

Preparation of Mother Plant Prebasic Apricot Material in the Czech Republic

Michal Adam

*Mendel University of Agriculture and Forestry Brno, Faculty of Horticulture
Lednice, Department of Pomology
Czech Republic*

Abstract: DAS-ELISA testing for ACLSV, ApMV, PDV, PNRSV, PPV virus presence on 15 apricot varieties, candidate plants for the certificate level virus-free material, has been performed with negative results. Candidate plants were tested on herbaceous and woody indicators with negative results both during visual testing and during subsequent DAS-ELISA testing.

The possible use of new herbaceous and woody indicators (*Helianthus annuus* L.; *Phaseolus vulgaris* L., GF-31) has been preliminarily proved.

Key words: Prebasic, herbaceous indicators, woody indicators, DAS-ELISA, apricot, ACLSV, ApMV, PDV, PNRSV, PPV.

Introduction

At present, there is a very low representation of stone fruit nursery material on certificate levels virus-free (VF) and virus-tested (VT) in the Czech Republic. It is possible to say that it is a mistake, which results in the lowered harvest and quality. It is impossible, even using all accessible agricultural engineering, to get such long term results as on virus-free plant. The Condition for the extension of the VF, VT material production is the availability of the basic material for propagation. It is possible to obtain foreign rootstocks VF, VT, however, it is not possible to obtain the varieties and rootstock with specific qualities for the conditions in the Czech Republic, e.g. different growing season, late vernal slight frost.

The aim of the research was to prepare the prebasic material of new Czech apricot varieties according to the philosophy EPPO (00/7845 (99/7214) PCF point 4.2), using new herbaceous and woody indicators.

Material and Methods

Variety grafts for certification VF material and for advances were taken from the experimental fields of the Department of pomology (Bergeron, Leala, Lebona, Ledana, Marlen, Legolda, Lejuna, Lemira, Lerosa, Leskora, Minaret, Svatava, Palava, Velkopavlovicka 12/2). In 2001, plants were prepared from the grafts, budding on rootstock Lesiberian and GF-305; the plants were planted into 24cm diametre containers and cultivated in the area of the Technical isolation. Herbaceous indicators were obtained, buying (*Cucumis sativus* L.; *Cucurbita maxima* Duchesne; *Helianthus annuus* L.; *Phaseolus vulgaris* L.), and asking virology institutes for (*Chenopodium foetidum* C. Schrad; *Ch. quinoa* Wild.; *Ch.album subsp. amaranticolor* H.J.Coste and A.Reyn).

Woody indicators were obtained abroad, buying (GF 31; Kwanzan, Shirofuga) and seeding the seeds under insectproof conditions (GF-305). Woody and herbaceous indicators were prepared for inoculation by virus in a green-house and in an outside environment according to the philosophy EPPO 1997 (00/7845 (99/7214) PCF point 4.2).

The philosophy of the Research and Breeding Institute of Pomology Holovousy Ltd. (KARESOVA 1993) was used to take the samples, which were taken in the vernal and autumn months into single-use PE bags.

The philosophy procedure for DAS-ELISA test was provided by the Department of immunological testing, microbiology and genetic diagnostics of the Central institute for supervising and testing agriculture Brno. The antibody from the firm Loewe Biochemica GmbH -Germany and Bioreba AG Switzerland was used. The Absorbance of the samples was measured on the spectrofotometre SLT 963 PR (SLT Labinstruments Austria) and Sunrise Remote (Tecan Austria) at 405nm. A positive sample is regarded the one with the absorbability higher than 0,100, decreased by the value of the blank (a clean extraction buffer used in stead of the sample), in two neighbouring sockets. When there was only one positive socket, the test was carried out again. The test for ACLSV; ApMV; PDV; PNRSV; PPV presence was performed twice after 21 days, on all tested plants from the Technical isolation.

The philosophy according to NEMETH (1986) and the philosophy EPPO 1997 (00/7845 (99/7214) PCF point 4.2) were used for the woody and herbaceous indicators. The herbaceous indicators were cultivated in a green-house in spring and in autumn. The transmission was carried out by a homogenate from leaves of tested plants with a phosphate buffer with the help of abrasiva carborundum. Young plants of size 10-15 cm were inoculated on three genuine leaves except for *C. sativus* L.; *C. maxima* Duchesne; *H. annuus* L.; *P. vulgaris* L., which were inoculated in the phase of primary leaves. All was acried out three times. Plants inoculated only by the phosphate buffer and plants inoculated by the homogenate phosphate buffer and leaves of plants with symptoms, which were proved by DAS-ELISA test as positive were used for the verification. Plants were examined twice visually after 21 days and twice serologically in bi-weekly interval, the whole procedure was performed twice (spring and autumn).

