Marker-assisted breeding in the peach breeding program of the University of Chile

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Abstract:

Peach is one of the most important fruit crops produced in Chile, being the 3rd most produced fruit in the territory with 332824 Tons (2017), and occupying the 9th place in the world production. Historically, local producers have employed cultivars generated in foreign regions (mostly USA and Europe), which has given good results in terms of quality and yield, but has limited the theoretical possibilities respect to the use of locally generated cultivars. By means of breeding work, hundreds of seedlings are generated, tested and selected, extending the possibilities of achieving accessions that completely expressed their genetic potential for quality and sensory traits, under the local environment and cultural practices. For that reason, in 2010 started officially the peach and nectarine breeding program of the University of Chile. The traits of major interest of the breeding program of the Univeristy of Chile are fruit size, sensory attributes (flavour and texture) and postharvest behaviour (resistance to chilling injury disorders). Since the beggining of the breeding program, we have launched a total of eight high-quality cultivars, five of them slow-softening nectarines with high flesh firmness sugar content, low acidity and an outstanding flavor. Yearly, the program performs 20-40 crosses, with a total of 3000 seedlings by year. From the seasons 2014-2015 and 2016-2017, a total of 12 advanced selections have been generated. However, since most of the production is exported to regions thousands of kilometers of distance, to allow a management the production calendar through the use of different cultivars, maturity date has been considered an additional prioritary trait, for which we are performing Marker Assisted Breeding.

Keywords:
peach, breeding, Chile, quality, sensory, marker, DNA, ripening date

Influence of selected parental forms on fruit and seed set in distant hybridization of *Prunus salicina Lindl.* (Japanese plum) and *Prunus armeniaca L.* (apricot)

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Abstract:

Distant hybridization is one of the important plant breeding techniques. This technique is used when there is no source of genus within a given species that determine the characteristics of cultivars, important for breeders and users. However, crossing different species of plants sometimes is difficult or even not possible to perform because of different crossability barriers – prezygotic and postzygotic (morphological, anatomical, physiological, biochemical or genetic). Pre-zygotic barriers prevent fertilization and embryo formation, while post-zygotic barriers disturb embryo development. The Research Institute of Horticulture in Skierniewice has been conducting research aimed at assessing the effectiveness of distant hybridization of selected Prunus genotypes belonging to the species *P. salicina* and *P. armeniaca*.

The research was carried out in 2015-2018 on trees growing in the collection of varieties in the Experimental Orchard in Dąbrowice and in a high tunnel in the Pomological Orchard in Skierniewice (Central Poland). Eight maternal genotypes of P. salicina and eight paternal genotypes of P. armeniaca were crossed. Relative to the number of pollinated flowers, the best at setting fruit, on average for the four years of the study, were the 'D 17-73' (25.1%) and 'Trumlar' (22.0%) genotypes (on average for the apricot paternal forms used), while the poorest were 'Angeleno' (3.3%) and 'OSL 57' (3.7%). The highest number of fruits was obtained by crossing 'D 17-73' × M I-7 (36.8%), 'D 17-73' × 'Early Orange' (31.9%) and 'Trumlar' × 'Early Orange' (30.0%). Apart from good fruit-setting, 'D 17-73' and 'Trumlar' also produced well-developed seeds with good germination capacity. The study showed that the 'D 17-73' and 'Trumlar' genotypes are good maternal forms in distant hybridization of *P. salicina* with *P. armeniaca*.

Keywords:
Interspecific crossing, *Prunus*, breeding, Japanese plum, apricot

Modern trends of breeding varieties for recreational areas of viticulture

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Abstract:

In modern conditions of global and regional climate change, there is a problem of recreational zones, which are considered both from the point of view of restoring human health and from the standpoint of restoring soil fertility and resuming agricultural production. One of the most plastic crops for cultivation in these conditions is grape. Considering the eastern Crimea as a recreational zone, several directions should be taken into account – the authenticity of the region's viticulture and the study of the salt tolerance of grape varieties.

A group of salt-tolerant autochthonous grape varieties of the Crimea has been identified, which can be cultivated at a high level of chloride salinity (NaCl) from 80 to 120 mM in root crops (cv. Asma). The data obtained during the vegetative experiments correlated with the data obtained in tissue culture in vitro. However, autochthonous grape varieties do not have a full range of valuable traits and properties for their effective cultivation, but it can be overcome by the method of generative hybridization. The effect of hybridization of the Crimean autochthonous grape varieties with varieties of different botanical origin has been established, the influence of maternal and paternal initial forms on cross ability has been revealed.

The observed variation coefficients of cross ability indicators as to the formation of hybrid seeds obtained from intraspecific crosses within the species of Vitis vinifera is almost twice lower than in interspecific crosses. The established mean value for cross combinations of the fully formed seeds differs insignificantly among intraspecific and interspecific crosses, with the difference 1.9%. It has determined a higher viability of hybrid seeds obtained as a result of cross-species hybridization. Using autochthonous variety ‚Krona', as a maternal form, and interspecific variety ‚Alminski', as paternal form, proved most efficient for obtaining vigorous progeny.

Keywords:
grapes, recreational zones, autochthonous varieties, breeding, generative hybridization, cross ability

New apple cultivar ´Antopa´

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Abstract:

It was selected upon evaluation of fruiting seedlings obtained after cross pollination of cultivars ´Angold´ and ´Topaz´. Slightly vigorous trees have still rather spreading canopies. They are fruting on spurs and acceptably productive. Till now seems to be resistant to scab and medium susceptible to powdery mildew. Fruits of obloid shape are medium to large. They are attractively nearly completely red coloured. Flesh is white, firm and very good. The harvest season is in the mean during the second week of October. Fruits are storable in a cold storage till April or May.

Keywords:
Tree characteristics, Fruit characteristics, Harvest time

New apple cultivar ´Semira´

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Abstract:

It was selected upon evaluation of fruiting seedlings obtained after cross pollination of cultivars ´Golida´ and ´Benet´. Trees of medium vigour are distinguished by spreading canopies.

They are fruting on spurs and very productive. The susceptibility both to scab and powdery mildew is on medial level. Fruits of medium size are attractive thanks to predominately dark red colour distributed flushed and mottled. Creamy flesh is firm and acidulated sweet. The harvest season is at the end of September to the first week of October. Fruits are storable in a cold storage till February or March.

Keywords:
Tree characteristics, Fruit characteristics, Harvest time

Parentage and inbreeding degree between apple cultivars of Epagri‘s Breeding Program - Brazil

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Abstract:

The Epagri has the largest Brazilian apple breeding program in activity under public funding, that completed 45 years in 2018. It was released 27 apple cultivars, including 15 hybrids that were developed from directed crosses presenting a few traditional cultivars in common along their genealogies. Unfortunately, it can lead to the narrow of variability of the program’s superior germplasm. So, the objective of this work was to estimate the parentage (Cpar) and inbreeding (Cinb) coefficients between the Epagri’s apple hybrids cultivars based on their genealogy and in relation to others traditional relative cultivars. The analyses were performed with PROC INBREED procedure of SAS® software. The Epagri’s cultivars show considerable predicted kinship, with the following mean values: Princesa (Cpar=0.31; Cinb=0.13), Condessa (Cpar=0.32; Cinb=0.13), Duquesa (Cpar=0.24; Cinb=0.09), Primícia (Cpar=0.18; Cinb=0.06), Imperatriz (Cpar=0.35; Cinb=0.14), Monalisa (Cpar=0.30; Cinb=0.12), Luiza (Cpar=0.32; Cinb=0.13), Fred Hough (Cpar=0.23; Cinb=0.08), Joaquina (Cpar=0.23; Cinb=0.08), Daiane (Cpar=0.35; Cinb=0.15), Baronesa (Cpar=0.28; Cinb=0.11), Venice (Cpar=0.35; Cinb=0.14), Catarina (Cpar=0.16; Cinb=0.05), Kinkas (Cpar=0.16; Cinb=0.05) and Elenise (Cpar=0.32; Cinb=0.13). The highest predicted relation was between Daiane and Princesa (Cpar=0.66; Cinb=0.33), since Daiane is daughter of Gala x Princesa (Princesa’s parents: NJ-56 x Anna). The lowest relation was between Princesa and the cultivars Catarina and Kinkas (Cpar=0.007, Cinb=0.003; respectively). These cultivars are full-sibs (parents: Fuji x PWR37TT133) and, in general, presented the lowest parentage degree with the others Epagri’s cultivars. The cultivars Jonathan and Golden Delicious make part the genealogy of 14 and 13 Epagri’s cultivars, respectively, however, with different mean kinship degrees (Cpar=0.09, Cinb=0.05; and Cpar=0.31, Cinb=0.16; respectively). The Malus floribunda 821 is present in the genealogy of the 07 scab resistant Epagri’s cultivars (mean Cpar=0.02; mean Cinb=0.01). Others genotypes can be highlighted by being part of the Epagri’s cultivars formation: Gala (mean Cpar=0.32; mean Cinb=0.16), Anna (mean Cpar=0.26; mean Cinb=0.13), Cripps Pink (mean Cpar=0.23; mean Cinb=0.11), Mollie’s Delicious (mean Cpar=0.21; mean Cinb=0.10), Fuji (mean Cpar=0.15; mean Cinb=0.07), Red Delicious (mean Cpar=0.12; mean Cinb=0.06) and Rome Beauty (mean Cpar=0.05; mean Cinb=0.02). The inbreeding coefficient represents the probability of alleles being identical by descent, and it is consequence of related parents. Considering the importance to decrease the genetic vulnerability and increase the biological diversity, the planning of the future crosses is very important in this context, since inbreeding can have adverse effects on traits associated with fitness, including reproductive and survivability characteristics, climate adaptation very important to the success of an apple cultivar.

Keywords:
Malus domestica Borkh., genetic improvement, genealogy, offspring

Designing successful apple crosses using novel self-incompatibility allele markers

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Abstract:

Apple breeding relies on design of compatible and productive crosses. The knowledge of S-genotypes of potential parents facilitates the selection of pairs of individuals carrying desirable traits and compatible S-alleles. Traditional incompatibility studies, based on costly and time consuming cross-pollination studies are now being replaced by molecular studies, using either allele-specific primers or restriction enzymes to digest PCR products of cleaved amplified polymorphic sequences (CAPS) or simple sequence repeats (SSR) markers. Allele-specific assays use agarose gels or capillary electrophoresis a single reaction per allele so do not provide information about other alleles in the gene pool. In addition, CAPS and SSR assays require a large quantity of costly restriction enzymes and numerous PCR reactions. Because of the complexity of the process, there are often delays in communicating genotypic information from the laboratory to the breeder. Nevertheless, thanks to previous assays, most of the S-genotypes of common commercial apple varieties have been already reported. However, each season any new genotypes being used as parents will need to be checked for compatibility.

We present here a high-throughput and practical set of markers for the identification of 13 different S-RNase alleles (S01, S02, S03, S05, S07, S08, S09, S10, S20, S23, S24, S25 and S28) using six allele-specific single nucleotide polymorphisms identified by high resolution melting technique (SNP-HRM) and two multi-allelic SSR markers. We used 55 commercial apple cultivars with previously reported S-genotypes as controls to validate the diagnostic accuracy of our markers in identifying the known S-alleles. We present the results of genotyping 80 advanced apple selections, representing some potentially valuable parents within the PFR apple breeding programme.

Keywords:
Self-incompatibility, apple, breeding, markers, marker-assisted-selection, S-RNase

Confirmation of intergeneric hybrids between apple and pear

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Abstract:
Malus and *Pyrus* are closely related, with highly co-linear genomes. However, the two genera are characterized by many specific differences, including disease resistances, secondary metabolites, fruit texture, flavour and shape. Hence, intergeneric hybrids between apple and pear provide a unique germplasm resource for genomic analysis, the application of advanced breeding strategies, and new cultivar development.

We utilized two sources of apple-pear hybrid plants, ’Zwintscher’s hybrid’ (Fischer et al., 2014) and its F2 progeny held at FEM, plus putative apple-pear hybrids developed at PFR. We determined which of the putative apple-pear hybrid genotypes were true hybrids by characterising the genomes for hybridity. We scanned the genomes of 47 putative hybrids with High Resolution Melting analysis (HRM) ‘apple/pear’ primers, developed following bioinformatics analysis, to detect SNP variants unique to the apple or pear genomes, respectively.

The initial primer set was optimised in a preliminary screen of DNA samples that included ‘Cox’s Orange Pippin’, ‘Old Home’, ’Zwintscher’s hybrid’, its parents and various mixtures. A set of 39 useful primers distributed along the genome was identified and used to screen the 47 putative hybrids. Results indicated these were true hybrids: 9-15 primers per genotype provided evidence for this. Interestingly, the apple and pear parents did not appear to contribute equally to the genomes of the progenies. Screening with a set of SSRs, as well as a preliminary screen of 6 samples over the 20K SNP array (Bianco et al., 2014) validated these results. We plan to screen all hybrid seedlings with the 9K apple/pear SNP array (Chagné et al., 2012; Montanari et al., 2013) to delineate their chromosomal structure.

Keywords:
intergeneric hybrids, *Malus, Pyrus*, apple-pear hybrid, HRM

Optimizing fruit breeding programs

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Abstract:

Fruit species are important crops worldwide for different purposes. In Vegenov, our projects aim at developing a range of tools (embryo rescue and molecular markers programs) to help breeders in optimizing peach and apricot breeding programs.

Plantlet growth after hybridization is one of the major bottlenecks for crop improvement in *Prunus* species. Seed germination requires stratification for a vigourous subsequent growth. Embryo immaturity of some early genotypes adds an additional difficulty to this process. Current seed stratification process, allow us to obtain 60 to 90% of plantlet development, depending on the crosses. For immature embryo rescue, the tissue culture technique developed leads to 50 to 80 % of plantlet development, depending mainly on the degree of maturity reached by the embryo in the fruit.

To better characterized the plantlets obtained by tissue culture technique and to help peach and apricot breeders to improve their selection programs, we are developing a range of molecular markers associated to genes or QTL controlling characters of interest : resistance to *Xanthomonas arboricola* pv. *pruni*, powdery mildew, monilia, sharka and green aphids and autoincompatibility.

We also developed genetic fingerprinting databases to control fruit plantlets production and to protect new varieties. For peach and apricot, we tested 22 SSR markers and we respectively selected a panel of 21 SSR markers that are the most polymorphic ones to optimize peach genotypes distinction and a panel of 7 polymorphic SSR markers to produce specific apricot genetic fingerprints.

These tools will help fruit breeders to (i) improve selection efficiency, (ii) reduce thetime and money needed to achieve breeding goals and (iii) secure the protection of new peach and apricot varieties.

Keywords:
Embryo rescue, marker assisted selection, fingerprinting, peach, apricot

New apple cultivar ´Kreja´

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Abstract:

It was selected upon evaluation of fruiting seedlings obtained after cross pollination of Melrose and Rubín cultivars. Trees of above medium vigour have spreading canopies. They are bearing both on spurs and long shoots and they are medium susceptible to scab and powldery mildew. Fruits of obloid shape are medium to large with mean weight of 200-250 g. Their red over colour is distributed flushed, striped and mottled on 60 - 75 % of surface area. The ground color is yellow green. Flesh is cream, crispy and juicy. Its taste is sweet or sub acid, more or less excellent. The harvest season is in the end of September or at start of October. Fruits are storable in a cold storage till March or April.

