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APPLE AND PEAR ROOTSTOCK RESEARCH IN LITHUANIA

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The paper presents ongoing apple and pear rootstock trials at the Lithuanian Institute of Horticulture. Rootstock research projects are established in following directions: rootstock and location interaction (Baltic fruit rootstock studies where Byelorussian, Estonian, Latvian, Lithuanian and Polish research institutions are involved); budding high effect on rootstock performance; interstock trials; rootstock effect on fruit quality, ripening time and fruit storage; rootstock and tree training system; virus status of apple planting material on different rootstocks (international trial with Applied Plant Research, Netherlands); rootstock and soil sickness in nursery; rootstock resistance to *Phytophtora*; rootstock response to irrigation; rootstock effect on tree physiological parameters and flower physiology; rootstock effect on tree nutrition; rootstock and dry matter accumulation; genetic engineering of *Cydonia oblonga* rootstocks. Following apple rootstocks are included in different research projects: M and MM series – M.9, M.26, MM.106; P series – P 2, P 22, P 14, P 59, P 60, P 61, P 62, P 66, P 67; B series – B.9, B.118, B.136, B.396, B.491, other rootstocks as PB.4, Bulboga, York 9, Pure 1 and Antonowka seedlings. Following pear rootstocks are included in different research projects: quinces – QA, QC, Sydo, BA-29, S 1, K.11, K.16, K.19, 1.2, pears – Pyrodwarf, OHF333, Mostbirne, Kazrausu. 14 scientists from Orchard technology department, Department of Genetics and Biotechnology of Orchard Plants, Plant protection laboratory, Laboratory of Biochemistry and Technology, Laboratory of Plant Physiology are involved in rootstock research.

Key words: *Cydonia* x *oblonga*, *Malus* x *domestica*, *Pyrus* x *communis*, rootstock, trial.

Introduction. Scientifically based rootstock research in Lithuania was started at the Vytėnai Horticultural Research Station (in 1987 reorganized into Lithuanian Institute of Horticulture) in 1960 (Kviklys, 1977). Apple rootstock research developed into different directions: propagation, nursery trials, rootstock vigour, winter hardiness, rootstock use as interstems, rootstock effect on fruit quality, storage capacity, ripening time, etc. (Kviklys, 1977, 1992; Švirinas, 1986; Šumskis, 1986a, 1986b, Kviklys et al., 1988; Kviklys, Kviklienė, 2002; Kviklys, 2004). During 1963–2002 more than 40 vegetative rootstocks and 20 seedling rootstocks were evaluated in the orchard for

their productivity and effect on fruit quality (Kviklienė, Kviklys, 2001; 2004; Duchovskis et al., 2000; Kviklys et al., 1999, 2000; Uselis, 2002, 2003, 2004, 2005). After long term of evaluation rootstocks MM.106 and B.118, semi-dwarf M.26, P 60 and B.396, dwarf M.9 and P 22 were recommended for propagation and growing on commercial scale (Kviklys, 2002).

At this moment rootstock research is being developed in following directions: rootstock and location interaction; budding high effect on rootstock performance; interstock trials; rootstock effect on fruit quality, ripening time and fruit storage; rootstock and tree training system; virus status of apple planting material on different rootstocks; rootstock and soil sickness in nursery; rootstock resistance to *Phytophthora cactorum*; rootstock response to irrigation; rootstock effect on tree physiological parameters; rootstock effect on tree nutrition; rootstock and dry matter accumulation; genetic engineering of *Cydonia oblonga* rootstocks.

The aim of our work is to present ongoing trials and achievements of rootstock research projects at the Lithuanian Institute of Horticulture.

Material and methods. All rootstock trials in the orchard are established in four-five replications with 3–5 trees in each. Replications are randomised. Variance analyses are performed and criteria are chosen according to trial schemes.

Tree vigour control. As a measure for the tree vigour control trunk circumference is measured annually; total shoot length is measured first two-three years after trial establishment; tree height is measured until it reaches technological allowed height. Additional measurements, as mean shoot length, canopy volume, number of buds on the shoot, are taken according to trial scheme.

Yield and productivity. Yield per tree and per hectare are the main measurements. Tree productivity is counted as kg cm^{-2} of trunk cross sectional area.

