Transcript for the Plant Virology course, week 5

5.1. (00:10 00:37) Welcome to the fifth lecture of the "Plant Virology" course, concerning detection, identification and control of plant viruses.

Many different methods and techniques are used for the detection and identification of plant viruses. We need them for diagnosis of a viral infection in fields, during many epidemiological and quarantine situations, as well as in plant virology basic research.

5.2 (00:37 01:06) Bioassay is the test involving the response of indicator plants to an inoculation (artificial infection). The test allows to measure the relative infectivity of a virus-pathogen. A rather long time is needed for the indicator plant to respond to infection (5-20 days up to 1 year).

5.3. (01:06 01:34) Serological methods are based on the immunological reaction of a virus (antigen) with specific antibodies (gamma globulins). There are many serological techniques but for large-scale routine plant testing Double Antibody Sandwich - Enzyme-linked Immunosorbent Assay DAS-ELISA is the most popular test. (See van Regenmortel, 1982)

5.4. (01:34 01:49) By electron microscopic techniques it is possible to observe directly virus particles and inclusion bodies, and sometimes to identify the possible genera of the plant virus.

5.5. (01:49 02:17) The following methods of molecular biology are often used in plant virology: polyacrylamide gel electrophoresis (PAGE), polymerase chain reaction (PCR), reverse-transcription polymerase chain reaction (RT-PCR), immunocapture reverse-transcription polymerase chain reaction (IC RT-PCR), Dot Blot hybridization (cDNA), Southern Blotting (DNA), Northern Blotting (RNA), Western Blotting (proteins) and many others.

5.6. (02:17 02:43) Generally, virus diseases of plants must be prevented. Until now, there is not direct chemical control of plant viruses. To reduce crop losses to acceptable levels the strategy of using many integrated methods has been introduced.

5.7. (02:43 03:00) Plant virus disease controlling should be based on application of healthy starting plant materials obtained from certificated virus-free vegetative stocks (e.g. after thermotherapy, meristem-tip culture or chemotherapy *in vitro* culture) or from virus-free seeds. The plant certification programs provide local growers with healthy, high quality propagules for starting their crops.

5.8. (03:00 03:21) The use of resistant plant varieties. Plant resistance is the reduction or elimination of plant viral infection genetically determined. It is a reduced potential for the disease caused by that virus. The resistance that exists in plants before infections, based on e.g. morphological structures of plant cells and tissues, is called preexisting resistance.

5.9. (03:21 04:24) The plant defense mechanisms evolving after infection include:

Hypersensitivity - a rapid host cell death response associated with defense mediated by "resistance genes R",
Systemic acquired resistance (SAR), largely mediated by salicylic acid-dependent pathways, with production of pathogenesis related proteins (PR proteins),
Induced systemic resistance (ISR), mediated by jasmonic acid-ethylene dependent pathways,
Gene silencing mechanisms mediated by RNA interference. RNA silencing is based on targeting specific sequences of RNA and degrading them. Plant viruses encoded proteins that suppress RNA silencing (https://en.wikipedia.org/wiki/Plant_disease_resistance).

5.10. (04:24 05:04) The level of plant resistance to virus inoculation in presented in the figure. Four different varietes of one plant species were inoculated with the same strain of plant virus.

The resistant plant variety has the inherent ability to overcome or delay the infection by the pathogen (virus). A plant may be slightly, moderately or highly resistant. Plants which belong to the taxonomic group that are outside the pathogen (virus) host range are called “immune plants”. It is also called “non-host resistance”.

5.11. (05:04 05:25) The susceptible plant variety has the inability to resist an infection by the pathogen (virus). A sensitive variety always reacts with severe disease symptoms to the infection. There are also tolerant varieties, which possess genetically determined reduction of potential disease and they are able to produce good crops even when they are infected.

5.12. (05:25 06:12) Breeding methods for resistance include:

Classical breeding methods e.g. selections, crossing with plants containing natural resistance genes (Rx1 – potato, N – tobacco, Tm1, Tm2 – tomato) – theory “gene-for-gene” and

Genetic engineering techniques for genetic transformation of plant cells for disease resistance. Transformation of plants with coat-protein coding sequences (e.g. expression of TMV coat protein in tobacco plants protects those plants against tobacco mosaic virus) (Pathogen–derived resistance, PDR).

Other methods include expression in plants of viral satellite RNAs, the application of artificial anti-sense mediated gene regulation to viral systems, ribozymes, and the expression of human interferon genes in plants (Gadani et al. 1990).

5.13. (06:12 06:44) Other rules in plant protection against viruses include: chemical control of insect, nematode and fungal vectors of plant viruses, chemical control of weeds – virus natural hosts, removal of sources of infection and use of any other agritechnical methods useful for plant protection and conformity to quarantine regulations. **Cross-protection** is a phenomenon that occurs when infection with a mild virus prevents or suppresses the harmful effects of a subsequent infection by a related severe virus.

5.14. (06:44 07:05) Thermotherapy and meristem tip culture are used for eradication of plant viruses. Plants in a growth chamber. For thermotherapy plants are treated with hot air (28-42^{O}C) for 4-6 weeks. Heat treatment reduces virus concentration in plants and newly grown shot tips are virus free.

5.15. (07:05 07:20) Most plant viruses have no ability to invade meristematic tissue. Apical meristems can be cut and cultivated aseptically “in vitro” on an artificial medium. Scheme of meristem (growing tip) with the line of cutting the explant.

5.16. (07:20 07:28) Meristem tip culture. Tip explants on filter bridges dipped in M/S medium.

5.17. (07:28 07:34) Meristem tip culture. Growing explant.

5.18. (07:34 07:43) Regenerated plants from meristem tip culture ready for transfer to sterile perlite or vermiculite substrates (ready for potting).

5.19. (07:43 08:00) Scheme for eradication of viruses from plant propagation material by thermotherapy followed by meristem- tip culture.

5.20. (08:00 08:04) Thank you for your attention.