Keywords:
apple breeding, apple variety

Use of traditional cultivars in the Pillnitz apple breeding program

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Abstract:

In 1928 fruit breeding started in Dresden-Pillnitz, Germany. Since that time, breeding programs have been continuously conducted for different fruit species regardless of the political changes. Most important fruit species were and are apple, sweet and sour cherry. Since the reunion of Germany in 1990, the fruit breeding program at Dresden-Pillnitz belongs to the German Federal Ministry for Food and Agriculture and became part of the Julius Kühn-Institut, Federal Research Centre for Cultivated Plants in 2008. Besides fruit breeding, the institute is responsible for the preservation of fruit genetic resources and their valorization. One possibility of valorization is the use of traditional cultivars as donors for breeding. In this poster, the use of traditional apple cultivars as parents for crosses in the Pillnitz apple breeding program will be summarized for the time from 1972 until 2016. This includes the number of seeds produced, as well as the number of seedlings and cultivars selected from crosses, where at least one traditional cultivar was used as parent. Traditional cultivars are differentiated from the origin before 1900 (old) and in between 1900 and 1931 (middle-aged). Over the years, around 530,000 seeds were produced in total of which 277,000 resulted from crosses with traditional cultivars. 552 seedlings were selected for further observations and seven genotypes got or were proposed for plant breeder's rights. The highest proportion of seeds produced with old cultivars resulted from crosses with ‚Golden Delicious', ‚Delicious', ‚James Grieve', Cox Orangenrenette', ‚McIntosh' and ‚Jonathan'. The highest proportion of seeds produced with middle-aged old cultivars resulted from crosses with ‚Clivia', ‚Helios', ‚Juno', ‚Alkmene', ‚Auralia' and ‚Carola'.

Keywords:

apple, breeding, genetic resources, traditional cultivars

Apple cultivar ´Idapaz´

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Abstract:

It was selected upon evaluation of fruiting seedlings obtained after cross pollination of selection HL 13 (HL 324 x ´Idared´) and cultivar ´Topaz´. Trees of slightly over medium vigour have spreading canopies. They are bearing both on spurs and long shoots and they are medium susceptible to scab and powdery mildew. Fruits of obloid shape are medium to large.

Their over colour is medium red distributed like solid flush with weakly defined stripes on 50 to 75 % of surface area. The ground color is yellow green. Flesh is cream, medium to firm, sweet to sub acid and excellent. The harvest season is in the first week of October. Fruits are storable in a cold storage till March or April.

Keywords:
Tree characteristics, fruit characteristics, harvest time

Breeding Apple Scab Resistant Cultivars in Estonia

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Abstract:

Apple tree selection began at the Polli Horticultural Research Centre of the Estonian University of Life Sciences in 1946. A total of 35 new apple cultivars has been obtained. A majority of them is more or less susceptible to apple scab. The breeding of apple scab resistant cultivars started in 1986, when Kalju Kask cross-pollinated cultivars ‘Cortland’ and ‘Tiina’ with apple scab resistant Crab No 23 (a seedling of unknown origin). From a family of 167 seedlings he selected cultivars ‘Kuku’, ‘Ritika’ and ‘Ruti’. The fruit of these cultivars is small, weighing about 20g, round or slightly oblong. The flesh is dense and sweetish sour. The trees start bearing fruit early. Breeding scab resistant cultivars with large fruit started in 1997 in cooperation with the Latvian breeder Laila Ikase. Seeds of scab resistant cultivars ‘Florina’, ‘Freedom’, ‘Imrus’, ‘Liberty’, ‘Siostra Liberty’, ‘Releta’, and ‘Remo’ and selection BM41497 were collected. Crosses were made between cultivars: ‘Bogatyr’ × ‘Fantasia’, ‘Freedom’ × ‘Auksis’, ‘Lobo’ × ‘Remo’ and ‘Merrigold’ x ‘Stars’. Of 1372 seeds sown in spring 1998, 875 seedlings were obtained (63.8%). The trees were monitored during 10 years and the ones with leaves that became infected were removed. Out of 875 seedlings, 269 (30.7%) remained uninfected in these years. Out of these seedlings, 93 (34.6%) bore fruit by 2007. Based on the quality of fruit, 20 promising seedlings were selected. The breeding process that lasted 20 years resulted in two apple scab resistant cultivars: ‘Virve’ and ‘Kalju’ (2018).

Keywords:
apple scab, resistant cultivars

The historical pedigree from the almond breeding program at CEBAS-CSIC, Spain

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Abstract:

In 1985, the Fruit Breeding Group of the Department of Plant breeding of CEBAS-CSIC started an Almond Breeding Program to improve the early flowering and self-incompatible autochthonous cultivars. Since then, based on controlled outcross pollination and followed by phenotypic selection over several generations, the breeding population has been improved for traits with moderate to high heritability (generally controlled by a major gene) and also for some traits such as harvest date and flowering time with a more complex control. The constructed pedigree is composed of ~ 13,000 individuals and it is derived from current and historic records dating back > 30 years.

Keywords:
almond, pedigree, breeding

Peach breeding at Clemson University: Combining disease resistance with horticultural quality

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Abstract:

Environmental challenges, changes in production systems and human preferences are driving the need for development and release of new peach cultivars for sustainable production. The South Carolina peach industry is the second largest producer of fresh market peaches in the United States, after California. Peaches in SC are produced on ~7,000 ha with an annual gross income of $80M. Most of the cultivars grown in the Southeastern US are developed in breeding programs that differ in environmental conditions, such as CA, emphasizing the need for new improved cultivars suited for our conditions. The peach breeding program at Clemson University was re-established in 2008, after 25 years of dormancy, to develop high quality, disease resistant peach varieties adapted to environmental conditions specific to Southeastern US. The emphasis of the program is on combining high quality and consistent productivity with improved resistance to bacterial spot and brown rot and with nutritional composition and abiotic stress tolerance being the latest focus. To accomplish this goal, both traditional and molecular breeding approaches are utilized. Sources of new and improved traits were sought via characterization and utilization of the peach genetic diversity and development and utilization of genomic technology to improve breeding efficiency. The program has been actively involved in development and application of modern technological tools in breeding programs (www.rosbreed.org; www.rosaceae.org) and 10 years of progress will be presented here.

Keywords:

bacterial spot, brown rot, marker-assisted breeding, breeding information management system, genomics

Breeding for large-sized, highly aromatic Saskatoon-berry (*Amelanchier alnifolia*) varieties

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Abstract:
Amelanchier alnifolia is a fruits species native to North America. Some cultivars are known but systematic breeding work has been missing. In 2003 a breeding program on Amelanchier alnifolia was set up at Bavarian Centre of Pomology and Fruit Breeding. 30 accessions were collected and described concerning fruit quality and pomological traits. Cross pollination started with the most promising accessions. Breeding aims are fruit size, aroma, high soluble solids content, skin colour, uniform ripening of the berries, high and regular yield as well as easy picking. 12 breeding clones covering a ripening period of three weeks were selected and propagated for further selection. Using the newly bred Saskanoon-berry varieties this fruit species could become an interesting fruit species for European berry producers due to their early ripening, ease of production and robustness of the plant. The fruits resemble blue berries.

Keywords:
Amelanchier alnifolia, breeding, fruit quality, ripening, cultivar

RubyS, new small apple

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Abstract:
'RubyS' is a new apple cultivar released from the apple breeding program conducted at the Apple Research Institute, National Institute of Horticultural and Herbal Science, R.D.A., Korea in 2014. 'RubyS' is a weight of 51–72.7 g; shape is round, ground color is yellow green, skin is without russet, in 80% to 100% covered with red, flushed blush. Soluble sugar content of fruit is 14.5° Brix, titratable acidity is 0.53% as malic acid, and firmness is 78 N by penetrometer equipped with 11.8 mm probe. The fruit is no preharvest drop and the storage ability is very good. It can be stored at least 6 months in normal atmosphere at 1 °C. In case of grafting on M.9 rootstocks, tree vigor is weak and tree height is about 2m. For that reason, it is recommended to use semi-dwarf rootstock M.7 for commercial orchard. In Korea, the price of large apples traditionally was higher for ancestral rites and gifts of Korean Thanksgiving day season. However, demand for small apples is increasing due to population aging and small family growth. The school meals, cake decorations and apples for the outdoors are attracting attention, and demand for these small apple markets is expanding. Therefore, we developed 'RubyS' to meet these demands. It is a good quality small dessert apple cultivar with high storage potential. Its fruit is very attractive and has very good flavor.

Keywords:
Malus domestica, Preharvest drop, Self-incompatibility, Self thinning

Prunus Alignment - an ECPGR project aimed at integrating data for European plum and cherry germplasm

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Abstract:

'Prunus Alignment' is a collaborative European project aimed at aligning genetic resources of plum and cherry towards the European Collaborative program for Plant Genetic Resources (ECPGR) AEGIS concept of 'A European Genebank Integrated System'. The project aims to build upon progress made in the ECPGR PRUNDOC and EU.CHERRY projects by expanding on the SSR analysis of accessions of local and nationally important plum cultivars, and aligning national datasets of SSR for cherry germplasm collections. Plum accessions were first analysed by SSR using capillary electrophoresis, in order to identify and confirm unique genotypes for potential inclusion in the European collection. A subset of samples will be analysed further using high resolution melt profiles, to evaluate the possibility of using this technique to differentiate genotypic (SSR) profiles within ECPGR. SSR data for cherry from national datasets produced in Italy, Germany, Sweden, France, Switzerland and the UK will be aligned by selecting additional standard accessions across these collections for genotyping at the site of analysis of the EU.CHERRY dataset. Phenotypic descriptor data and all SSR data will be submitted to the ECPGR *Prunus* database and formatted for future inclusion in EURISCO.

Keywords:

ECPGR, *Prunus*, plum, cherry, SSR, high resolution melt, AEGIS

Classification of a Texan wild grapevine population within the genus Vitis

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Abstract:

The major challenge in rootstock development is to achieve highest possible protection against the most devastating viticultural pest, grape phylloxera (*Daktulosphaira vitifoliae* Fitch), while meeting the manifold site-specific demands within the multiplicity of winegrowing regions. For European conditions, adaptation to calcareous soils is required primarily. So far, only Vitis berlandieri has been the breeding source for limestone tolerance, but only a small amount of its gene pool is represented in the world's rootstock inventory of today. In Geisenheim, a germplasm collection of Texan wild specieswas established from previously collected seeds of different mother plants native to the Edwards Plateau in central Texas – a region mostly consisting of limestone related soils. Within this collection, the population which was given the name "Dawn View" (DV) attracted attention, not only for its high tolerance against phylloxera and fungal diseases, but also for its phenotypic deviations from the majority of the collection. The position of DV within Vitis was clarified in this project by phenotypic and genetic approaches comparing DV with different Vitis species originatingfrom America, Europe and Asia. The phenotypic approach included an assessment of 16 traits involving four shoot tip and twelve mature leaf characteristics. Most of them were evaluated according to the OIV descriptor list. The genetic evaluation included microsatellite-analysis and a next generation sequencing (NGS)-based method, double digest restriction-site associated DNA sequencing (ddRADseq). All applied methods were evaluated for their efficiency as tools for successful determination of DV's classification within the genus Vitis. According to our results it can be ruled out that DV belongs to the species *V. berlandieri.* Out of all evaluated species *V. monticola* shows highest similarity to DV, making it a species candidate. As *V. monticola* was not widely used in rootstock breeding, DV might represent a valuable genetic resource for future breeding programs.

Keywords:
Vitis berlandieri, Vitis monticola, genetic resources, double digest restriction-site associated DNA sequencing, ddRADseq

Preservation of genetic diversity of sweet cherry in Czech Republic

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Abstract:

From the point of view of human diet, sweet cherry provides a delicious fruit, which is rich in sugars and minerals. In the Czech Republic, sweet cherries are eaten seasonally out of hand or traditionally used in sponge cakes, desserts, fruit salads, juices, sorbets, ice cream, yoghurts or in distilled beverages. In Central European conditions, the earliest sweet cherries are the first fresh seasonal fruits. On the other hand, late sweet cherry cultivars are gaining popularity in commercial growing systems due to higher return. There were 850 ha of commercial sweet cherry plantations (mostly late varieties) registered in the Czech Republic in 2018. Numerous landraces of sweet cherry for example ´Chlumecka´, ´Horicka Pumra´, ´Libejovicka rana´, ´Techlovicka´, ´Sychrovska chrupka´, ´Vitovka molitorovska´ arose in different regions of the Czech Republic from medieval times. They were named after locality of distribution and typical for certain areas. These landraces represent the most valuable part of genetic diversity. They are well adapted to the soil-climatic conditions of a particular region. Displacing traditional landraces in modern agricultural systems and continuing destruction of old plantations and alleys contributed to a considerable loss of genetic diversity in Central Europe. Because of the possible extinction of these valuable genotypes, the programme for collecting and long term conservation of sweet cherry genetic resources has been carried out in Research and Breeding Institute of Pomology in Holovousy (RBIPH). Rescue of sweet cherry landraces is described in this presentation. Collecting missions were directed to the regions not influenced by modern agricultural production. Important accessions were localized by Global Positioning System (GPS) and in situ registered. Sweet cherry landraces showed a high variation in tree size, productivity, ripening time, fruit size, quality and disease resistance. The most important cultivars found were: ´Karesova´, ´Kastanka´, ´Napoleonova´, ´Thurn Taxis´, ´Velka cerna chrupka´, ´Ladeho pozdni´. Selected accessions were characterised and transferred to field genebank of RBIPH. Germplasm preservation of sweet cherry in field conditions is supplemented using biotechnological methods (*in vitro* cultures, cryopreservation).

Keywords:
Prunus avium, genetic resources, landrace, in situ, rescue, biodiversity

On farm rescue and preservation of fruit genetic resources

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Abstract:
The evolution of fruit cultivars is a spontaneous process influenced by many factors and growing traditions. It consists of initial historic phase of unconscious selection by growers for several hundred or thousand years, followed by intentional selection by breeders in successive breeding steps. Traditional fruit landraces represent a valuable part of genetic diversity, because they differ from their wild progenitors morphologically and genetically and they are not influenced by narrowing of genetic base in modern breeding programmes. Old landraces are usually specific for a county and represent also a cultural heritage of particular nation. The security of fruit genetic resource collections requires their conservation by several techniques. On farm conservation is an alternative method for preservation of fruit genetic resources in indigenous areas. This method is currently investigated and employed in Research and Breeding Institute of Pomology (RBIP) Holovousy Ltd. Four on farm plantations KRNAP Vrchlabi, “Orchard of Reconciliation” Neratov, National park Podyji – Znojmo and National park Sumava were successfully established in cooperation with RBIP Holovousy Ltd. At present, there are mostly apple, pear, plum and sweet cherry landraces in these plantations. Because of longevity of the plantation, seedling rootstocks were used for production of planting material. On farm collections comprise plants representing a diversity of genes and gene combinations for particular traits for each preserved fruit species. For efficient use of this biodiversity source, comprehensive evaluation of phenotypic and genotypic characters is carried out in RBIP Holovousy Ltd.

Keywords:
landraces, cultivars, ex situ, biodiversity, conservation

Results from testing of yellow-green apple cultivars

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Abstract:

The present study was carried out during the period 2015-2019 at the Institute of Agriculture - Kyustendil with poorly distributed in the production yellow-green apple cultivars. The purpose was to offering the most suitable of them for wider use in Bulgaria.The trees were grafted on MM 106 rootstock and planted in spring of 2002 at distances of 4.5 x 2.5 m. Control cultivar was 'Golden Delicious'. The blooming and ripening time, growth, productuvity and chemical characteristics of the fruit were evaluated. First flowering occurred on 15 April ('Rosana', 'Ginger Gold', 'Arkcharm') and the latest on 19 April ('Golden Delicious'. Fruit of 'Arkcharm' and 'Ginger Gold' ripened the earliest - between 11 and 23 August, while 'Charden' and 'New Jonagold' the latest - from 28 September to 04 October. Average yield per tree ranged from 17.0 kg ('Belgolden') to 37.6 kg. ('Rosana'), the fruit weight was from 128 g ('Belgolden') to 253 g ('Charden'), fruit flesh firmness - from 7.1 kg/cm2.('Arkcharm') to 11.3 kg/cm2 ('Rosana'). The fruit of 'Golden Delicious' and 'New Jonagold' are characterized with the highest content of soluble solids (18.0 and 16.6%) and total sugars (9.1 and 8.5%). The results showed that the cultivars studied are suitable for the agro-climatic conditions of Kyustendil region and deserve a wider spread in the production of apples.