Fruit quality, harvest time. From each replication 100 fruits are weighed and sized in 5 mm intervals. Fruit colouring is expressed by percentage of red colour covering fruit surface. Ten fruits from each replication are taken for laboratory measurements. Fruit firmness is measured with an Effegi penetrometer. Starch conversion is estimated after treatment with 0.1n iodine and potassium iodine solution (scale 1–10). Concentration of soluble solids is determined with refractometer. Maturity index is calculated as F/RS, where F – firmness, R – concentration of soluble solids, S – starch conversion.

Flowering time, T-phase, days after full bloom are counted for harvest time evaluation.

Natural mass loss, fruit rots, storage diseases, transpiration is recorded for storage trials.

Hormones. Analyses of gibberellic acid (GA_3), indolyl-3-acetic acid (IAA), abscisic acid (ABA) and zeatin are performed using HP 1050 Series liquid chromatography system with variable wavelength UV-VIS detector (Agilent Technologies, Waldbronn, Germany). Intersil ODS-2 column (150 x 4.6 mm^2) (Alltech, Deerfield, USA) was used for phytohormones separation. Mobile phase: 45% methanol containing 1% acetic acid. Flow rate: 1 mL/min. The wavelengths of 254 nm for GA3 and ABA detection, 270 nm for zeatin and 280 nm for IAA detection were set.

P i g m e n t s. Total chlorophyll and carotenoid content in green mass is determined in 100% acetone extracts using spectrophotometrical Wettstein method [13]. Spectrophotometer – Genesys 6 (ThermoSpectronic, USA).

S u g a r s. Carbohydrate samples are prepared by grinding ~ 1g of fresh weight (FW) material and extracted with 4 mL hot bidistiled water. After 24 h extract is filtered through cellulose and membrane (pore diameter 0.2 µm) filters. Chromatographic analysis was carried-out using Shimadzu 10A HPLC system with refraction index detector (Shimadzu, Japan) and Adsorbosil NH_2 – column (150 mm x 4.6 mm; Alltech, USA). Mobile phase: 75% acetonitrile. Flow rate: 1 m/min.

M o r p h o p h y s i o l o g i c a l analysis of buds is performed according to F. Kuperman methodology. Bud development is analyzed by microscope and organogenesis stage is established.

Resistance to Phytophthora cactorum (Lebert & Cohn) Schroeter. In vegetation trial isolates of *Phytophthora cactorum* are tested on one year old apple rootstocks grown in pots in a greenhouse. Inoculations are made *via* insertion of small agar plugs from actively growing cultures into bark flaps in two sides. Length of necrosis is measured after five weeks after inoculation. Total plant weight, fresh and dry root, leaf, trunk weight are measured.

In laboratorial trial current season shoots are used. They are inoculated mycelia disks of *Phytophthora cactorum*, taken from seven-day-old culture grown on PDA. Basal and middle rootstock shoot parts are incubated on moist blotting paper in foil-covered polystyrene boxes. Length of necrosis is measured after 3 and 8 days of incubation.

D r o u g h t s t r e s s. Biometric measurements are done at the end of experiment. Total plant weight, fresh and dry root, leaf, trunk weight are measured. Leaf area is measured by Leaf area meter WinDias (U.K).

Budding height trials. Trial with P 60 and B.396 rootstocks budded at 5, 10, 20 and 30 cm planted in the spring of 2000. Planting distances – 4 x 1.5 m.

Trial with M.9, M.26, P 22, P 59 rootstocks budded at 5, 10, 20 and 30 cm planted in the spring of 2002. Planting distances – 4 x 1.5 m for M.26 and 4x1 m. for M.9, P 22, P 59.

'Baltic fruit rootstocks studies'. Location effect on rootstock performance**.** Trial with apple cv. 'Auksis' on P 22, P 2, M.9, B.9, Pure 1, B.491, B.146, York 9, B.396, P 60, M.26 and Bulboga rootstocks planted in the spring of 2001. Planting distances – 4 x 1.5 m. Under the same scheme trials were established at Babtai, Lithuanian Institute of Horticulture (Lithuania), Pure Horticultural Research Centre (Latvia), Polli Research Centre of the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (Estonia), and Pruzany, Brest Agricultural Experimental Station (Byelorussia).

