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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE

**Thirty-Fifth Session
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**PHYTOPLASMAS IN EUPHORBIA: OVERVIEW AND COMMENTS ON
ARGUMENTS PRESENTED DURING THE 1998 SESSION OF THE TECHNICAL
WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST TREES (TWO)**

*Document presented by experts from the Netherlands in cooperation with
the Community Plant Variety Office (CPVO)*

OVERVIEW OF PHYTOPLASMAS IN EUPHORBIA

1. Phytoplasmas are one of the major plant pathogens and they are associated with diseases in several hundred species of plants. Diseases associated with the presence of phytoplasmas in phloem are usually influencing the balance of plant hormones. Symptoms that are often observed are sterility of flowers, proliferation of axillary shoots or auxiliary shoots resulting in witches-broom appearance and bunched appearance of growth at the stems.
2. Poinsettia, the common name for *Euphorbia pulcherrima*, is a tropical plant indigenous to Central America and tropical Mexico. These poinsettias are unlike our today's commercial cultivars, they grow into straight and tall shrubs. Since the introduction of the first cultivars in the 1920's, an unknown factor is present in the plant, causing free-branching and a shorter plant. Only in 1997 was it proved to be a phytoplasma that is present in the *Euphorbia pulcherrima*. The phytoplasma is introduced into a plant by grafting that plant on an infected plant (or can be transmitted by leaf-hopping insects).
3. Recent studies show that it is possible to remove the phytoplasma from the plant by heat treatment & meristem tissue culture, or embryogenetic tissue culture. The phytoplasmas are extremely small, phloem-limited prokaryotes that lack a cell wall. It is not possible to culture the phytoplasmas in vitro, but they can be transmitted into healthy periwinkle plants, that show after a while bushy symptoms. The viruses, sometimes present in Poinsettias, do not survive such a transfer. Infection of non-branching (virus-free) poinsettias gives free-branching of that plant after 3-4 months. All these studies provide us the evidence that the phytoplasma is not a part of the plant genome, but an addition to the plant genome.
4. Recent studies provide evidence that there is one predominant type of phytoplasma that is present in all free-branching poinsettia cultivars. But there are also some secondary phytoplasmas present in some infected plants. When mixed infection occurs the phenotype is different (different degree of branching).
5. It is possible to detect phytoplasma by PCR, using phytoplasma specific primers. There are also possibilities of using ELISA or Electron Microscopy.
6. The above described situation creates problems in relation to the DUS testing of varieties that contain these phytoplasma(s). There are very large economic interests involved. After *Euphorbia pulcherrima* there are also *Euphorbia fulgens* varieties carrying phytoplasma. One could foresee that also other species will follow (or exist already, without our knowledge). It is also not impossible that existing cultivars, without phytoplasma, are treated and applied for PBR. What about the novelty of these applications?
7. List of Documents related to this subject:
 - (a) Report of the Thirtieth Session of the Technical Working Party for Ornamental Plants and Forest Trees in Denmark 1997, TWO/30/12, pages 9-10 point 35 "Judgements of vectors."
 - (b) Technical Committee, 1998, TC/34/7 "Phytoplasmas and viruses: Influence on the phenotype of Ornamentals in relation to the expression of their genotype" (document presented by experts from the Netherlands).

- (c) Technical Committee, 1998, TC/34/3 “Matters arising from the sessions of the technical working Parties to be dealt with by the technical committee” point 46, page 16, “Judgements of Vectors”.
 - (d) Technical Committee, 1998, TC/34/10 Prov., draft report of the thirty-fourth session of the Technical Committee, page 15, point 37 and 38 “Judgement of Vectors (Phytoplasm).
8. Furthermore we like to mention the most important literature that is to our knowledge:
- (a) Euphorbia pulcherrima, methods to eliminate Poinsettia Mosaic Virus (PNMV) and reinfection by different methods to reveal the “nature” of the branching factor”, K. Bech and K. Rasmussen, Danish Institute of Plant and Soil Science, Aarslev, DK. In: Proceedings IX Int. Sym. Virus Dis. Ornam. Plants 1996 (Abstract).
 - (b) “Phytoplasma induced free-branching in commercial poinsettia cultivars”, Ing-Ming Lee, Michael Klopmeier, Irena M. Bartoszyk, Dawn E. Gundersen-Rindal, Tau-San Chou, Karen L. Thomson and Robert Eisenreich in NATURE BIOTECHNOLOGY VOLUME 15, February 1997.
 - (c) “Genetische afwijkingen door weefselkweek”: Veredeling via Somaklonale variatie verloopt moeizaam”, Geert-Jan de Klerk en Han Bouman, Prophyta, March 1998 page 17-19 (in Dutch).
9. Further information obtained through personal communications with the three scientific groups who are working on this subject:
- Dr. Ing-Ming Lee, Molecular Plant Pathology Institute, Plant Sciences Institute, USDA/ARS, Beltsville, USA
 - Dr. James W. Moyer, Department of Plant Pathology, NCSU, Raleigh, North Carolina, USA.
 - Dr. Walter Preil, Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Ahrensburg, D.
10. As the last source of information, a Website, prepared by Dr. Ing-Ming Lee, gives the best overview of the subject: <http://www.scisoc.org/feature/poinsettia/Top.html>

PHYTOPLASMA IN EUPHORBIA

Some Comments on Arguments Presented During the TWO 1998 (TWO 31/19, page 9)

11. This document has to be read in addition to documents TC/34/7 and TWO 31/19.

(a) TWO/31/19, paragraph 37:

“37. The comparison with the virus infection was wrong. The virus would weaken the plant. It would use the protein of the cell and control the protein synthesis and in many cases would finally destroy the plant. There was often not only one single pattern but several patterns. It would not cause uniform changes but plants would have different degrees of virus infection. The endophyte would produce hormones that finally would produce branches and create one single and uniform expression in all plants.”