Keywords:
Malus × domestica, blooming, yield, fruit weight, fruit flesh firmness

Genetic diversity of apple landraces from VIR collection based on SSR markers

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Abstract:

VIR Collection of Plant Genetic Resources is the largest in Russia and includes about 3800 Malus Mill. accessions. The collection is maintained at six institution branches located in regions with different climatic conditions. At Pushkin and Pavlovsk Laboratories of VIR, near St. Petersburg, a collection of 531 apple species, cultivars and landraces from the Northwest and Central Russia and Northern Europe is maintained. Many of these landraces have high ecological plasticity and increased adaptation to abiotic stress.

To study genetic diversity of 108 apple landraces from VIR collection ten SSR markers were used. Analysis was performed on ABI Prism 3130xl (Applied Biosystems). As a result 116 alleles were detected. The number of alleles per locus varied from 9 (CH03d01, CH01f03b, CH02c09) to 20 (CH02c02a) and averaged 11.6. High frequency of rare (57.76%) and unique (20.69%) alleles was found. The polymorphism information content (PIC) was rather high 0.59 (CH01h01) - 0.87 (CH02c02a) with an average value of 0.74. The expected heterozygosity (He) was 0.76. For each of the studied accessions specific alleles combinations were revealed. Dice genetic similarity coefficient varied from 0.97 (for Arkad Zimnyj - Pudovka and Skryzhapel‘ (klon) - Pudovka) to 0 (for 11 pairs of studied landraces) and averaged 0.32. Cluster analysis did not reveal a clear differentiation of the studied samples, but allowed to suggest some relationships based on putative pedigrees and origin.

Thus the analyzed collection of apple landraces showed high genetic diversity which indicates its potential for research and breeding purposes. The results will provide valuable information for proper characterization and effective management of the plant material preserved in VIR collection.

Keywords:
apple, landraces, genetic resources, microsatellite markers

Classification for the Genus Sorbus by Multivariate Analysis of Morphological Characteristics

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Abstract:

Genus Mountain ash (*Sorbus commixta, Sorbus sambucifolia, Sorbus amurensis, Sorbus aucuparia*) is a deciduous and taller tree that is widespread in China, Japan, and Korea. In those countries, it has been used as medicinal resources for treating various ailments, as well as food, an industrial, and an ornamental tree. This study was conducted to an analysis of bud, leaf, flower and fruit characteristics among the four mountain ash species and then consider its relationship using the multivariate analysis on the basis of raw data among 8 populations. From the principal component analysis of 28 characteristics, three principal components were obtained, which explained the variation of inter-specific relations among the genus of mountain ash. The first, the second and the third components contributed 58.95%, 29.79%, 11.26% of the variations, respectively. The first principal component was related to Fruit width (FW), Length of a terminal leaflet (LTL), Petal length (PL) and Diameter of a winter bud (DWB). The second principal component was related to Number of the leaflet (NL), Number of the fruit/fruiting lateral (NFL), Length of a upper rachis (LUR). And the third principal component was related to the Diameter of a rachis (DR), Length of a winter bud (LWB). According to the cluster analysis based on 28 characteristics, the Sorbus spp. populations were classified into two groups with an average distance greater than 7.0 between the group. Group I was *S. amurensis, S. aucuparia* and group II was *S. sambucifolia* and *S. commixta.*

Keywords:
characteristics, cluster, Mountain ash, multivariate analysis, medicinal resource

Definition of the optimized core in the Spanish apple conservation collection

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Abstract:

The Spanish apple conservation collection (SACC) is composed of 285 genotypes unequivocally assigned to the “Iberian genepool” in a previous study, as well as 172 others that are admixed within foreign genepools. The preservation strategy of the traditional Spanish apple germplasm aims to rationalize the conservation, characterization and evaluation of this material by assigning priority levels to the entries of the collection. The goal of this work was to determine the most appropriate strategy for the selection of the minimum set of accessions (optimized core) that efficiently represents the genetic variation within the SACC. This optimized core, selected by genetic criteria, will constitute the “first priority” level of genotypes for phenotyping and evaluation purposes. The efficiency of advanced stochastic local search (ASLS) selection strategies that differed both in the final size of the core and in the combination (and relative weight) of the measures of genetic distance and allelic richness to be optimized has been evaluated. The strategies employed have provided optimized cores with large differences in preserved diversity, as well as in the representativeness of the overall genetic structure. Taking into account the main use of this optimized core, the strategy that offers the best balance between representativeness and suitability for use is the one combining the optimization of the average distance between each genotype of the entire collection and the nearest entry in the core, with the Shannon-Weaver diversity index and the allele retrieval.

Keywords:

Malus x domestica Borkh., core collection, genetic resources, SSR markers, optimization.

Genomic architecture of the Swedish National collection of mandate apple cultivars

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Abstract:

Previous studies of national germplasm collections at the European level has given insight into the patterns of diversity and gene flow between countries. However, these studies relied on SSR-markers, while being highly informative for assessments of genetic diversity and haplotype clustering they are lacking the resolution needed for assessments of genome-wide IBD and tracking of horticulturally important haploblocks. Furthermore, previous studies has only investigated a minor proportion of the Swedish National apple collection. Therefore, we are now genotyping the Swedish National apple collection with a 20k SNP array in order to get an insight into the genomic architecture of the entire collection. This collection is expected to be at least partially adapted to a northern climate, and thus the genotypic data will improve the collection's usefulness in breeding for improved hardiness. By that, we intend to turn the national apple gene bank into a true genetic resource.

Keywords:
apple, germplasm collection, SSR markers, genetic diversity

Definition of the optimized core in the Spanish apple conservation collection

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Abstract:

The Spanish apple conservation collection (SACC) is composed of 285 genotypes unequivocally assigned to the "Iberian genepool" in a previous study, as well as 172 others that are admixed within foreign genepools. The preservation strategy of the traditional Spanish apple germplasm aims to rationalize the conservation, characterization and evaluation of this material by assigning priority levels to the entries of the collection. The goal of this work was to determine the most appropriate strategy for the selection of the minimum set of accessions (optimized core) that efficiently represents the genetic variation within the SACC. This optimized core, selected by genetic criteria, will constitute the "first priority" level of genotypes for phenotyping and evaluation purposes. The efficiency of advanced stochastic local search (ASLS) selection strategies that differed both in the final size of the core and in the combination (and relative weight) of the measures of genetic distance and allelic richness to be optimized has been evaluated. The strategies employed have provided optimized cores with large differences in preserved diversity, as well as in the representativeness of the overall genetic structure. Taking into account the main use of this optimized core, the strategy that offers the best balance between representativeness and suitability for use is the one combining the optimization of the average distance between each genotype of the entire collection and the nearest entry in the core, with the Shannon-Weaver diversity index and the allele retrieval.

Keywords:

Malus x domestica Borkh., core collection, genetic resources, SSR markers, optimization

Conservation of fruit genetic resources in Sweden

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Abstract:
Since 2016, Sweden’s pomological heritage is the responsibility of the National Gene Bank for Vegetatively Propagated Horticultural Crops. In this article we describe the background to the establishment of the gene bank and how the preservation system for fruit cultivars is organized. The system focuses on so called mandate cultivars. A mandate cultivar is a cultivar that is either of Swedish origin or that has a long growing tradition within the country. Today some 340 mandate cultivars are preserved within the gene bank preservation system, of these about 220 are apples, 55 pears, 45 plums and 30 cherries. Recently the planting of a central collection at the Swedish University of Agricultural Sciences in Alnarp was started with the aim to assemble all mandate cultivars in the same field. Along with this collection, the gene bank preservation system also has back-ups at 14 local clonal archives hosted by botanical gardens, outdoor museums and other similar types of establishments. Each cultivar is therefore preserved with two trees in the collection at Alnarp and two trees in a local clonal archive. The allocation to clonal archives reflects the geographic origin of the cultivars. For example, the apple cultivar ‘P. J. Bergius’ is preserved at the Bergius Botanic Garden in Stockholm, while the Gotlandic apple cultivar ‘Stenkyrke’ is preserved by the local museum on the island of Gotland. Besides their responsibility to safeguard mandate cultivars the clonal archives are also partners in disseminating knowledge about the local pomological heritage to the general public.

Keywords:
Keywords: fruit gene bank, heirloom cultivars, apple, pear, plum, cherry

Genetic Diversity and Population Structure of the Spanish Pear Resources

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Abstract:

European pear (*Pyrus communis* L.) is one of the most important fruit crop of the temperate regions worldwide. Though some previous studies about the genetic variability of pear genetic resources from Spain were already performed at the regional scale, a complete analysis is needed in order to evaluate the whole diversity and structure of this species at the national level. For doing that, the Spanish Government funded the project "Optimization of apple and pear germplasm management and application of biotechnology in the analysis of their suitability for breeding programs". In the present study, we analyzed a set of 1041 pear accessions from Spanish germplasm collections using a common set of 11 SSR markers. SSR profiles were obtained independently and allele sizes between the different datasets were subsequently aligned using a common set of cultivars selected as references. First preliminary results allowed identifying 510 unique genotypes and 119 duplicated groups of accessions. Bayesian model-based clustering combined with multivariate and molecular variance analyses were also applied to investigate the genetic diversity at the Spanish national-level for this species for the first time, as well as to shed light about the distribution of this germplasm into population subdivisions.

Keywords:
germplasm collections, local cultivars, *Pyrus communis* L., SSR markers, variability

EUCARPIA

Physicochemical and sensory evaluation in apple diversity from mountainous areas in Spain

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Abstract:

Ancient apple cultivars grown in local areas have so far been largely unexplored and might attract a large share of consumers oriented towards natural food evoking ancient flavors. In this work, 37 traditional apple cultivars of the Pyrenees and 14 commercial varieties were analyzed in terms of genetic diversity, acidity profile, ºBrix and sensory traits. The results show a great variability observed in local accessions in parameters such as acidity, brix, firmness and color. A great correlation has been observed between different factors, such as acidy and sourness, over color, skin flavor, russet, weight, or Juicy with flesh, sweetness, flavor and eating quality. Between commercial and local cultivars, the analysis showed a very wide range of variability for the values of acidity, brix, bitterness and astringency. The results show the variability found in the group of local varieties, as well as the different correlations between different characters analyzed, which open the studies to select the most suitable cultivars in order to promote the intrinsic value of local food products of quality and differential value with interest in the market.

Keywords:
Local germplasm, *Malus domestica*, fruit quality, sensory traits

Association of ParSOC1 genotype with dormancy and flowering time among sweet cherry genebank accessions

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Abstract:

Early spring frost damage and extreme temperature fluctuation after their dormancy period is a serious problem for growers. Prunus species are very sensitive to sudden rises and drops in temperature in spring, when dormancy ends and rising temperature causes bud break and leads to flowering of the trees. Extreme weather conditions are getting more common, thus new cultivars tolerant to such conditions are needed. In order to breed new cultivars adopted to the changing climate, understanding the dormancy and flowering of fruit trees, and knowing their genetic background is important.

Some evidence supports that ParSOC1, which encodes a MADS-box gene, is involved in the control of dormancy break, and controls flowering time. Studies identified 13 alleles of the ParSOC1 gene in apricot, and 3 alleles in peach. In our studies, 32 sweet cherry accessions were analysed. Nine alleles of ParSOC1 gene were identified, the majority of the accessions were heterozygous, only 9 were found to be homozygous. The 200 bp allele was the most common; it has been carried by more than 66% of the investigated accessions. The second most common was the allele 246 bp, it was present in 19 accessions.

To identify flowering time of the accessions, phenological data were recorded in three consecutive years, and also chilling requirements of the selected accessions were determined. Comparison of genotypic and phenotypic data revealed possible correlation between observed traits and specific alleles present in accessions. Allele 224 was only present in early flowering accessions, with low chilling requirements, while allele 232 occurred in accessions with higher chilling requirements. These two newly identified alleles might predict a sweet cherry cultivar's chilling requirement and flowering time, thus might be used as a functional molecular marker for these traits in breeding programs.

Keywords:
dormancy, sweet cherry, ParSOC1 gene

EUCARPIA

Tracking down the changes in olive intra-varietal genetic diversity for an efficient use and conservation: the ‚Galega vulgar' case study

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Abstract:
Olive oil and olive trees are symbols of the Mediterranean diet and agriculture with a high economic impact. In Portugal, 'Galega vulgar' also called 'Galega' is the main national variety. This variety is of major importance due to its high-quality olive oil (under a PDO label — Protected Designation of Origin). However, 'Galega' has been replaced by more productive foreign varieties, leading to an intense reduction on its cultivation, which might endanger the survival of this national high-quality genetic resource. The enrichment of the current understanding of the ‚Galega' genetic diversity is a key aspect for its efficient use and conservation. In this context, we traced down the ‚Galega' genetic variability evolution by comparing 335 centenary accessions, representative of germplasm still in production at the Portuguese orchards, with 260 ancient accessions (500-2,000 years old) collected from the same locations. Fourteen microsatellite markers were used to calculate a variety of population diversity indices in these two groups of accessions. Out of 595 accessions, 95 distinct genotypes were identified. The two most frequent genotypes of ‚Galega' were found in 254 and 104 individual trees, respectively, combining both centenary and ancient accessions. Ancient accessions showed a higher clonal richness (58.0 vs 48.9), private clonal richness (38.0 vs 30.1) and genotypic richness (0.22 vs 0.17) as compared to centenary accessions, while gene diversity was almost equal (0.397 vs 0.402) in the two groups of accessions. Thus, while there are some indications of genetic erosion that took place during the transition from ancient to centenary ‚Galega' accessions, the overall level of gene diversity has been maintained. These results will enable further investigations of the agronomic value and breeding potential of ancient olive trees representing rare ‚Galega' clones.

Keywords:
Olea europaea; genetic diversity; SSRs; ancient olive trees; centenary olive trees

Simple sequence repeat markers enable genetic characterization of mulberry germplasm preserved in the CREA's collection of Padua (Italy)

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Abstract:

Sericulture, silkworm rearing to produce silk, relies primarily on mulberry (Morus spp) leaves to feed the insect. Today, mulberry is also gaining attention due to the nutritional quality of its fruits, in particular the high levels of ascorbic acid and anthocyanins, especially abundant in the black-fruited varieties. Silk industry was an important economic resource in Italy from Middle Ages to the first half of XX century, and a number of traditional varieties were locally selected in distinct cultivation areas. Today, although sericulture in Italy has been almost completely abandoned, a remarkable amount of the genetic diversity of germplasm has been preserved since the '60s of the last century at CREA, Padua, Italy.

This Mulberry Germplasm collection is composed of 15 accessions of traditional germplasm developed in distinct Italian areas where sericulture was once thriving, 25 accessions imported from Japan and 30 of various and often unknown origin. This genetic material shows great variability in terms of phenotypic characters of fruit, leaf and general plant behavior.

As a complete genetic characterization of this germplasm is still missing, we analyzed the molecular profiles and the genetic relationships of a part of the mulberry collection, using single sequence repeat (SSR) corresponding to 13 previously characterized microsatellite loci. The preliminary results show different clusters, with Italian cultivars partially separated from the rest of accessions with the presence of some accession of unknown origin, probably the results of hybridization events. Despite the number of used markers, not all accessions could be differentiated; this result could be attributable to the scarce efficiency of selected markers, which were developed on different genetic material. Alternatively, homonyms might be present, i.e. the same genotypes (genetic material) individuated under different names in the different Italian areas where they were originally cultivated.