Trial with apple cv. 'Beloruskoje malinovoje' on P 22, M.9, B.9, Pure 1, B.146, B.396, P 60, M.26 and Bulboga rootstocks planted in the spring of 2001. Planting distances – 4 x 1.5 m. Under the same scheme trials were established at Lithuanian Institute of Horticulture, Pure Horticultural Research Centre, Polli Research Centre, and Brest Agricultural Experimental Station.

Trial with pear cv. 'Suvenir' on Quince BA29, QA, QC, *Pyrus* Pyrodwarf, *Pyrus* OHF333, *Pyrus* Kirchensaller Mostbirne, and *Pyrus* Kazrausu planted in the spring of 2001. Planting distances – 4 x 3 m for seedling rootstocks and 4 x 2 m for clonal rootstocks. Under the same scheme trials were established at Lithuanian Institute of Horticulture, Pure Horticultural Research Centre, Polli Research Centre, and Brest Agricultural Experimental Station.

Trial with apple cvs. 'Auksis' and 'Ligol' on B.396, B.9, PB-4, Pure 1, M.9, M.26, P 22, P 59, P 61, P 62, P 66, P 67 in the spring of 2005. Planting distances – 4 x 1.5 m. Under the same scheme trials were established at Lithuanian Institute of Horticulture, Pure Horticultural Research Centre, Polli Research Centre, and Skierniewice, Research Institute of Pomology and Floriculture (Poland).

Interstock trial includes rootstocks B.9, P 22, B.396, 3-3-72, apple cvs. 'Summered', 'Kaunis', 'Lietuvos pepinas', columnar apple 109 and planted in the autumn of 2002. Planting distances – 4 x 1.5 m.. Interstock length 30 cm, rootstock B.396.

Virus status of planting material. International trial planted in the spring of 2003 together with Applied Plant Research, Research Unit Fruit, Randwijk, Netherlands. Cvs. 'Sampion' and 'Jonagold decosta' on M.9 and M.26 rootstocks. Planting distances – 3 x 1 for M.9 and 3 x 1.5 for M.26. Virus free and not tested material is evaluated.

Replant trial in the nursery established in 2005. Cvs. 'Auksis' and 'Sampion' budded on M.9, M.26, MM.106, P 2, P 22, P 59, P 60, B.396, B.118, Antonowka seedling rootstocks and planted in fresh soil and at the place where apple stoolbeds were cultivated for 10 years.

Rootstock and orchard constructions**.** Trial with cv. 'Auksis' on P 22 and P 60 rootstocks planted in 2001. Different planting schemes and tree training systems are evaluated.

Trial with cv. 'Rubin' on P 22 and P 60 rootstocks planted in 2001. Time and crown training systems are evaluated.

Rootstock and planting distances. Trial with cv. 'Ligol' on P 22, P 2 and P 60 rootstocks planted in 1999. Different planting schemes are evaluated.

Trial with cv. 'Lodel' on M.26, P 2 and P 60 rootstocks planted in 1999. Different planting schemes are evaluated.

Trial with cv. 'Delikates' on M.26, MM.106 and seedling rootstocks planted in 1999. Different planting schemes are evaluated.

Rootstock effect on tree physiology. Trial started in 2004 with cv. 'Auksis' on P 22, P 2, M.9, B.9, Pure 1, B.491, B.146, York 9, B.396, P 60, and M.26 rootstocks.

Drought stress trial established ex-situ in 2005 with M.9, M.26, MM.106, P 2, P 22, P 59, P 60, B.396, B.118, Antonowka seedling rootstocks. Three soil moisture regimes are created: 20–30 kPa, 40–50 kPa, and >70 kPa.

Rootstock resistance to *Phytophthora cactorum* trials established ex-situ and in laboratory in 2006 with M.9, MM.106, P 60, B.396 and B.118 rootstocks.

Rootstock effect on fruit quality and harvest time. Trial with apple cv. 'Auksis' on P 22, P 2, M.9, B.9, Pure 1, B.491, B.146, York 9, B.396, P 60, M.26 and Bulboga rootstocks started in 2004.