Comments

In document TC 34/7, the effects of phytoplasma and virus on the phenotype in relation to the expression of their genotypes were simply enumerated. They were not compared. When comparing the influence of both organisms it is too general to state that virus infection would result in heterogeneous plants and finally in the destruction of the plant since there are quite a lot of examples of the opposite: symptomless virus for instance in Lily, resulting in homogeneous plants but of only half the size of the virus-free plants, mottle-virus for example in abutilon or colour breaking virus in gladiolus. On the other hand, in crops other than Euphorbia, phytoplasmas can cause very destructive diseases, for instance in: apple, aster, ash, elm, faba-bean, lilac, lime, palm, pea, peanut, pigeon pea, prunus, rice, tomato, sugar-cane etc. (1).

(b) TWO 31/19, paragraphs 38 to 40:

“38. The endophyte was more comparable to a gene introduced into the cell by genetic engineering, although it was not in the nucleus but in the plasma.

“39. After crossing the endophyte would be found back in the seed. It was thus behaving as other genetic material of the cell and would be inherited by the offspring. Only heat or chemical treatment of the seed might remove it. The endophyte might be compared to chimera varieties. In both cases the variety consisted of two genotypes, in the case of chimera of two different cells, in case of an endophyte of one genotype in the cell of another.

“40. The criterion of easy removal was not correct. An endophyte could not be removed as easily as a virus. In the end also a gene introduced by genetic engineering could be removed from the cell. A chimera could also be separated easily in a cell culture.”

Comments

- The only similarity between genes introduced by genetic engineering and phytoplasmas is their artificial introduction into the plant.

- Phytoplasmas will not be inherited generatively (3 and 4 cited in 2), as is truly the case with genes introduced by genetical engineering.
- The endophyte as a separate organism can be removed quite easily (2).
- There is no evidence that genes once incorporated in the genome by genetic engineering can be removed (personal communication CPRO experts).
- Chimera versus phytoplasma: Theoretically we are dealing with 2 genotypes indeed but in the phytoplasma-euphorbia, both genotypes are effective in addition giving one expression in the plant. In the case of chimeras the one OR the other genotype is effective giving more than one expression in the plant. This can be illustrated by for example flowers with a spotted or striped flower colour.
- Chimeras can not be seed reproduced by nature!

(c) TWO 31/19, paragraph 42

“42. The Working Party agreed that all depended on the definition of variety. In the UPOV Convention a variety was defined in such a way that it could have one or more genotypes. Most experts in the drafting procedure of the text of the convention might have had in mind cross-fertilized varieties, but others might have thought also of chimeras. Therefore at present endophytes might be a further example. The UPOV definition of variety: “a plant grouping within a single botanical taxon of the lowest rank, which grouping, irrespective of whether the conditions for the grant of a Breeders’ Right are fully met, can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes etc.”

Comments

- It is clear that Euphorbia and the Phytoplasma involved belong to TWO different taxa with no relationship at all, irrespective of whether the taxon to which this Phytoplasma belongs is known or not.
- It is out of the question that we are dealing here with any form of hybridization. It is more a kind of symbiosis of two taxa. Consequently the conclusion can be drawn that the requirement: “within a single botanical taxon” has not been fulfilled. The (next) conclusion—according to the UPOV variety definition—is that the phytoplasma-infected Euphorbia does not meet the requirements of the UPOV Convention.
- Now the discussion on the meaning of “several genotypes” is no longer interesting for this case. It is obvious, however, that the experts preparing the 91 Convention had cross-pollinated varieties in mind, where each plant has a different genotype.
- Otherwise the definition would have been accordingly different!

Literature

1. Sinclair W.A., Griffith, H.M., and Davis R.E., 1996. Ash Yellows and Lilac Witches'-Broom: Phytoplasmal Diseases of Concern in Forestry and Horticulture. *Plant Diseases* 80-5 = 468-475
2. Lee, I.M., Klopmeier, M., Bartoszyk, I.M., Gundersen-Rindal, D.E., Chou, T.S., Thomson, K.L., Eisenreich, R., 1997 Phytoplasma induced free-branching in commercial poinsettia cultivars, *Nature Biotechnology* 15: pp. 178-182
3. Dole, J.M., Wikins, H.F., and Desborough, S.L. 1993. Investigation on the nature of a grastransmissible agent in poinsettia. *Can. J. Bot.*71: 1097-1101
4. Ruiz-Sifre, G.V. 1993. Further studies in the transmission of poinsettia branching agent. Ph.D. thesis. Oklahoma State Univ. Stillwater Oklahoma.

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