This study integrates other genetic analyses on the Italian mulberry germplasm and could represent a starting point to broaden the study to other collections. Investigating the genetic variability and relatedness among European accessions is relevant for breeding purposes; indeed, the mulberry tree is exploitable both for sericulture and functional food as well as for its rusticity and tolerance to dry and hot Mediterranean climatic conditions, which suggest its use in areas susceptible to erosion and desertification for environmental purposes.

Keywords:
M. alba, germplasm, sericulture, SSR

Phenotypic analysis of fruit quality traits and effect of climate in an apple germplasm bank of Aragón, Spain

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Abstract:

Apples are among the most widely consumed fresh fruits in the world. They constitute a major contribution of phytochemical compounds to the human food. The total phenolic contents (TPC) are associated with a reduced risk to develop degenerative diseases. In this work 155 apple accessions (*Malus x domestica* Borkh) from a Germplasm Bank established at the Experimental Station of Aula Dei, located in the Ebro Valley (Zaragoza, Northeastern Spain) were studied. The TPC were determined for 5 consecutive years (2014-2018) using a spectrophotometer photodiode array detector. TPC values ranged from 3.3 to 116.7 with a mean of 39.6 mg Gallic acid equivalents (GAE) 100g FW-1. Significant differences were observed among accessions. A set of mixed-effects models were fitted to the data with the TPC as the dependent variable, the climate features of each growing season as independent variables or fixed effects, and the variety as random effect. Minimization of the Akaike Information Criterion (AIC) statistic was used for feature selection, using a manual forward procedure. The best model included precipitation (although non-significant), minimum daily temperature and solar radiation. The R2 of the model was 0.85, and the mean absolute error (MAE) was 5.75. A cluster analysis was performed on the model coefficients space to classify the 155 accessions, using the Ward's D method based on the Euclidean distance. A solution with five groups was selected as allowing for the best interpretation. The local traditional grown varieties were concentrated in three of the five established groups. TPC values tended to decrease with temperature and increase with solar radiation. Statistical analyses highlighted accessions and climate (minimum temperatures and solar radiation) as the main factors affecting metabolite profile (TPC) and fruit characteristics.

Keywords:
Malus x domestica, total phenolic contents, antioxidants, temperature, solar radiation

Genetic variability of a geographically diverse almond germplasm

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Abstract:

To compare genetic diversity parameters and characterize genetic differentiation in almond (*Prunus dulcis* (Mill.) D. A. Webb), eighty-six accessions of different geographic origin were genotyped in 15 SSR (simple sequence repeat) loci. All genotypes clustered according to their pedigree and geographic origin. Nine genetically distinct subgroups were clearly separated by STRUCTURE analysis including the Kyrgyz, Akdamar, Bademli, Hungarian, Monor, Italian, Moroccan, Californian accessions, wild species and an admixed group. A considerable genetic variation within populations was detected using AMOVA analysis, and genetic variation among populations was also significant. The mean values of the fixation index (FST) indicated marked genetic differentiation among the populations. Significant correlation between the geographic distance and the genetic distance was not found. Our data showed that that geographic distance among the assessed populations has little influence on their genetic differentiation. Important components of genetic differentiation were drift, mutations, and massive gene exchange between several wild almond species and cultivated P. dulcis. A moderate loss of genetic diversity is caused by geographic isolation, human selection and recent occurrence of self-compatibility. Almond could efficiently preserve its genetic variability along its dissemination routes from the center of origin and throughout the continents. There was no indication of a major decrease in genetic diversity in almond germplasm from Asia to Europe. Our results proved that almond domestication avoided the occurrence of a genetic bottleneck although its risk is present in many subpopulations.

Keywords:
Almond Genetic variability Genetic differentiation Microsatellites *Prunus dulcis* SSR

Unravelling genetic diversity and phylogenetic relationship of a collection of Malus species based on SSR markers

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Abstract:

The apple plants of genus *Malus* Miller is an economic crop of great value. The *Malus* species plays all important role not only in practical use in fruit cultivation, breeding and ornamental plant production, but also in the research of apple origination, evolution and genetic diversity. A collection of 312 apple accessions, including 37 *Malus* species maintained at the Horticulture Experimental Station of the Northwest A&F University, Yangling, China, was genotyped using 15 SSRs with the aim of assessing the genetic diversity within this panel of accessions, evaluating relationships among them and determining their genetic structure. Genotyping made the identification and clarification of the 15% redundancy, involving 47 accessions from 10 groups with different names but identical SSR profiles. A total of 265 unique genotypes amplified 445 alleles in total with a mean number of alleles per locus (A) of 29.73, ranging between a minimum of 21 (Hi05e07) and a maximum of 43 different alleles (CH01a09), demonstrating a much more diversity when compared to a previous investigated *M. domestica* collection. In this *Malus* species collection, we identified that 0.75% accessions are pentaploid, 4.15% are tetraploid and 20% are triploid, which support that *Malus* species are polyploids in origin and can reproduce both sexually and apomictically. Based on the genetic distance analysis of DICE coefficient, the unweighted pair group method average (UPGMA) clustered the 265 accessions into five groups. Group I contained only *M. florentina* which had the fastest relationship to the remaining population; Group II consisted of mainly the Yunnanensis Series, including *M. honanensis, M. yunnanensis, M. pratti* and *M. ombrophila* together with *M. kansuensis*; Group III contained mainly Chloromeles Series, including *M. coronaria, M. angustifolia* and *M. platycarpa* ; Group IV contained mainly Gymnomeles Series, including *M. baccata, M. rockii, M. hupehensis, M. halliana* and M. sieboldii and M. toringoides; and the Group V consisted of *M. sieversii, M. prunifolia* and *M. domestica* subsp. *Chinenesis.*

Keywords:
Malus species, SSR, genetic diversity, phylogenetic relationship, evolution

Characteristics of pest occurrence on jujube by cultivation types in Korea

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Abstract:

In Korea, jujube (*Zizyphus jujuba* Mill.) has been attracting attention as a fruit, and its cultivation in greenhouse is increasing to produce the high quality fresh fruit. In this study, we investigated the pest occurrence of Bokjo cultivar cultivated in open field and 4 types of greenhouse, multi span greenhouse with roof and side vent, multi span rain shelter house with roof vent, single span rain shelter house with a column in the center and roof vent and single span rain shelter house without roof vent, at Boeun region of Chungbuk province located in the central part of Korea where jujube is mainly produced as a fresh fruit. In this study, the main disease was anthracnose by *Collectotrichum sp*. and bacterial fruit rot by *Xanthomonas arboricola*. Anthracnose and bacterial fruit rot occurred from July and June in open field, repectively, but almost not in greenhouse where *Alternaria sp*. and *Cladosporium sp*. that were not confirmed pathogenic were isolated from flower in a humid environment. The main pests are Miridae which is *Apolygus sp., Dasineura sp*. and *Tetranychus urticae*. The incidence of Miridae was relatively high in open field compared with that in greenhouse. On the other hand, Dasineura sp. and Tetranychus urticae started to occur earlier and more occurred in the greenhouses, especially with roof and side vent where no. of fruit per branch was decreased due to Dasineura sp.. And *Pseudococcus comstocki* occurred only in the greenhouses. As a result of this study, the occurrence pattern of pests was different according to the cultivation type of jujube. Therefore, depending on the type and purpose of production, it is necessary to establish target pest and control strategies for pest resistant breeding and stable production of jujube.

Keywords:
jujube, pest, *Apolygus sp., Dasineura sp., Collectotrichum sp., Xanthomonas arboricola*

Selection of submergence tolerant pear rootstock germplasm and related root growth habit changes under waterlogged condition

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Abstract:

Major climate change issues observed in Northeast Asia is drought in early spring and sudden heavy rainfall in summer. Recently, as rainy season in summer is becoming longer and intensive for short periods, Korean growers are concerned about excessive moisture injury for the fruits. Waterlogged soil make anaerobic environments for root system, and that cause abnormal growth and necrosis accompanying root rot in fruit trees. Genetic resources composed of various species contributes to have great genetic diversity for pear (*Pyrus* spp.). Generally, it has been known that pears have better submergence tolerance than other fruit crops. However, as most pear root stocks using in Korea are chance seedlings generated from harvested fruits (*P. pyrifolia*), it is hard to expect they have reliable growth stability for waterlogged or harsh soil condition. In this research, we screened submergence tolerant *Pyrus* germplasm by seasonal investigation and excess irrigation. In conclusion, *Pyrus betulifolia* line displayed the most tolerance under waterlogged condition. Though axial root growth was decreased, vitality was rarely affected by submergence. Rootlet and root hair growth was increased and their vitality was dramatically decreased, vice versa. As an abiotic stress, submergence is considered to affect hormonal changes in pear. It leads to morphological modification in root system which is advantageous for oxygen uptake in the waterlogged soil.

Keywords:
pyrus betulifolia, abiotic stress, adventitous root

Venturia nashicola (Asian pear scab) full-genome sequencing and gene annotation resource

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Abstract:
Venturia nashicola is a fungal pathogen that causes Asian pear scab disease. This pathogen is of particular importance in Northeast Asian countries, where Asian pears are growing industrially. Scab disease in Asian pear is currently controlled by fungicide spraying and this situation calls for developing scab resistant cultivars. Therefore, high quality genome data is required for in-depth comparative genome analysis of different isolates of *V. nashicola* and *V. pyrina*, a closely related *Ventruia* species. We report the high contiguity whole genome asembly of V. nashicola isolates. Two different strains MAFF615029 and PRI2 collected from Fukuoka, Japan and Naju, Korea, respectively, were employed for producing sequence information. PacBio single Molecule Real-Time (SMRT) platform was applied to MAFF615029 for the employment of reference genome and Illumina Hi-Seq 3000 platform were applied to PRI615029 for analysing genetic variation. Each performances generated 198 contigs totaling 41,878,255 base pairs and 1,849 contigs totaling 33,716,288 base pairs for MAFF615029 and PRI2, repectively. We expect that genome comparisons for various *Venturia spp*. would help to identify the genes involved in determination of host range of V. nashicola., and provide more reliable tools for disease resistance breeding in pears.

Keywords:
scab, sequence, genome, effector

Breeding value of selected apple cultivars (*Malus domestica*) for the resistance to apple scab and powdery mildew

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Abstract:

Suitability of several apple cultivars for breeding of new genotypes resistant/low susceptible to apple scab and powdery mildew based on their general (GCA) and specific (SCA) combining abilities.

Infection degree of apple seedlings, being the progeny of 11 cultivars crossed in the model: ♀4 x ♂7 – 4 maternal cultivars (‘Alwa’, ‘Golden Delicious’, ‘Free Redstar’, ‘Gold Milenium’) and 7 paternal cultivars (‘Glogierówka’, ‘Kronselka’, ‘Kosztela’, ‘McIntosh’, ‘Oliwka Żółta’, ‘Malinowa Oberlandzka’, ‘Koksa Pomarańczowa’) by both apple pathogens was evaluated in July 2018. Results of the statistical analysis (SERGEN programme) have shown the significant GCA effects for the infection degree by apple scab and powdery mildew for some cultivars. Significantly negative values for the GCA effects for the infection degree by apple scab were estimated for ‘Free Redstar’ and ‘Gold Milenium’. It indicates that both cultivars are good donors of genes responsible for resistance to apple scab in the progeny seedlings. Negative value of GCA effects for susceptibility to powdery mildew was stated for ‘Free Redstar’ only. It means this cultivar may serve as good donor of genes determining resistance/low susceptibility to powdery mildew in its progeny.

The SCA effects estimated for 28 cross combinations have shown both positive and negative values for the studied characteristics. The negative SCA effects for resistance/low susceptibility to apple scab were stated for 5 cross combinations: ‘Free Redstar’ x ‘Koksa Pomarańczowa’, ‘Gold Milenium’ x ‘Kosztela’, ‘Alwa’ x ‘Oliwka Żółta’, ‘Golden Delicious’ x ‘Glogierówka’ and ‘Golden Delicious’ x ‘Malinowa Oberlandzka’. The negative SCA effects for powdery mildew resistance/lowsusceptibility was stated for one cross combination only: ‘Alwa’ x ‘Koksa Pomarańczowa’.

Keywords:
apple, *Malus domestica* L., apple scab, powdery mildew

Candidate loci identification for resistance to *Venturia nashicola* in interspecific hybrid pear (*Pyrus pyrifolia × P. communis*)

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Abstract:

‘Greensis’ (*Pyrus pyrifolia × P. communis*), one of the Korean bred pear cultivars, has resistance to Venturia nashicola, which causes scab. Although a gene conferring resistance to V. nashicola, was identified in an indigenous Japanese pear cultivar, it showed susceptibility to V. nashicola recently. Therefore, re-identification of scab resistance loci is important. Scab resistance was evaluated in 93 seedlings derived from a cross ‘Greensis’ (resistance) × ‘Whasan’ (susceptibility). Single nucleotide polymorphisms (SNPs) derived from genotyping-by-sequencing and simple sequence repeats were used to construct two genetic linkage maps. The position of resistance loci was identified in terminal region of linkage group (LG) 6 of ‘Greensis’ and twelve SNPs were linked with the region. The candidate SNP loci for scab resistance cover pseudo-chromosome 6 of pear reference genome from 17,037,120 to 21,079,665 bp. In the genomic region, we could find 25 genes related with disease resistance. Using the sequences of candidate region for scab resistance, construction of fine map for LG 6 will be possible to identify new scab resistance gene.

Keywords:
genotyping-by-sequencing, genetic linkage map, molecular marker, scab, single nucleotide polymorphism

EUCARPIA

Sensitive, fast and easy detection of grapevine fleck virus from crude extracts by reverse transcription loop-mediated isothermal amplification (RT-LAMP)

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Abstract:

Grapevine fleck virus (GFkV) is the causing agent of fleck disease and occurs in grapevine worldwide. Infection is latent in most scion and rootstock cultivars but can lead to several symptoms such as translucent spots and clearing of veins in leaves as well as problems when grafting with rootstocks. Symptoms typically increase when infections with multiple virus species occur. Therefore the EU directive 2005/43/EC demands that rootstock propagation material must be tested negatively. The virus spreads primarily through infected propagation material. Thus, excluding infected grapevines from propagation is the only possibility to control the spread of this disease. Consequently, sensitive, inexpensive and fast methods for its detection are needed.

In the present study, a detection method for GFkV was developed employing reverse transcription loop-mediated isothermal amplification (RT-LAMP). A method to reverse transcribe viral RNA to DNA and amplify DNA in one step. Primers were designed to bind all known variants of the coat protein gene. The RT-LAMP works without time consuming RNA extraction. In contrast it can be performed on a crude homogenate, made from wooden disks mixed with water and beaten with a sledge to destroy the fibers. This homogenate is roughly filtered and directly used to set up the RT-LAMP reaction which is incubated at 64 °C on a fluorescence measurement system. The method takes approximately two hours from sample preparation to result. Sensitivity of RT-LAMP was compared to ELISA, a method widely used for routine diagnosis of grapevine virus. The comparison was performed by diluting extracts from infected material in extracts prepared from healthy plants. While ELISA gave positive results after diluting infected samples 1:10, detection with RT-LAMP was possible after diluting up to 1:10.000. Thus, it is possible to identify also those plants with lower virus titres, which then can be excluded from further propagation.