The woody indicators were cultivated under the insectproof conditions provided by covering of the green-house construction with a linear PE net (mesh size 0,42 x 0,79 mm). The indicators were planted into containers 12x12cm. The inoculation was effected by chip-budding in summer. There were two buds inoculated on one indicator. Three indicators from each variety (GF31; GF-305, Shirofuga) were

used for one tested plant. Bud survival was checked after three weeks. Virus symptoms, virus-like organisms symptoms and, by DAS-ELISA testing, ACLSV; ApMV; PDV; PNRSV; PPV presence were visually checked during spring months the following year.

Results and Discussion

Serological detection of five viruses DAS ELISA testing proceeded from 22. April till 1. June 2002. All plants' tests were evaluated as negative as to the presence of five fundamental viruses.

Table 1. Brief survey of testing performed on candidate plants in the Technical isolation
(Faculty of Horticulture, Lednice)

[illegible]

N-negative sample V/V/E visual check/visual check/ ELISA test

On May 2003 a new DAS-ELISA test on the viruses presence was carried out with negative results for all tested plants. Indexing using herbaceous and woody indicators.

Budding on woody indicators was carried out on 10. and 11. July 2002 for 15 varieties of the prebasic material from the Technical isolation. Visual testing was performed in May and June 2003 DAS-ELISA test early in June 2003.

The following plants were used for indexing on herbaceous indicators: *Chenopodium foetidum*, *Chenopodium quinoa*, *Cucumis sativus*, *Cucurbita maxima*, *Phaseolus vulgaris*, *Heliantum annuum*. The plants genus *Chenopodium* were seeded in a green-house and inoculated after transplantation and into-planting to the size 10-15 cm. Inoculation was effected 19. 6. 2002, for 15 varieties from the Technical isolation. The plants were checked visually twice in terms 29.6. and 8.7. and DAS-ELISA tested 20 days after inoculation. Visual and serological testing was negative.

Indexing was repeated 30. 8. 2002 on the plants genus *Chenopodium* in the phase of five genuine leaves and on other plants 3. 9. 2002 in the phase of primary leaves, for 15 plants from the Technical isolation. Visual check was performed 10 and 20 day after inoculation, which means 9. and 13. 9. 2002 and 19. and 23.9. 2002 ELISA test was made 17.9. and 30.9. 2002. The visual and also serological check focused on the five viruses' presence was negative.

At present, testing on ESFY presence using NESTED PCR has been initiated.

Conclusion

Testing on ACLSV, ApMV, PDV, PNRSV, PPV presence, using DAS-ELISA test, was made on 15 apricot varieties, candidate plants for the certificate level virus-free material, with negative results. Candidate plants were tested on herbaceous and woody indicators with negative results both during visual testing and during the following DAS-ELISA testing.

After this testing performed in 2002 and 2003 it was found out that all tested apricot varieties (Bergeron, Leala, Lebel, Lebona, Ledana, Marlen, Legolda, Lejuna, Lemira, Lerosa, Leskora, Minaret, Svatava, Palava, Velkopavlovicka 12/2), can preliminarily be classified as corresponding to the certification level virus-free. Newly tested indicators (*Helianthus annuus* L.; *Phaseolus vulgaris* L., GF-31) have given equivalent results as the indicators recommended by the philosophy EPPO.

References

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PRIPREMA SADNOG MATERIAJALA MATIČNE BILJKE KAJSIJE U ČEŠKOJ REPUBLICI

-originalni naučni rad-

Michal Adam

*Mendelev Univerzitet Poljoprivred i Šumarstva Brno
Fakultet Povrtarstva Lednice, Odsek Pomologije
Češka Republika*

Rezime

Rad se bavi DAS-ELISA testiranjem ACLSV, ApMV, PDV, PNRSV i PPV, virusa kod 15 sorti kajsija, inače kandidata za dobijanje sertifikata kao bezvirusni materijal. Ove biljke su ispitivane sa zeljastim i drvenastim uzorcima ali su rezultati bili negativni, kako u toku vizuelnog tako i za vreme kasnijeg DAS-ELISA ispitivanja.

Moguća primena novih zeljastih i drvenastih primeraka (*Helianthus annuus* L. i *Phaseolus vulgaris* L. GF-31) se već pokazala u praksi.