Trial with apple cv. 'Auksis' on B.396 and P60 budded at 5, 10, 20 and 30 cm started in 2004.

Trial with pear cv. 'Conference' on QA, QC, Sydo, QS1, K.16, K.11, 1.2 rootstocks and *Pyrus* x *communis* seedlings started in 2000.

Rootstock choice to columnar apple varieties. 24 columnar apple selections from the breeding program at the Lithuanian Institute of Horticulture are tested on P 60, B.396, M.26, MM.106, B.118 and seedling rootstocks.

Genetic engineering of *Cydonia oblonga* rootstocks. Quince rootstocks K.11, K.16 and K.19 from Lithuanian rootstock breeding program are included. *RolB* gene (~ 800 bp) was isolated from *Agrobacterium rhizogenes* in PCR with rolB5kod-rolB3kod primers. Binary constructs basing on pART27 and pNOV2819 plasmids and containing *rolB* gene under own promoter sequence, 35S CaMV, and stress-induced PR promoter were prepared and used to transform quince using *A. tumefaciens.*

Results. Some published and more important preliminary results of ongoing rootstock trials at the Lithuanian Institute of Horticulture are presented.

'Baltic fruit rootstocks studies'. Location effect on rootstock performance**.** The strongest tree growth of cv. 'Auksis' was recorded in Lithuania and Byelorussia. The highest yields were obtained in Lithuania. All tested rootstocks according growth vigour control can be grouped in the following way: less vigour than M.9 – P 22, the same as M.9 – Pure 1, B.396, York 9, P.60, B.9 and P 2, between M.9 and M.26 – B.491, the same or more vigorous as M.26 – Bulboga and B.146. Trees on rootstocks Pure 1 gave the highest cumulative yield and were the most efficient although there was no significant difference in cumulative fertility index with trees growing on rootstocks P 22, P 2, M.9, B.9 and York 9. The least efficient were trees on rootstocks Bulboga and B.146. Rootstock and location interaction was recorded for B.491 and P.60 in growth vigour control, York 9 in total yield, and B.9 in yield efficiency (Kviklys et al., 2006).

The evaluation of vegetative growth showed that the most vigorous trees of apple cv. 'Belorusskoye Malinovoye' were on rootstock Bulboga, followed by B.146, M.26, P 60, B.396, M.9, B.9 and Pure 1. The smallest trees were on P 2. Highest cumulated yield was harvested from the trees on rootstocks M.26 and B.396, the smallest – on P 22 and B.9. The highest yield efficiency (kg cm^{-2} TCSA) was observed on Pure 1 and B.9 rootstocks, the smallest – on B.146 and M.26. These results were observed in all trials in all four scientific institutions, although some differences were noted (Bite et al., 2006).

In Lithuania pear trees grew more vigorously than in two other localities. The trees on the QC were founded to be smaller and the trees on *Pyrodwarf*. In Latvia, the trees on all *Pyrus* rootstocks were more vigorous than these on *Cydonia* rootstocs. In Estonia, the trees on *Pyrodwarf* and OHF 333 were found to be weaker than these on *Cydonia* rootstocks. The first crop in Lithuania appeared in the fourth year after planting only on *Cydonia* and *Pyrodwarf* rootstocks. In Estonia and Latvia, the first crop appeared in the fifth year on all rootstocks due to the entire killing of flowers in the fourth year by spring night frosts; the first crop was bigger on *Cydonia* rootstocks. In Lithuania, the fruit mass was bigger on OHF 333 than that of BA 29 (Haak et al., 2006).

Rootstock effect on fruit quality. High yielding trees on dwarf rootstock Pure 1 have the smallest fruits. Largest fruits were on strong growing Bulboga, B.146 and dwarf rootstock M.9. Pure 1 and P 22 determined better fruit colouring. Fruits on Bulboga were firmer, and these on rootstock P 2 – softer. P 2 determined higher content of soluble solids (Kviklienė, Kviklys, 2006).