Keywords:
virus, grapevine fleck virus, GFkV, loop-mediated isothermal amplification, LAMP, reverse transcription loop-mediated isothermal amplification, RT-LAMP

Pyramiding fire blight resistances Fb_E and FB_MR5 in Malus domestica

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Abstract:

Fire blight, caused by the bacterium *Erwinia amylovora*, is the most destructive bacterial disease in apple production. Strong resistances are present in wild *Malus* species, e.g. *M. × robusta* 5, *M. baccata* and *M. fusca* (Peil *et al.* 2009; Emeriewen *et al.* 2014). To date only a few partially resistant cultivars are available for farmers (e.g. ‘Ladina’, Kellerhals *et al.*, 2011) and more research is needed to understand the complex host-pathogen-interaction (Silva et al. 2019). Following the devastating disease outbreak in Switzerland in 2007, a specific fire blight resistance breeding approach was established at the Agroscope apple breeding programme, Waedenswil. In 2008, a speed breeding approach introducing major resistances originating from wild *Malus* species and partial resistances from modern cultivars was established under greenhouse conditions, called „Fast Track“. This method reduces the long apple generation cycle of 4 to 5 years under field conditions, to approximately 2 years, without using GMO techniques. The method includes marker assisted selection, artificial winter simulation and the use of growth regulators. To achieve commercial fruit quality after the introgression of wild material, several pseudo-backcrosses (pBC) with high quality parental lines are required. After ten years, the most advanced generations carrying the resistance gene FB_MR5 (*M. × robusta* 5, pBC’4) or Fb_E (Evereste, pBC’3) are presented, including results of the artificial shoot and flower inoculation tests, population studies, breeding achievements and fruit quality development. Keeping in mind that Peil *et al.* (2011) showed that a single point mutation in the pathogen genome is sufficient to bypass the resistance mechanism of *M. × robusta* 5, pyramiding different resistances is of great importance. In the last year, several crosses were performed to combine the resistance genes in the most advanced generations. The pedigree of the first plant carrying Fb_E (pBC’3) and FB_MR5 (pBC’4) are presented.

Keywords:
apple breeding, *Malus*, fire blight, *Erwinia amylovora*

GWAS of Fruit Quality and Marssonina Blotch Disease Resistance in Apple Germplasm

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Abstract:

The Apple (*Malus × domestica* Borkh.) is a highly valuable fruit crop worldwide and get a lot of attention as one of model plants in Rosaceae family. The primary purpose of apple breeding program is to combine high fruit quality with disease resistance and to breeding new apple cultivar that have high commercial value. Genomic selection has strong potential to efficiently advance fruit tree breeding system. For apply this, it is necessary to confirm association between genomes and targeted traits. Genome wide association study (GWAS) is useful method that analyze association between genome and traits in fruit tree crops. Using apple germplasm, we evaluated major traits including fruit height, fruit diameter, and apple blotch disease resistance. GWAS analysis was performed with SNP data based on Genotyping-by sequencing (GBS) and identified significantly SNPs related to apple traits. Related to fruit size, significant SNPs associated with fruit height showed up on chromosome 8, 4, 15, and 9, whereas those associated with fruit diameter were confirmed on chromosomes 3, 15, 8, and 9. Highly significant SNPs relative to marssonina blotch disease resistance were detected on chromosome 3, 9, 10, and 17. This study will be able to propose candidate genes and markers including SNPs associated with apple traits. The genomic selection using these results will contribute to improve breeding system of apple tree efficiently.

Keywords:
GBS, GWAS, Apple, Fruit quality, Marssonina blotch disease resistance

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The effector protein AvrRpt2EA from Erwinia amylovora induces salicylic acid response in apple

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Abstract:

The AvrRpt2EA effector protein of *Erwinia amylovora* is important for pathogen recognition in the fire blight-resistant crabapple Malus × robusta 5; however, little is known about its role in susceptible apples. To study its function in planta, we expressed a plant-optimized version of AvrRpt2EA driven by a heat shock-inducible promoter in transgenic plants of the fire blight-susceptible cultivar Pinova. After induced expression of AvrRpt2EA, transgenic lines showed shoot necrosis and browning of older leaves, with symptoms similar to natural fire blight infections. Transgenic expression of this effector protein resulted in an increase in the expression of the salicylic acid (SA)-responsive PR-1 gene but, also, in the levels of SA and its derivatives, with diverse kinetics in leaves of different ages. In contrast, no increase of expression levels of VSP2 paralogs, used as marker genes for the activation of the jasmonic acid (JA)-dependent defense pathway, could be detected, which is in agreement with metabolic profiling of JA and its derivatives. Our work demonstrates that AvrRpt2EA acts as a virulence factor and induces the formation of SA and SA-dependent systemic acquired resistance.

Keywords:

Malus, fire blight, resistance, AvrRpt2EA

Characterization of drought response and nutraceutical value in Mediterranean almond cultivars

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Abstract:
Almond is an important fruit crop species worldwide cultivated for its appreciate kernel for both processed food industry and as a functional food with and medical (nutraceutical) properties including nutrients, vitamins, healthy blood lipids or anti-inflammatory and hypocholesterolemic properties. This fruit tree species, compared to other nut crops grown in Mediterranean climates, is relatively drought resistant. Climatic conditions, in particular climate variability and water availability, require the development of production systems able to cope with risk and uncertainty. Therefore, rusticity and flexibility of the different components of the production systems (including varieties) should be improved together with a suitable kernel quality including nutraceutical values. A new experiment using wild relatives and commercial genotypes have been used to understand drought response in almond. In addition, a characterization of drought resistant almond cultivars from the Mediterranean Sea (Morocco, Tunisia and Algeria) with high nutraceutical values has been carried out. These studies allowed the preliminary identification of several drought-resistant cultivars and ecotypes. In addition, the evaluation of nutraceutical properties (lipid and fatty acid contents, vitamins, phytosterol content, minerals, protein and aminoacids, phenols and fiber) can result in an increase of the add value of these cultivars. A better understanding of the physiological mechanisms associated with drought resistance in almond will be reached.

Keywords:
drought, nutraceutical, almond, resistance, quality

Response of apple genotypes to UK Phytophthora cactorum isolates in cut-shoot tests

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Abstract:

Phytophthora cactorum is a water-borne oomycete pathogen, responsible for economically-significant losses in the commercial production of apple, strawberry and a number of other important horticultural crops. In cultivated apple (Malus x domestica), P. cactorum causes bark rots on the scion (collar rot) and rootstock (crown rot), as well as necrosis of the fine root system (root rot) and fruit rots. Reproducibly characterising plant genetic resistance in controlled environments can be difficult; most reports of inheritance in apple have looked at segregations following inoculation of young seedlings whilst cultivar performance is often confirmed in field plantings.

This study aimed to test the usefulness of inoculating detached shoots to determine the response of apple genotypes to a range of UK P. cactorum isolates. We tested 29 apple genotypes to assess the feasibility of this method for large scale phenotyping of germplasm, breeding lines and mapping populations to inform UK breeding. Of the genotypes assessed, 13 showed different degrees of susceptibility to P. cactorum isolates, while the rest showed no lesion development. In addition, to support the results obtained in this study and start to elucidate the genetic basis of the inheritance of resistance, seedling populations of a subset of the genotypes employed will also be tested for resistance/susceptibility to P. cactorum. There are also plans to expand this testing to non-UK isolates and opportunities for collaboration are most welcome.

Keywords:
Apple rootstocks, germplasm phenotyping,

EUCARPIA

Resistance to mildews in the genome and in the vineyard: more than 100 grapevine accessions at a glance

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Abstract:

The development of novel varieties resistant to mildews and other fungal diseases is commonly considered as one of the most promising steps towards a more sustainable viticulture. Different resistance genes have been described having origin in wild grapevine species and conferring a barrier to downy (DM) and powdery mildew (PM). Several of them have been introgressed into Vitis vinifera background resulting in the connection of sustainability and excellent wine quality. Despite the discovery of many resistance factors, it is common knowledge that disease resistances are not necessarily stable traits and could be overcome by evolutionary developments of the pathogen. Thus, pyramiding resistance genes is considered to be an effective strategy to stack various defense mechanisms and hence increase resistance durability.

A collection of 102 grapevine accessions descending from crosses between Vitis species and vinifera cultivars was studied. Their leaf and cluster level of DM and PM resistance was evaluated in an untreated field at veraison and harvest time in three consecutive years. Besides a true-to-type analysis, an exhaustive genetic characterization was carried out at eleven exploitable loci associated with mildew resistances (R-loci) available in the literature to date.

The majority of the genotypes carried two or more R-loci associated with DM and/or PM. Interestingly, 8% of the genotypes showed phenotypic resistance scores with no known R-loci, indicating putative novel sources of resistance. The effect of single or pyramidized R-loci on phenotypic resistance is described, as well as the impact of variations within and between years.

The aim of this study was to evaluate the response of grapevine genetic resources to mildews resistance in an untreated field in combination with the R-loci characterization. The three years experiment shed light on the effect of different R-loci, and combinations thereof, on mildew disease outbreaks under the same environmental conditions.

Keywords:

Disease resistance loci, Erysiphe necator, grapevine, Marker-Assisted Breeding, *Plasmopara viticola*.

Dormancy release monitoring in almond by metabolomic analyses

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Abstract:

Dormancy release is a prerequisite for flowering in temperate fruit trees as Prunus species. It is known that metabolites play a key role in dormancy release. Therefore, in this study, dormant flower buds from four different almond (*Prunus dulcis* (Mill.) D.A. Webb) cultivars (Desmayo Largueta, Antoñeta, Penta and Tardona), with different chilling requirements and flowering times, were analysed in an UPLC-QTOF equipment, obtaining the metabolite fingerprinting of every single sample. The spectra of those metabolites with the most significant variation along the different states during dormancy release were subjected to the Metlin, HMDB and KEGG databases. These analyses allowed us to know the putative identification of the compounds and the different pathways involved. Interestingly, some of those pathways, such as the abscisic acid biosynthesis, just varied in one of the late-flowering varieties. Nevertheless, it was the glutathione metabolism that varied in all the varieties. This pathway is related with reducing species and is modulated by the abscisic-gibberellin acid ratio. Moreover, some fatty acids suffered a sharp decrease in the earliest-flowering varieties, while some oligopeptides showed a huge decrease in the late-flowering species. This work is a stepping stone in the development dormancy-associated biomarkers in temperate fruit tree species.

Keywords:
almond, breeding, dormancy, flowering, metabolomics, *Prunus dulcis*, UPLC-QTOF

EUCARPIA

Screening of citrus germplasm to identify tolerant and resistant genotypes to Plenodomus tracheiphilus (Petri) Gruyter, Aveskamp & Verkley

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Abstract:

“Mal secco” is one of the most harmful disease of citrus and it is widespread in different Mediterranean countries. It’s a tracheomycotic disease caused by the fungus Plenodomus tracheiphilus (Petri) Gruyter, Aveskamp & Verkley. Besides lemon (*Citrus limon* L. Burm.) “Mal secco” pathogen infects, with high yield losses, other citrus species, like as citron (*C. medica* L.), lime (*C. aurantifolia* Christ.), bergamot (*C. bergamia* Risso), sour orange (*C. aurantium* L.) and volkamerian lemon (*C. volkameriana* Ten. Et Pasq.). With the aim of identifying sources of tolerance to “Mal secco”, we started a phenotypic survey in the CREA lemon and lemon-like germplasm located in Acireale, Italy. The germplasm was planted in 2002 and included 25 lemon clonal selections and autotetraploids, as well as 17 citron and lemon hybrids. Other citrus species reported as tolerant or resistant were also included in the survey as reference. All genotypes were replicated twice or three times and were planted in the same field block with high pressure of P. tracheiphilus. A few very susceptible lemon clones died within 3 to 5 years after planting, and some others declined slowly without a significant ability to recover from the disease. Phenotyping for “Mal secco” symptoms started in 2018 and was performed twice on the remaining clones. A molecular screening by PCR was also performed to detect possible sources of P. tracheiphilus in old flushes, new flushes and branches.

The results revealed important sources of tolerance in the germplasm. The pathogen was detected by PCR in all lemon clones, many lemon hybrids and citron hybrids, also in plants without clear symptoms, suggesting the ability of some genotypes to recover from the disease. Interestingly, an autotetraploid lemon seems to be immune to the disease under natural pressure, since P. tracheiphilus was not diagnosed by PCR and visual screening. The survey was also helpful to identify the most suitable parents for the development of new segregating populations.

Keywords:
Lemon, breeding, mal secco, phenotyping

Dormancy of sweet cherries in climatic conditions of two different locations

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Abstract:

Fruit species including cherries (*Prunus avium*) are adapted to climatic conditions in medium latitudes and are known as temperate fruits. These fruit species require a certain amount of cold called chilling period to complete their life cycle. Without this period, cherry trees would not finish their development (endodormancy), trees would produce less pollen, which would affect the fruit set and total crop. Duration of the chilling period varies among cherry cultivars. Chilling requirement is a limiting factor for growing sweet cherries in countries with mild winter. It could be recorded as a chilling hours (CH), an accumulation of hours with temperatures 0 - 7.2 ° C. The objective of this study was to determine the chilling requirements of sweet cherry cultivars in conditions of locations Pillnitz (Germany) and Holovousy (Czech Republic). In the locatin Holovousy, from the 34 genotypes, the lowest chilling requirement, lower than 500 CH, was recorded for cultivar ´Adélka‘. Medium chilling requirement with 500 – 750 CH was shown by cultivar ‚Kasandra‘. Medium high requirement (1000 – 1500 CH) and high requirement (over 1500 CH) was observed in all other tested cultivars. Collection of selected cultivars bud samples were also done for evaluation of genetics response of sweet cherry cultivars.

Keywords:
temperate fruits; *Prunus avium*; mild winter; chilling requirements, climatic change

Improvement of salt tolerance and resistance to Phytophthora gummosis in citrus rootstocks through controlled hybridization

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Abstract:
The sustainability of Mediterranean citriculture depends largely on the use of rootstocks that provide a better adaptation to biotic and abiotic constraints, as well as a good graft-compatibility with commercial cultivars. In the absence of rootstocks meeting all these criteria, the management of the available diversity and the selection of the desirable traits are necessary. In the present work, we conducted a controlled pollination program involving four different rootstock genotypes that belong to the germplasm collection of INRA Morocco. These genotypes included citrumelo cv. 'Winter Haven' (*Citrus paradisi Macf. x Poncirus trifoliata L. Raf.*), Sunki mandarin (*Citrus sunki*), Cleopatra mandarin (*Citrus reshni*), and pomelo (*Citrus grandis*). The breeding program resulted in the development of a population of 199 seedlings, 31 of which expressed marked morphological traits of the male parent. Molecular characterization of these 31 genotypes using flow cytometry and microsatellite markers helped to determine their ploidy level and to confirm the parental origin of the zygotic seedlings. On the other hand, the phenotypic characterization of the potential hybrids by early screening tests revealed heterogeneous responses to salinity and Phytophthora attacks. An interesting combination of salt tolerance traits (chloride exclusion, high relative growth, high stomatal conductance) with resistance to Phytophthora gummosis was observed in two citrumelo hybrids, H56 and H92, which seem therefore to be qualified for use in salt and Phytophthora affected regions as a replacement for the CTV-susceptible rootstock, sour orange.

Keywords:
Citrus, salinity, *Phytophthora diseases*, conventional breeding, phenotypic characterization

Evaluation of the tolerance of seven citrus rootstocks to Phytophthora gummosis under saline conditions

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Abstract:

The combination of biotic and abiotic stresses in semiarid regions results in severe losses in citrus production. Neglecting the interactions that may occur between these factors would lead to the over or underprediction of the performance of rootstock species under natural conditions and therefore to their erroneous ranking. In this study, seven citrus rootstocks, including two new Poncirus trifoliata hybrids, were evaluated for tolerance to Phytophthora gummosis after a short exposure to salt stress. The inoculation was made at stem level with a pathogenic isolate of Phytophthora citrophthora collected at the experimental field of INRA in Kenitra, Morocco. The results of this study highlighted a significant influence of salinity on the length of stem necrosis caused by Phytophthora attacks. Interestingly, the seedlings of the new Poncirus trifoliata hybrids, F6 and F18, expressed a high level of tolerance to gummosis under saline conditions similar to that observed in the 'Gou Tou' sour orange (Citrus aurantium L.) and Sunki mandarin (Citrus sunki Hort. Ex. Tan.) checks, whereas pomelo (Citrus grandis) and citrumelo cv. 'Winter Haven' (Citrus paradisi Macf. x Poncirus trifoliata L. Raf.) were ranked as sensitive. The length of stem necrosis was positively correlated with the accumulation of chloride in the leaves, but was independent of stem water content. This finding supports the hypothesis that the fungal infection is favored by the specific effects of salts rather than their osmotic effects.

