in Evira's LIMS database and sample bags were marked with the codes from that recording (codes for the sample and for the accompanying letter). Separate samples were all marked with their own codes and numbers and in addition to that, with the name of the variety.

Evira has the right to take samples free of charge and to get all necessary information that is needed for supervision purposes (Gene Technology Act 27 §)

The results

Samples were analysed in Evira's accredited laboratory. When planning the analyses, it was presumed that possible transformation was made using vector p35A1 described in Meyer et al. (1987). In addition to the regulatory elements this vector contains maize A_1 gene coding for dihydroquercetin 4-reductase (DQR), which, when expressed in suitable genetic background in petunias is resulting in orange colour. By using the information about regulatory elements in

p35A1, it was concluded that screening elements commonly used in GM analyses could be used here too: 35S promoter (p35S), NOS promoter (pNOS), NPTII gene (NPTII), and junction between NOS promoter and NPTII gene (pNOS-NPTII). Presence of even one of these GM elements suggest that plant is genetically modified, since none of these GM elements are naturally present in plant genome. Six analyzed varieties (Pegasus Orange Morn, Pegasus Orange, Pegasus Table Orange, Potunia Plua Papaya, Go!Tunia Orange, Bonnie Orange) contained all above mentioned GM elements, showing that they were genetically modified, and indicated that vector that was used could be p35A1. Two of the varieties (Sanguna Patio Salmon, Sanguna Salmon) were positive with p35S element, although the amplification required more cycles than other varieties to reach the threshold cycle. It requires further analyses to investigate if this is due to different copy numbers of GM genetic elements in the plants genome. One of the samples was negative (Cascadia's Indian Summer). The seed sample delivered by Evira's seed inspection unit gave also a positive result and was (African Sunset).

If the result is negative regarding GMO elements, no measures are taken, but if it is positive, Evira will take measures in accordance with the Gene Technology Law 24 a §.

Origin of petunia plants

Finnish producers (for greenhouse growers)	**Source**	**Petunia varieties**
Koroisten Puutarha Oy	1) Volmary GmbH, Postfach 2721, 48014 Münster, Germany 2) GASA Young Plants, Denmark	1) Pegasus Orange, Pegasus Orange Morn, Pegasus Table Orange 2) Potunia Plua Papaya
Huiskulan Puutarha Oy	Florensis B.V., Langeweg 79, 3342 LD Hendrik-Ido-Ambacht, P.O. Box 32, NL3330 AA Zwijndrecht, The Netherlands	Go!Tunia Orange
Huiskulan Puutarha Oy (agent Tillmann/Finland)	Selecta Klemm GmbH & Co. KG, Hanfäcker 10, 70378 Stuttgart, Germany (producer?: Van Zaal, Poleweg 12, 142 PB De Kwakel, The Netherlands)	Bonnie Orange
Ansari-yhtymä Oy/Oy Schetelig Ab	Syngenta Seeds B.V:, Westeinde 62 / Postbus 2, 1600 AA Enkhuizen, The Netherlands	Sanguna Salmon and Sanguna Patio Salmon

The number of plants delivered to Finland is known in part for some of the varieties, but there have been several consignments of each variety. In the marketing bans the consignees (Finnish plant producers) will be obliged to clarify for the total number received and delivered to growers.

The measures

Those petunia varieties that have been found positive in the GM analysis will be removed from the market and from the production (Gene Technology Act 24 a §). Evira will give a decision that forbids growing and marketing of these illegal petunia plants and orders the illegal plants to be destroyed. This decision will be given to those three plant deliverers that have delivered illegal petunia plants. In addition the plants that have already been sold and delivered to growers have to be withdrawn and destroyed. The destruction has to be done in accordance with an instruction given on 3/6/2009 by the Board for Gene Technology and the National Product Control Agency for Welfare and Health *Instruction on waste disposal of genetically modified plant material in a closed environment*. The plants have to be made unviable through any of the methods recommended in the instruction for petunias (burning, autoclaving, steaming, boiling, freezing, desiccation, composting, crushing or by herbicides). If the plant material is destroyed through composting, the composting may be done on the company's own grounds or the material may be sent to a

composting facility once it has been made inviable (e.g. by shredding, crushing or desiccation). The growth medium has to be inactived if it can contain non-germinated GM-petunia seeds.

Evira reports the results of the analyses and the given decisions on marketing bans to the Board for Gene Technology, which in turn reports these to the Commission. The results of the investigation will be published by Evira and/or the Board for Gene Technology.

The following varieties were tested by Evira: 'Pegasus Orange', 'Pegasus Table Orange', 'Pegasus Orange Morn', 'Cascadias Indian Summer', 'Go!Tunia Orange', 'Potunia Plua Papaya', 'Sanguna Salmon', 'Sanguna Patio Salmon', 'Bonnie Orange'

References: Meyer et al. (1987) Letters to Nature 330: 677-678.