The biggest 'Conference' fruit weight were from trees on rootstock QS1, K.11 and K.16. These rootstocks and QA increased fruit diameter. *Pyrus* x *communis* seedlings negatively effected fruit mass. 'Conference' fruits on the *Pyrus x communis* seedling rootstock were firmer at harvest and as well as QS1 and 1.2 rootstocks had higher starch content. There were no rootstock influence on the soluble solids content and maturity indices among *Cydonia oblonga* rootstocks (Kviklys, Kviklienė, 2005a).

Rootstock effect on fruit maturity. The maturity index indicated that 'Auksis' apples were more mature from trees on rootstock Pure 1. Rootstock Bulboga caused later ripening of apples.

The maturity indices indicated that 'Conference' pears were more mature from trees on *Cydonia oblonga* rootstocks (Kviklys, Kviklienė, 2004).

Rootstocks choice for cv. 'Conference'. Rootstock 1.2 significantly reduced growth of a pear tree (stem diameter and total shoot length during the first two years), whereas trees on Sydo and seedling rootstocks had the strongest growth. Significant differences of stem diameter were not established between other tested rootstocks after five years in the orchard. Quince MC, Sydo, K11 and K16 were the earliest to start cropping. Trees grafted onto them started to yield in the second year after planting. The highest cumulative yield was obtained on Quince MC followed by Sydo. Other rootstocks gave statistically lower yields. Significant differences were not found among Quince MA, K.16, K.11 and S1. The lowest yield was on 1.2 and *Pyrus x communis* seedling rootstocks (Kviklys, 2005).

Virus status of planting material. Virus free trees had stronger vegetative growth (total shoot length and stem diameter) at planting year than not tested ones. During second and third year in the orchard vegetative and generative development of apple trees and fruit weight did not depend on health status of planting material. During the second season virus free trees of cv. 'Šampion' had higher yield, but lower growth, when not tested trees – lower yield and stronger growth. During the third year opposite tendencies were established. Correlation between vegetative growth and generative development of cv. 'Jonagold' was not established. Health status of planting material had no effect on scab incidence on leaves and fruits. Not tested trees were more sensitive to bark diseases (Kviklys, Stankienė, 2005).

Budding height. The differences among rootstocks were recorded already in the second year. Trees on P 60 grew stronger than on B.396. Stronger growth of trees was recorded at lower budding height too. Trees planted at the height of 0 and 10 cm significantly differed from trees at the height of 20 and 30 cm in total shoot growth, stem diameter and tree height. First crop was the same on both rootstocks and there were no significant difference between budding height. In following two years significant differences occurred for both rootstocks and budding height. Rootstock B.396 gave higher yield and trees on it were smaller. Influence of budding

height on crop load differed between years and rootstocks. Significant differences were found with B.396 budded at different height. Less pronounced differences were with P 60 rootstock. Trees, which budded higher, had smaller stem diameter. Apple fruit weight did not depend on budding height (Kviklys, Kviklienė, 2005b).

Genetic engineering of Cydonia oblonga rootstocks. The aim is to transform quince rootstocks to increase their rooting ability. *RolB* gene was isolated from *Agrobacterium rhizogenes.* 1992 explants derived from clones K.1, K.16 and K.19 were transformed using *A. tumefaciens* (Ražanskienė et al., 2006). Transgenic plants were obtained with good rooting ability. Root morphology was typical to plants transformed with *rolB* gene.

Discussion. The choice of rootstocks depends mostly on climatic conditions, which are usually more unsatisfactory in northern countries. The length of the vegetation period, sum of temperatures and rain precipitations have significant effects on the rootstock performance. The vegetation period in Lithuania is shorter than in other European countries and the sum of temperatures is also lower. Winter could be a limiting factor of growing sensitive apple and pear rootstocks. Apple and pear rootstocks are released in different countries and have their own quality parameters and often exhibit unequal performance in different countries (Wertheim, 1998), mainly due to ecological conditions. Therefore rootstock research is relevant in Lithuania in order to develop commercial fruit growing and scientific knowledge.