Keywords:
Citrus, environmental stress, salinity, *Phytophthora citrophthora*, chloride

Public Private Partnership project NORDFRUIT: meeting the future challenges in Nordic apple and strawberry production

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Abstract:

Nordic countries are situated at the edge of the growing region for horticultural crops, which seriously limits fruit and berry production. Still, consumers ask for locally produced fruit and berries and thus their demand is met only partly leaving a market space for increased local production. The germplasm adapted to northern conditions is highly specialized and unique. To develop cultivars competitive on the fresh fruit market and resistant against dominating diseases, the germplasm adapted to our conditions has to be crossed with germplasm from other regions in the world. Scientists from seven Nordic and Baltic countries and The Netherlands joined their forces within a Public Private Partnership project with the target of providing resistant, high quality germplasm and efficient breeding tools to their fruit and berry breeders. The focus of NORDFRUIT is to strengthen the pre-breeding cooperation among the partners in line with previous PPP-projects (NORDAPP). It evaluates germplasm resources and creates genetic and phenotypic information, in order to widen the parental pool to be used in the breeding for targeted traits. Phenotyping protocols are standardized and validated for evaluating both disease resistance, e.g. apple canker and fruit rots in apple and crown rot in strawberry, and fruit quality traits. Specially designed core and diversity collections well representing the most important cultivars as breeding parents and covering broad genetic variation, respectively, will be characterized phenotypically and genetically by high-density molecular markers, to validate marker-trait associations found elsewhere as to find new ones. Within this project we also enhance and develop the genetic competence among breeders and associated scientists as well as initiate the process of integration of modern genetic tools into the breeding programs. The project is coordinated by Graminor (Norway) and operates from 2018 to 2020.

Keywords:

Pre-breeding, apple, strawberry, genetic diversity, disease resistance, fruit quality

EUCARPIA

Understanding genetic resistance to Armillaria root rot in Prunus

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Abstract:

Throughout the U.S., stone fruit and nut crops (peach in South Carolina, Georgia, and California; cherry in Michigan; almond in California) are under threat from Armillaria root rot (ARR), for which there are no long-term control or mitigation strategies. ARR is the main factor in mature orchard decline, and the causal fungi are comprised of multiple Armillaria species, which infect and reside in the root system and kill trees long before they reach their maximum productivity. There is one commercially available rootstock with resistance to ARR for peach (‘MP-29’), but it is not widely accepted by growers. Furthermore, ARR resistant rootstock development is hampered by the difficulties associated with inconsistent infection, time and other complexities in the greenhouse and field hotspots, respectively. To make advancements toward our understanding of resistance mechanisms expressed in the’ MP-29’ rootstock, we developed a uniform population of ‘MP-29’ clonal propagules and a population of P. persica susceptible controls (GF 305) and co-cultivated them with Armillaria mellea for a period of 12 weeks. We observed rapid canopy decline after 3 weeks in the inoculated ‘GF 305’ genotype; while ‘MP-29’ showed no signs of decline. Furthermore, the ‘GF 305’ inoculated lines were completely dead at 8 weeks, while ‘MP-29’ persisted well beyond 12 weeks, suggesting validation of resistance to heavy pathogen pressure in the in vitro system. To develop an understanding of the genetic mechanisms endowing resistance to ARR, we sequenced complete transcriptomes of both the GF305 and ‘MP29’ genotypes under inoculated and non-inoculated conditions. The in vitro co-culture system and gene expression analysis will be presented.

Keywords:
replant disease, rootstock, genomics, breeding, tissue culture

Inheritance of resistance to Plum pox virus in apricots

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Abstract:

Since 1981, the aim of the apricot breeding programme at the Faculty of Horticulture, Mendel University, in Lednice has been to obtain new adaptable cultivars that combine valuable biological traits. Only few apricot cultivars, which does show resistance, 'Stark Early Orange' 'Harlayne' and 'Orangered' were chosen for a study of the genetics of PPV resistance. In order to determine the inheritance of resistance to PPV in apricots between resistant and susceptible cultivars resp. resistant x resistant parents were performed. The seedlings were inoculated with the PPV-M strain by an infected bud. PPV infection was evaluated over 3–5 consecutive growth periods through visual symptoms, ELISA and in some cases reverse transcriptase PCR assays. Chi-square analysis of each progeny was performed to determine if the segregation ratio differed from the expected ratio. PPV resistance segregated in apricot B1 progenies in a 1:7 (resistant:susceptible) ratio, where SEO was used as resistant genitor. The results show that PPV resistance present in the donor 'Harlayne' was transmitted to its descendants. Within families, the resistant: susceptible ratio was 1:1. The progeny are being screened to confirm the hypothesis of inheritance of the resistance after gene pyramiding. The observed segregation ratio (153:30:16) for the progeny of two resistant parents 'Harlayne' × 'Betinka' was not significantly different from the predicted 12:3:1 segregation, in the case of 'Orangered' × 'Harlayne' was obtained ratio 9:7 when we expected two- gene hypothesis of resistance or 27:37 in tree -gene hypothesis of resistance.

Keywords:
apricots, *Prunus armeniaca L., Plum pox virus*, progeny, chi-square test, breeding

Identification of scab resistance genes in new Hungarian apple hybrids using SCAR and SSR markers

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Abstract:

Disease-resistant apple varieties are ideal selections as raw material for processing apple purée. The best apples for purée are those that are sweet and less acidic with a flesh that breaks down easily. For production of baby food apple purée yellow-skinned and yellow-fleshed fruits are preferred. Some promising scab resistant apple hybrids suitable for this purpose have been selected as part of a new Hungarian private apple-breeding program. These are also resistant to powdery mildew and could be grown with only a few pesticide applications each year. Considering the reports that certain scab races overcome the resistance of the scab resistant cultivars carrying the Rvi6 (Vf) gene only, within this apple-breeding program the main aim is pyramiding of resistance genes in the newgenotypes. In current study resistance genes of seven new promising hybrids were detected. Rvi1 (Vg), Rvi2 (Vh2), Rvi4 (Vh4), Rvi6 (Vf), Rvi8 (Vh8) and Rvi15 (Vr2) resistance locus were analyzed using SCAR (AD13, OPL19, OPB18 and AL07) and SSR (Ch05e03 and Ch01d03) markers. The results of these examinations will be shown on the poster.

Keywords:
Malus x domestica, scab, marker analysis

New scab resistant apple cultivars registered in Hungary: Bellona, Damara and Isolda

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Abstract:
An apple breeding program has been carried out at the Dept of Pomology, University of Horticulture and Food Industry and at successor institutions from 1990 until 2016. The aim of this program was to widen Hungarian apple assortment by resistant and tolerant cultivars with high fruit quality. As a result of this program, previously four multiresistant (‘Artemisz’, ‘Cordelia’, ‘Hesztia’, ‘Rosmerta’) and two tolerant cultivars (‘Karneol’ and ‘Rodonit’) were introduced into the cultivation in Hungary.This year three new cultivars (‘Bellona’, ‘Damara’ and ‘Isolda’) were registered in Hungary and are marketable in the EU. Damara was developed from a cross between ‘Freedom’ and ‘Florina. Mature around the harvest time of ‘Golden Delicious’ and are good for fresh eating and juice. The bright to dark red fruit is medium to large in size and has a light-colored, mildly acidic, crispy flesh. While resistant to scab it is slightly susceptible to powdery mildew and fire blight. Bellona was also developed from ‘Freedom’ and ‘Florina’ cross. Ripen just after ‘Golden Delicious’ and are suitable for dessert or juice and concentrate purposes. The fruits are medium or large size with fine texture, juicy, and have a good acid-sweet balance. In addition to apple scab, it is resistant to fire blight and moderately resistant to mildew. Isolda is a bicolor sweet dessert apple selected from the progenies of ‘Fuji’ and ‘Freedom’. The fruit is medium-large and the skin color is yellow overlain with orange stripes. Flesh is light cream, juicy with rich flavor. Mature about two weeks before ‘Golden Delicious’. Trees are resistant to apple scab, powdery mildew and fire blight, too. The most important characters of the cultivars will be shown.

Keywords:
Malus x domestica, scab, mildew, fire blight

Physiological and transcriptomic analysis of the effect of ethylene biosynthesis regulator Ethrel® application in the postharvest behaviour of Japanese plum (*Prunus salicina L.*) fruits

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Abstract:

Japanese plum (*Prunus salicina* L.) is one of the most important worldwide stone fruit trees, and there is great commercial interest not only for fruit quality but also for its shelf life period, which largely determines the plum varieties commercialization to faraway countries. In this context, the main aim of this work is to study the postharvest behaviour of two contrasting varieties (‘Angeleno’ and ‘September King’), analysing the main differences at fruit gene expression level after maturation induction using ethylene biosynthesis regulator Ethrel®. ‚Angeleno‘ is characterized by a long shelf-life period having a suppressed climacteric stage unlike to ‚September King‘. The results showed that ‚Angeleno‘ didn’t show differences between Ethrel® treatments for softening, respiratory rate or ethylene production, while ‚September King‘ showed a greater softening and respiratory rate at higher Ethrel® doses. In addition, ‚September King‘ started the ethylene emission on the fourth day for the treatment of 1,000 ppm and on eleventh day for the 300 ppm treatment. However, ‚Angeleno‘ didn’t initiate a climacteric stage in any case. Regarding the results of RNAseq, almost 89% of the filtered counts aligned with the peach reference genome, around 7% didn’t align, while almost 4% of the counts aligned in multiple positions. The results of differential gene expression showed important differences between varieties and treatments especially for genes involved in cysteine-methionine, flavonoid biosynthesis, pentose-glucuronate, starch-sucrose and plant hormone signal transduction.

Keywords:
Postharvest, Transcriptomic, RNAseq, Ethrel, *Prunus salicina*

Physiological and transcriptomic changes derived of the application of ethylene biosynthesis regulators 1-MCP® and Ethrel® in different apricot (*Prunus armeniaca* L.) and Japanese plum (*Prunus salicina* L.) cultivars

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Abstract:
Japanese plum (*Prunus salicina* L.) and apricot (*P. armeniaca* L.) are together with peach (*P. persica* D.A. Webb.) the most worldwide commercialized stone fruits. Japanese plum and apricot breeding programs are mainly focused on fruit quality traits. Therefore, in the current context, the analysis of postharvest traits related to the fruit shelf-life period is a new breeding aspect of special interest. Thus, the aim of this work is the physiological and transcriptomic analysis of postharvest behaviour of different Japanese plum (‚Angeleno', ‚Black Splendor', ‚Golden Japan' and ‚Santa Rosa') and apricot (‚Moniquí', ‚Goldrich' and ‚Dorada') cultivars using ethylene biosynthesis regulators 1-MCP® (ethylene inhibitor) and Ethrel® (ethylene precursor). Regarding the study of apricot cultivars, first results showed that ‚Moniquí' was the most climacteric variety since it showed a fast softening and high ethylene emission in all the treatments, whilst ‚Goldrich' and ‚Dorada' showed a lower softening rate, being ‚Dorada' the lowest climacteric variety. In the case of plum cultivars, ‚Santa Rosa' produced the highest ethylene emission, whereas ‚Angeleno' was characterized by a longer shelf-life period and didn't start its ethylene emission until day 21 after treatment. Therefore, apricot ‚Goldrich' and Japanese plum ‚Santa Rosa' were selected on day 4 as the most contrasting varieties for transcriptomic analysis using RNA-Seq. A de novo transcriptome assembly was performed by Trinity in order to reconstruct the transcript sequences without reference genome sequence. A total of 51,481 unigenes from a total of 86,685 were annotated. Differential expression gene analysis showed a list of candidate genes involved in the response to the application of 1-MCP® and Ethrel® in apricot and Japanese plum.

Keywords:
Postharvest, Transcriptomic, 1-MCP, Ethrel, *Prunus salicina, Prunus armeniaca*

Pesticide residues and their influence on the total antioxidant activity of apricot fruits

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Abstract:

The monitoring the dynamics of degradation of residues of selected pesticides in apricots in fresh and stored fruits and the effect of post-harvest treatment with 1-methylcyclopropene (1-MCP) and ozone on total antioxidant activity (TAA) was monitored. Residues of 9 active pesticides (pyraclostrobin, boscalid, myclobutanil, fenhexamid, fenpyrazamine, thiacloprid, acetamiprid, trifloxystrobin and pirimicarb) were analyzed in fresh fruits of the variety ´Betynka´ (28 days after the first application of pesticides) as well as after 10, 20, and 30 storage days. None of the samples tested exceeded the maximum residue limit (MRL) for the active substance for fresh fruit or after removal from storage. The degradation of the active substances in apricot fruits occurred already in the pre-harvest period, depending on the number of days since the application of the individual preparations. Fruits treated with ozone and 1-MCP had a slight decrease in their content during storage. The total antioxidant activity of fresh fruits and fruits treated immediately after ozone harvest and 1-MCP was compared. During evaluating the total antioxidant activity of fresh fruits in 1-MCP treatment, the values were very similar for all variants. When using post-harvest ozone treatment, it can be stated that every fruits picking date (after 10, 20 and 30 days), all variations of TAA were increased. The main influence on the TAA value is in particular the storage method, whereas the spray type is rather a negligible factor.

Keywords:
apricots; rezidues pesticides; storage; ozone; 1-methylcyklopropene

Yield and fruit quality of some promising apple hybrids selected in Bulgaria

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Abstract:
In 2015-2019 the blooming and ripening time, productivity and fruit quality of six apple hybrids, selected at the Institute of Agriculture in Kyustendil were evaluated. The trees grafted on rootstock MM 106 were planted in the spring of 2007 at distances of 4.5 x 2.5 m. ‘Golden Delicious’ was used as a standard cultivar for comparison. According to the ripening time, selection 1/7 (‚Winesap‘ x ‚Prima‘) is mid-ripening while the rest selections are late ripening. The greatest average yield (31.3 - 32.0 kg/tree) was obtained from selections 1/18 (‚Winesap‘ x ‘Erwin Baur’) and 1/3(‘Mollie’s Delicious’ – open pollinated). The trees of hybrid 8/25 (random seedling) had the most yield efficiency (1.07 kg/cm2 of trunk cross-sectional area). The greatest average fruit weigh (242 g) had 8/25 and the smallest 1/3 (112 g). The fruit of hybrid 1/18 had the highest flesh firmness, followed by hybrids 2/30 and 1/3 (11.35; 10.47 and 10.12 kg/cm2, respectively). The highest soluble solids content (18%) and total sugars (8.9 - 9.0%) was found in 1/3 and ‘Golden Delicious’.

Keywords:
Malus × domestica, selection, ripening time, yield, fruit weight, firmness

Gene expression of Mal d 1 allergen isoforms in apple fruits

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Abstract:

Apples are one of the most consumed fruit species, although in a certain percentage of people eating fresh apples may cause an allergic reaction. We evaluated the gene expression of Mal d 1, a major apple allergen for Central and Northern Europe. Gene expression was evaluated in four cultivars: ‘Idared’, ‘Opal’, ‘Topaz’ and ‘Rubín’. Samples of cultivars ‘Idared’, ‘Topaz’ and ‘Rubín’ were freshly harvested, samples of cultivar ‘Opal‘ were stored for four months in two ways, either under ULO conditions (ultra-low oxygen; 2% O_2, 1% CO_2, temperature 1,5 - 2°C, humidity 99%) or cold storage conditions (without controlled atmosphere, temperature approx. 2°C). Total RNA was isolated from the samples and transcribed into cDNA. To quantify the gene expression of all 31 allergen isoforms, we used the NGS method, specifically the Ion Torrent PGM sequencer. In summary, in the five tested samples, relative expression above 1% was observed only in seven genes, namely Mal d 1.01, Mal d 1.02, Mal d 1.05, Mal d 1.06A + 1.06D , Mal d 1.13A and Mal d 1.13C genes. The expression of these genes accounts for 95% of all allergens contained in the sample at varying rates. However, only four genes, Mal d 1.01, Mal d 1.02 and Mal d 1.06A + 1.06D, dominate in all cultivars, representing 88-98% of all allergens by cultivar. In contrast, almost zero expression at background level, was observed in three genes: Mal d 1.04, Mal d 1.12 and Mal d 1.14.

Keywords:
Malus domestica, NGS technology, apple allergy

Mechanical and anatomical investigation to assess the peel contribution in grape berries

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Abstract:

In Japan, people usually peel grape berries before eating them. Because the texture of peeled berries has a dreadful mouth feel. ‚Shine Muscat' grape berries has been recently introduced and their production increase rapidly. This cultivar can be eaten with the peel on. In the present study, we conducted mechanical and anatomical investigation to assess the peel contribution in grape berries. Eleven cultivars were used, and compression and penetration tests with and without peel were done to evaluate berry firmness. The peelability of berries was measured by a tensile test. The weight and thickness of the peel were also measured after peeling enzymatically. In addition, cross section of fresh peel was observed using a microscope. The peelability was less correlated with the hardness, thickness and weight of the peel, and higher correlated with the flesh hardness. 'Shine Muscat' had characteristics, such as hard flesh, thin peel and high resistance to peeling. It was found by microscopic observation that cultivars with peelability had the larger area difference between adjacent subepidermal cells and outer wall cells near the pericarp. These results show that less peelability, less flesh firmness, thin peel, and larger area difference between adjacent subepidermal cells and outer wall cells near the pericarp are involved in the characteristics of eating grape berries with the peel on.

Keywords:
grape beery, peelability, texture

Effect of sweet cherry rootstocks on harvest and fruit quality in non-irrigated orchards

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Abstract:
The hot and dry weather of recent years has had a significant impact on cherry growing in Central Europe. Research on the influence of the weather on the quality and yield of cherries in the non-irrigated orchard has been launched in Research and Breeding Institute of Pomology Holovousy. The sweet cherry varieties ‘Burlat’, ‘Jacinta’, ‘Early Korvik’ and ‘Kasandra’ were chosen to evaluation. These varieties were grafted on rootstocks Weiroot 720, PiKu 1, PHLC, Gisela 3, Gisela 5 and Krymsk 6 in a plant spacing 4 x 1.5 m. Trees grafted on Gisela 6, Krymsk 5, Colt and Alkavo rootstocks were planted in a spacing 5 x 1 m. Harvest, flowering set, fruit set, flowering date, fruit maturity date, average fruit size (mm), soluble solids (° Brix.), fruit firm (DURO), TCSA (cm2), length of shoots (mm) were evaluated. The highest harvest was 4.88 kg/tree, 8.4 kg/tree, 4.6 kg/tree, 6.18 kg/tree on cvs. ‘Burlat’ on Gisela 5, ‘Early Korvik’ on Gisela 6, ‘Jacinta’ on Gisela 5 and ‘Kasandra’ on Krymsk 6, respectively.

Keywords:
Prunus avium, drought, fruit size, yield, cultivar

Study on Anti-oxidant and Cosmeceutical Activities for New Cultivar from Selected Individuals of Wild Pear (*Pyrus* spp.)

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Abstract:

Wild pear species widely distributed in Korea are Ussuri pear (*Pyrus ussuriensis*) and Oriental pear (*Pyrus pyrifolia*), which are used for food, and Korean sun pear (*Pyrus calleyana*) used for ornamental tree. *Pyrus* spp. is a deciduous broad-leaved tree belonging to *Rosaceae*. The fruit has been used as a folk remedy for cough, hangover, constipation, and diabetes. Recently, many researchers have conducted studies on physiologically active substances of pear. As a result, it has been reported that it contains a phenolic component such as chlorogenic acid having excellent antioxidant effect and arbutin excellent in skin whitening. The antioxidant activity of natural substances is mainly effected by metabolites. It is known that wild species exposed to environmental stresses contain more secondary metabolites than cultivars. So, It is considered that wild pear trees in Korea have a more functional effect than cultivars. This study was conducted to investigate the anti-oxidant and cosmeceutical activities from selected 17 individuals of pyrus spp. (*P. pyrifolia, P. ussuriensis*) and provide basic data for the cultivation which has more functional ingredient than the local variety. As a result of the analysis for physiologically active substances show that chlorogenic acid and arbutin were detected and there was a difference in individuals. DPPH radical scavenging of 70% EtOH extract from 'U6' was highest, and ABTS radical scavenging was higher than 70% activities from all samples. In 1,000 ug/ml concentration of hot-water and 70% EtOH extract, hydrogen peroxide scavenging activity was highest at 93.0% and 88.4%, respectively. Tyrosinase inhibitory activity was the highest at 18.5% in P. ussuriensis ‚U6', but other individuals showed less than 10% activity. Elastase inhibitory activity was higher in the 70% EtOH extracts than hot-water extracts and P. ussuriensis ‚U6' individual was highest at 65.4% in 70% EtOH extracts. These results suggest that the antioxidant activity and cosmetic pharmacological activity of Pyrus spp. individuals were different from each other, and *P. ussuriensis* 'U6' individual was the most likely new cultivar.

Keywords:
wild pear, Cultivar, Arbutin, Physiologically active substance, antioxidant activity

Flesh browning phenotyping in apple through image analysis

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Abstract:

Apple flesh browning is a significant problem in fresh cut and processing industries. Phenotyping flesh browning in a quick and objective manner can assist in breeding and characterizing efforts. Flesh browning is typically measured using subjective categorization with color scales or quantified with colorimeters, which only measure a small proportion of the fruit surface. This work evaluates the viability of phenotyping apple flesh browning using digital images of the entire surface of the fruit. Fifteen traditional apple cultivars from the UPNA germplasm bank were harvested in 2018. Sixty apples were selected from each cultivar representing a wide range of maturity stages, assessed by flesh firmness and starch-iodine testing. The browning phenotype was assessed in the bottom half surface of fruits with digital images obtained using a mirrorless camera. Ten photographs per fruit were taken at regular intervals from immediately after slicing up to an hour later. The raw images were transformed into CIE Lab images and the average values of L^*, a^* and b^* extracted individually for each fruit surface using image analysis software. The total extent of browning was calculated fitting regression curves of the total color change ($\Delta E^*=\surd((\Delta L^*)2+(\Delta a^*)2+(\Delta b^*)2$) from the time of slicing. Moreover, the relationship between browning and maturity level for each cultivar was explored by regression curves with firmness and starch-iodine index levels. Most (> 90%) of the browning occurred in the first 30 minutes after slicing, and browning intensity was affected by maturity differently depending on the variety. The analysis of digital images has been shown a suitable method for phenotyping apple flesh browning. Quantitative repeatable measures allow genotype comparisons and the definition of their expression for this character in a clear and unambiguous way. A standardized characterization protocol for apple flesh browning has been provided, enabling to comparing properly phenotypes within germplasm collections.

Keywords:
Malus x domestica Borkh, phenotyping, image analysis, characterization

Differences in qualitative parameters of apple cultivar ´Golden Delicious´ depending on geographical origin

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Abstract:
Apples as a kind of fruit and vegetable are biological material and show a great variability among individual fruit from the same tree. And more at the same time there are bigger differences among fruits originating from different plants. And the biggest differences are among fruits from several orchards even though it is the same cultivar. In the Research and Breading Institute of Pomology Holovousy Ltd. a research has started to find out and describe differences in apples of cultivar ‘Golden Delicious’ coming from six various geographical and climatic area of the Czech Republic, namely Drahoraz, Holovousy, Velké Bílovice, Synkov, Klapý and Soběnice. As difference indicators physical and analytical methods were used to determine weight and diameter of apples as well as soluble solid content, firmness of the flesh, pH and titratable acidity right after 2018 harvest.

Keywords:
Fruit; qualitative parameters; fruit cultivation area; analysis; SSC; firmness

Evaluation of internal quality of apple selections and cultivars bred in Holovousy

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Abstract:

The evaluation of internal quality parameters of new apple genotypes is an important step of selection and registration the new excellent cultivars. The overall taste of apples consists of the ratio of sweetness and acidity. Therefore, the soluble solid content, acidity, flesh firmness and value of pH are most often monitored. Parameters of internal quality were observed in new breedings HL 32, HL 1579, HL 2010, HL 1592, HL 2350, HL 53, HL 1304 and cultivars 'Julia', 'Judita', 'Rubinstep', 'Rucla', 'Reluga', 'Lady Silvia', 'Andera', 'Artiga', 'Fragrance' and 'Meteor' that were bred in the Research and Breeding Institute of Pomology Holovousy Ltd. The quality of apple genotypes varies year-to-year depending on climatic conditions. Thus, the same apple varieties were repeatedly harvested and evaluated in consumer maturity in 2016, 2017 and 2018.

Keywords:
the soluble solid content, acidity, flesh firmness, pH, new breedings

Phenological, pomological and fruit analytical analysis of elderberry (*Sambucus nigra* L.) genotypes grown in Hungary

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Abstract:

Elderberry is one of the most widely grown berry species in Hungary today. Practically, Hungarian growing is based on one cultivar ’Haschberg’ which is risky. Our aim is the breeding of varieties, which adapt well to our ecological circumstances, have reliable productivity, and meet the requirements of the food industry. In the genebank of NARIC Research Station in Fertőd 35 wild genotypes were collected from different parts of Hungary, 300 seedlings of some wild genotypes and 3 cultivars (’Haschberg’, ’Sampo’, ’Samidan’) are maintained. According to the health status of the leaves and the fruit yield in 2017, 11 wild genotypes and 26 open pollinated seedlings were selected for our analysis in 2017 and 2018. Soluble solid content ranged from 9.6 to 20.9 oBrix, and titratable acid (as citric acid) was 0.6 to 1.2%. The ferric reducing antioxidant power (FRAP) values were 30.6 to 58.6 µmol TE/g. Total anthocyanin content ranged from 16860 to 89656 mg Cy3-GE/kg. Total phenolic contents were from 6311 to 21418 mg GAE/kg. All analytical parameters are expressed on a fresh weight basis. According to our results, among the wild genotypes, ’K.11’ and ’K.34’ (first decade of August), the mid-ripening ’K.16’ (second decade of August) and the mid-late ripening ’K.15’ (third decade of August) were the most valuable. After the repeated selections the most valuable types were found among the progenies of ’Sampo’. Their fruit cluster weight remained below the weight of the female partner, but their water soluble dry matter content, anthocyanin and total polyphenol content exceeded their parents.

Keywords:
Sambucus nigra, FRAP, acid, polyphenol, anthocyanin, ripening time, fruit weight

Yield evaluation of some plum cultivars from 4. to 13. year after planting in the Czech Republic

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Abstract:
In the experimental station of Czech University of Life Sciences Prague (location Troja, altitude below 200 m), twenty-three spindle-grown plum cultivars grafted on clonal (St. Julien A and Wavit) and seedling (Wangenheim and WaxWa) rootstocks were evaluated. The trial orchard was established from one-year-old trees in autumn 2005 with the spacing of 4.5 x 2.0 m, with drip irrigation and cultivating in the system of integrated fruit production. During evaluated ten years period (2009-2018) the average annual temperature was 9.35 °C and the average annual precipitation 588 mm. In a long-term evaluation of same new cultivars in comparison with older one, we found great differences among them, namely in aspect of yield, fruit size, regular bearing and ripening time. The highest mean fruit yield (expressed in yield per area) among tested plum cultivars we detected in cultivar ´Tophit´ grown on Wavit (46.62 t/ha) and ´Jojo´ grown on same rootstock (44.16 t/ha). On the other hand, cultivars ´Samek´ and ´Čačanska Rana´ had the lowest yield, both on St. Julien A rootstock. Based on the specific yield in relation with crown volume, cultivar ´Tophit´ on Wangenheim rootstock was the most productive (6.48 kg/m3). A mean fruit weight ranged between 25 and 75 g according to particular cultivar. The highest mean fruit weight was observed at cultivars ´Čačanska Rana´, ´Tophit´ and ´Haganta´, on the contrary the cultivar ´Top 2000´, ´Stáňa´ and ´Katinka´ had the smallest fruits with mean weight below 30 g. High yield and a good fruit size should be mentioned in cases of cultivars ´Tophit´, ´Topend Plus´, ´Valor´, ´Haganta´ and ´Jojo´. Cultivar ´Čačanska Rana´ had very big fruit but its disadvantage was small yield in both ways of expressions.

Keywords:
Prunus domestica, productivity, fruit weight, cultivar, rootstock

Overcoming the internal browning problem of apple fruits in breeding for red fleshed apple varieties

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Abstract:

Since 20 years, breeding for red fleshed apple varieties is a subject of worldwide breeding activities. In case of type 1 red fleshed apples, the browning of the fruit flesh during storage is a major problem. Especially when stored under low temperature conditions (1-2 °C), browning of fruit flesh starts within eight weeks. The more intense the red colour of the fruit flesh is the higher is the tendency to internal browning, unfortunately. This hampers the use of type 1 red fleshed apple varieties for commercial fruit production.

Ten white fleshed apple breeding clones the fruits of which store well were selected and used for cross pollination with red fleshed apple selections. First fruits of the seedlings were harvested in 2017. During 2017 and 2018, storage tests with fruits of 30 different seedlings were performed under conditions forcing the development of internal browning. Only genotypes with dark red fruit flesh were used. Fruits of three out of those 30 breeding clones showed no internal browning even when stored until May. All three were descendents of the same white fleshed parent. These results show that the problem of internal browning of fruits of type 1 red fleshed apple genotypes can be overcome by choosing appropriate parents in breeding.

Keywords:
type 1 red fleshed apple, storage conditions

Characterization of molecular diversity and transcriptional response of wild Iranian cherries under drought conditions

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Abstract:

Knowledge of genetic potential in wild species of cherries is important for recognizing gene pools in germplasm and developing effective conservation for breeding programs. In this research, wild cherries were collected from different parts of Iran. 20 genotypes belonging to Prunus avium (L.) L., Prunus microcarpa (C.A. Mey.), and Prunus incana (Pall.) Batsch were selected. Seedlings were investigated in terms of morphological traits such as seedlings height, diameter, leaf shape, length and number of internodes and leaf area were measured in each genotype. Water stress was imposed by water withholding. Cherry seedlings were irrigated to pot water capacity before stress. Then, two different water groups were used, so that half of the seedlings were water-stressed by withholding for a 3-week drying cycle and the other half of the seedlings continued to be watered to pot capacity. After applying drought stress treatment, an initial screening was carried out at which some parameters such as photosynthesis and chlorophyll fluorescence were measured. Then, according to the results of primary screening, genotypes were grouped into susceptible and tolerant. Sampling from each group was performed for certain periods. Molecular characterization of genotypes was performed by SSR markers and drought tolerance candidate genes were analysed by quantitative qPCR to evaluate the transcriptional responses. This study specifies the importance of some genes studied to create drought resistance in P. incana and P.microcarpa seedlings. It also helps to determinate the relationship between phenotypic genotypic data for identification of molecular markers related to drought tolerance. Our results also provide a significant contribution to the understanding of how P. incana and P. microcarpa respond to drought stress, which may help to explain molecular mechanisms associated with the response to drought of cherries.