At the Lithuanian Institute of Horticulture 14 research workers from Orchard technology department, Department of Genetics and Biotechnology of Orchard Plants, Plant protection laboratory, Laboratory of Biochemistry and Technology, Laboratory of Plant Physiology are involved in rootstock research. Research collaboration and mutual rootstock trials take place with Applied Plant Research, Research Unit Fruit, Randwijk, (Netherlands), Research Institute of Pomology and Floriculture, Skierniewice, (Poland), Pure Horticultural Research Centre (Latvia), Polli Research Centre of the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (Estonia), and Brest Agricultural Experimental Station, Pruzany (Byelorussia) (Bite et al., 2004; 1999).

24 apple and pear rootstock research projects are being carried out at the Lithuanian Institute of Horticulture in 2006: 15 projects at the Orchard Technology Department, 2 projects together at the Orchard Technology Department and Laboratory of Plant Physiology, 2 projects together at the Orchard Technology Department and Plant protection laboratory, 1 project together at the Orchard Technology Department and Department of Genetics and Biotechnology of Orchard Plants, 1 project together at the Orchard Technology Department and Laboratory of Biochemistry and Technology, 2 projects at the Department of Genetics and Biotechnology of Orchard Plants, 1 project together at the Department of Genetics and Biotechnology of Orchard Plants and Laboratory of Plant Physiology.

Following apple and pear rootstocks are included in different research projects: M and MM series – M.9, M.26, MM.106; P series – P 2, P 22, P 14, P 59, P 60, P 61, P 62, P 66, P 67; B series – B.9, B.118, B.136, B.396, B.491, other rootstocks as PB.4, Bulboga, York 9, Pure 1 and Antonowka seedlings; quinces – QA, QC, Sydo, BA-29, S 1, K.11, K.16 and 1.2; pears – Pyrodwarf, OHF333, Mostbirne, Kazrausu.

In 2003–2005 research workers of the Lithuanian Institute of Horticulture have published 25 scientific publications on apple and pear rootstock research.

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Parengta spausdinti
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OBELŲ IR KRIAUŠIŲ POSKIEPIŲ TYRIMAI LIETUVOJE

D. Kviklys

Santrauka

Straipsnyje analizuojami šiuo metu Lietuvos sodininkystės ir daržininkystės institute atliekami obelų ir kriaušių tyrimai. Tiriant obelų poskiepius, nustatoma poskiepių ir aplinkos sąlygų (geografinių vietovių) sąveika (Baltijos poskiepių studija, kurią atlieka Baltarusijos, Estijos, Latvijos, Lenkijos ir Lietuvos mokslo įstaigos), poskiepio reakcija į akiavimo aukštį, įtaka vaisių kokybei, sunokimo laikui ir laikymuisi, obelų su skirtingais poskiepiais formavimo sistemos, poskiepių naudojimas tarpiniam skiepijimui, poskiepių sveikumas (tyrimai atliekami su Nyderlandų mokslininkais), reakcija į gentinį dirvos nualinimą medelyne, atsparumas šaknų kaklelio puviniui, sausrai, įtaka vaismedžių fiziologiniams rodikliams, sausųjų medžiagų pasiskirstymui ir maisto medžiagų pasisavinimui, atliekami paprastojo svarainio poskiepių genų inžinerijos tyrimai. Atliekant įvairius bandymus, tiriami šie obelų poskiepiai: M ir MM serijos – M.9, M.26, MM.106; P serijos – P 2, P 22, P 14, P 59, P 60, P 61, P 62, P 66, P 67; B serijos – B.9, B.118, B.136, B.396, B.491, kiti poskiepiai – PB.4, Bulboga, York 9, Pure 1 ir 'Paprastojo antaninio' sėjinukai, ir kriaušių poskiepiai: Paprastojo svarainio – QA, QC, Sydo, BA-29, S 1, K.11, K.16, K.19, 1.2, kriaušių – Pyrodwarf, OHF333, sėkliniai – Mostbirne ir Kazrausu. Lietuvos sodininkystės ir daržininkystės institute poskiepių tyrimus įvairiu lygiu atlieka 14 Sodų technologijų skyriaus, Genetikos ir biotechnologijos skyriaus, Augalų apsaugos, Augalų fiziologijos bei Biochemijos ir technologijos laboratorijų mokslo darbuotojų.

Reikšminiai žodžiai: *Cydonia* x *oblonga*, *Malus* x *domestica*, moksliniai tyrimai, *Pyrus* x *communis*, poskiepis.