Keywords:
Drought, SSR marker, qPCR, transcript, wild cherries

Molecular and phenotypic characterization of interspecific *Prunus salicina* Lindl. × *Prunus armeniaca* L. (plumcot) hybrids

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Abstract:

A Japanese plum (Prunus salicina Lindl.) breeding program coordinated by CEBAS-CSIC and IMIDA is being conducted since 2011 in Murcia (Southeast of Spain). Self-compatibility and resistance to sharka disease (Plum pox virus, PPV) are some of the main objetives for the new released cultivars. For that purpose and due to the lack of resistance in Japanese plum species, numerous interspecific crosses between Japanese plum and self compatible, sharka-resistant apricot (Prunus armeniaca L.) cultivars have been performed during these years in order to obtain resistant and self-compatible plumcots (interspecific hybrids). In addition, these new plumcots are characterized by a new fruit typology with a great agronomic and market potential. In this work, the inheritance of the most important agronomical and phenotypic traits have been studied in these interspecific hybrids. An initial screening of more than 650 seedlings was carried out for interspecific hybrid verification using SSR (Simple Sequence Repeat) markers. S-allele genotyping and PPV resistance evaluation in controlled conditions were performed in the true interspecific genotypes. In addition, a complete phenotypic characterization was performed including pollen viability, androsterility, leaf and fruit morphology, flowering time, flowering intensity, flower color, pubescence of ovary, ripening date, pubescence of fruit skin, productivity, fruit color, flesh color, firmness and organoleptic characteristics such as soluble solids content and acidity.

Keywords:
Plumcot, inheritance, breeding, interspecific hybrids, floral compatibility, sharka resistance, SSRs.

Identification of S-alleles in Algerian autochthonous apricot (*Prunus armeniaca* L.) cultivars using PCR markers

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Abstract:

Gametophytic self-incompatibility, governed by the S-locus, functions in Prunus species including the apricot to protect self-pollination of flowers. Knowledge of the S-genotypes in apricot cultivars is essential to establish productive orchards, defining combinations of compatible cultivars. In this study, 84 apricot cultivated accessions from the regions of Aurés (semi-arid) and Hodna (semi-arid to arid) in Algeria have been evaluated. These cultivated accessions included local Algerian cultivars and French and Spanish introduced cultivars with known S-alleles. To identify the S-alleles associated with self-incompatibility, two pairs of specific primers were tested using PCR. This PCR-based molecular tool allows S-genotypes to be characterized quickly and efficiently. Results showed a great diversity in terms of S-alleles in the assayed Algerian germplasm. In addition, some of these apricot cultivars are self-incompatible, and so to obtain a yield they need to be pollinated with cross-compatible cultivars. Finally, determination of the S-genotype might be considered as an important addition to a set of identity markers of apricot genetic resources, what is of particular interest in the characterization of the germplasm diversity.

Keywords:
S-alleles, Self-incompatibility, apricot, genetic diversity

Practical application of molecular markers in fruit plants breeding

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Abstract:

Classic breeding methods based on long-term selection of the best genotypes are costly and time-consuming. These types of studies are supported by methods based on molecular markers application and allow accelerating the breeding program by identification of genotypes with the most favorable alleles.

The purpose of breeding-genetic work carried out at the Research Institute of Horticulture as part of the Multi-annual Program financed by the Ministry of Agriculture and Rural Development is to produce, and implement for production, new varieties of fruit plants, as well as to increase profitability, competitiveness and innovation of fruit production in Poland.

The main goal of performed studies was molecular description of the most important species of fruits plant germplasm and newly developed genotypes. In this venture molecular markers (ISSR and SSR) were used for analysis of: (1) genetic relationships of parental forms (apple, black currant, gooseberry and blueberry), (2) genetic identification of selected genotypes (apple, plum, cherry, apricot, peach and strawberry) as well as for (3) elimination of undesired genotypes being the results of uncontrolled cross pollination to confirm their origin (apple). In two seasons of the studies (2017 and 2018), 550 of genotypes were tested in 29 120 PCR tests, using 186 ISSR's and 117 SSR's. The DNA fingerprints were prepared for 16 clones/hybrids of strawberry, 26 - of apples and 10 - of Saskatoon berry. Creation of a molecular markers database gave the opportunity for characterization of newly developed plant varieties, justifying the phenotypic effect of the inherited traits.

Additionally, DNA fingerprinting, applied also in our study, is nowadays commonly used tool for protection of intellectual property of varieties and breeders.

Keywords:
molecular markers, genetic relationships, DNA fingerprinting

Molecular diversity of selected Prunus genotypes determined with microsatellite markers

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Abstract:

In recent 20-years, a number of microsatellite markers have been developed and used in different Prunus species, including peach, apricot, plum, or cherry plum for genotype characterization, and studies of genetic diversity. Often, microsatellite markers developed in one species have been used in a different species in the same genus, demonstrating their ability to detect polymorphism.

The aim of the presented study was to evaluate diversity of 31 representatives of Prunus genus used in inter specific crossing using microsatellite-based SSR technique. Among 45 primers applied in the tests only 31 generated clear and reproducible polymorphic bands. In total, 137 alleles with size 50-800 bp were obtained. The mean value of polymorphism information content (PIC) was 0.874.

Cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) showed that analyzed genotypes of the genus *Prunus* possess abundant genetic diversities. The degree of genetic similarity was ranging from 12 to 65%. In addition, the obtained dendrogram contains four main clusters: I cluster - genotypes *Prunus salicina* L. (Japanese plum); cluster II- *Prunus cerasifera* (Cherry plum) varieties; cluster III- *Prunus domestica* L. (plum) genotypes; cluster IV- *Prunus armeniaca* L., apricot varieties.

This study confirms the high transferability of SSR markers developed for peach genome and theim utilization for detection of polymorphism and the characteristics of other *Prunus* genotypes.

Keywords:
diversity, *Prunus*, SSR, UPGMA

Construction of genetic linkage map based on SSR markers for blackcurrant (*Ribes nigrum* L.) population `Ceres´ x `Bona´

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Abstract:

The fruit plant genome maps, built on the basis of molecular markers, are one of the main tools for studying quantitative traits. Molecular markers linked to important functional traits, can be used at an early stage of assessment and selection of appropriate breeding material and selection of parental forms for crossing programs, as well as for the selection of appropriate breeding individuals and clones can be achieved. An extensive blackcurrant breeding program conducted at the Institute of Horticulture in Skierniewice is aimed to release new cultivars that are well adapted to Polish climate and soil conditions, high yielding, with high fruit quality as well as with increased plant resistance to main pathogens and pests. In recent years, several compacted blackcurrant genetic maps have been published, but no advanced map has been constructed for Polish cultivars recommended for cultivation in the Central European climate.

In connection with the above, the aim of the presented research was to construct a genetic map for a cross of two Polish blackcurrant cultivars (‘Ceres’ and ‘Bona’) using microsatellite markers (SSR).

Twenty SSR markers located on reference maps of the Ribes genus were selected for the skeleton structure of the map. The integrated genetic map was based on the results of segregation of 177 identified microsatellite alleles. The skeleton map contains 11 groups of joins, which are fragments of seven chromosomes (I-VII) with a total length of 537 cM. The integrated genetic map constructed for Polish cultivars (‚Ceres‘ and ‚Bona‘) is the basis for further research of the inheritance of traits and for work on an identification of marker assisted selection (MAS) as one of the modern methods of blackcurrant breeding.

Keywords:
blackcurrant, genetic map, genome, marker, microsatellite

Evaluation of genetic diversity of tamarind accessions from Eastern parts of Kenya using ISSR markers

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Abstract:

Tamarind (*Tamarindus indica*) is a highly valued fruit and forest tree in tropical and subtropical parts of Africa and Asia. Genetic characterization of Kenyan tamarind has not been done. A study was carried out in Kibwezi, Mwingi and Masinga to determine the genetic differences among accessions. Molecular characterization was done using young apical leaves. DNA was extracted using modified CTAB method. DNA was amplified using 12 ISSR markers and 7 markers produced scorable reproducible bands that ranged from 100-1000bp. Levels of polymorphism ranged from 54-95%. Cluster analyse was done using DARwins software and revealed 3 major clusters: Cluster one and two had accessions from all the three regions of study while cluster three had two accessions from Kibwezi. The results of this study revealed ISSR markers showed differences in the accessions and this region

Keywords:
Tamarind, accessions, ISSR, markers

Using edgeR software to analyze complex RNA-Seq data

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Abstract:

RNA-Seq is now commonly used to identify differentially-expressed genes (DEGs) between one treatment and another. However, the data analyses can be challenging when there are multiple genotypes and treatments. This study proposes a methodology to discriminate gene expression from a complex RNA-Seq experiment. Data were from an experiment comparing genotypes from a 'Honeycrisp' x MN1974 Malus x domestica cross whose fruit were either not crisp at harvest, or that retained or lost crispness after storage. 'Honeycrisp' fruit retained crispness after months of storage, while MN1974 fruit softened. Statistical analyses of gene expression were performed using edgeR software. To identify genes related to postharvest change in crispness, the expression abundances between the 'retain' and the 'lose' group, the 'retain' and the 'non-crisp' group, and the parents were compared. The DEGs found in all three comparisons with FDR < 0.05 were further selected based on 1) the log2-fold change > 1, and 2) the expression threshold (CPM > 1). A total of 567 genes were identified that could be associated with crispness retention.

Keywords:
apple, fruit crispness, postharvest, transcriptome

EUCARPIA

The use of SSR method for identification of unknown pear varieties

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Abstract:
Pear species belong to one of the longest-cultivated temperate species. Large number of varieties is spread worldwide, that have different names in different countries. Probably the most famous is the variety Williams, which is known in the USA as Bartlett. However, not all synonyms are always known. Molecular methods, namely the SSR method, allow reliable identification of varieties. In this study, the SSR method was applied to 31 Asian pear varieties, 4 interspecific hybrids and 3 botanical species from the East China gene center. Six SSR primer pairs were used to determine the genetic relatedness. SSR primers were labeled with fluorescent dyes and the PCR products were evaluated using a genetic analyzer. Based on the obtained results, the varieties were divided into two main clusters. Cluster I was divided into three subclusters, subclaster I contained *P. pyrifolia* and varieties derived therefrom, subcluster II. contained P. ussuriensis and several ussiriensis-type varieties and Chinese white pear varieties and subcluster III. contained varieties of Chinese white pears. Cluster II. was formed by only 4 varieties probably being interspecific hybrids. Further, combinations of varieties Man San Gill and Pung Su; Early and Zaosu Li; Snow Flower, Shon Shu and Dangshanshu Li had identical SSR profiles, which indicates, that they are probably identical varieties or possibly different clones.

Keywords:
molecular genetic, PCR, finger printing, Oriental pear

The apple REFPOP, a population dedicated to multi-trait genomic selection in a multi-environment design

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Abstract:

Genomic selection has the potential to increase the efficiency of breeding programs in perennial crops. In apple, genomic predictions have been reported only in a limited number of studies and to our knowledge genomic selection is hardly applied. The very first step to build up an efficient genomic selection program is to develop an experimental design with adequate plant material to test predictions. In apple, this is now made possible with the joint efforts of the European FruitBreedomics Consortium in developing the apple REFPOP, a population dedicated to genomic predictions and genome wide association analyses, along with appropriate genotyping and phenotyping tools. Here, we present the long-term genomic selection apple project built around the apple REFPOP. This population is composed of 570 replicated genotypes planted in six countries and was phenotyped for the first time in 2018 at all six sites for yield, phenology and fruit quality traits. Phenotyping for these and additional traits will be repeated in the coming years. Genotyping data were available through FruitBreedomics for most of the genotypes and were obtained with the combination of a medium, 20K SNP array, and the high-density Axiom® Apple487K SNP Affymetrix array via imputation. We provide insights into the genetic features of the apple REFPOP with a specific focus on linkage disequilibrium and structural patterns. By combining several phenotypic variables recorded across countries and years and high-density genotyping, our goal is to use the apple REFPOP as a training population to predict the potential of applied populations in multiple environments and for multiple traits. To face the increasing complexity of the data collected, machine learning methods will be tested. Finally, we also discuss different strategies to calibrate and implement genomic prediction models into modern, climate-ready breeding programs. This work is partially funded by the EU-H2020 project N°817970 INVITE.

Keywords:

Malus domestica, genomic predictions, genotype by environment interactions, training population

Phenolinc components profile of grapevine cultivars with a complex genetic structure

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Abstract:

Phenolic components profile of grapevine cultivars obtained as a result of interspecific hybridization and its peculiarities have been studied. HPLC analysis was used to identify 42 phenolic components of the flavonoid and non-flavonoid structure in the berries. The results showed that the majority of components in the qualitative composition of polyphenolic compounds of cultivars with complex genetic structure did not differ from those of Vitis vinifera L. species, however, the quantitative composition demonstrated significant differences. Cluster analysis of the data revealed phenolic complex similarities in black berry grapevine cultivars ‚Pamyati Golodrigi‘ and ‚Alminskiy‘ (Ed = 680); grapevine cultivar ‚Antey Magarachskiy‘ differed from the cultivars mentioned above (Ed =1000), but was closest to them, while ‚Cabernet Sauvignon‘ differed significantly from the rest of the cultivars (Ed =1600). Analysis revealed similarities among white berry grapevine cultivars ‚Pervenets Magaracha‘, ‚Tsitronnyy Magaracha‘ and ‚Chardonnay‘ (Ed = 157-350); the phenolic complex of ‚Risling Magaracha‘ cultivar singled it out into a separate group (Ed =1220). Among the monomeric flavonoids of the anthocyanin group, glycosides of delphinidine, malvidin, cyanidine, petunidin, and peonidine and their derivatives were identified in selection varieties of black berry grapevines. It was established that the total anthocyanidin content made 1467 mg L-1 (‚Pamyati Golodrigi‘) - 1585 mg L-1 (‚Antey Magarachskiy‘), which is twice as high as the same parameter in ‘Cabernet Sauvignon’. Among components of the anthocyanin profile, we identified anthocyanin 3,5-0-diglycosides, for example, malvidin-diglucoside and its derivatives, concentration of which varied among cultivars within 7-423.8 mg L-1. Cluster analysis revealed similarities in the anthocyanin complex of grapevine cultivars ‚Cabernet Sauvignon‘ and ‚Antey Magarachskiy‘(Ed = 1000). ‚Pamyati Golodrigi‘ (Ed =1940) and ‚Alminskiy‘ (Ed =2430) cultivars differed significantly from this group of cultivars and made separate clusters.

Keywords:

cultivar, HPLC, phenolic acids, flavonols, flavan-3-ols, procyanidins, anthocyanins

Screening of an apple germplasm collection by a newly developed Ap17in1 SSR Genotyping Kit

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Abstract:

An apple germplasm collection at Research and Breeding Institute of Pomology Holovousy, Ltd. has been screening using a newly developed Ap17in1 SSR Genotyping Kit. The kit combines 17 SSR markers (ECPGR-recommended markers included), covering all chromosomes in one reaction, and is thus suitable for genotyping of large apple germplasm collections. Up to date, 577 Holovousy's germplasm entries have been analyzed; 530 samples (91.85%) being diploid, 42 samples being triploid (7.28%), and five samples being tetraploid (0.87%). Among diploid samples, seven showed the presence of three alleles in one chromosome only; among triploid samples, two showed the presence of four alleles in one chromosome only. Within the diploids, the variety "Slovakia" had the lowest number of heterozygous markers (8), and 24 varieties (4.5%) had all 17 markers heterozygous; a variety had 14 heterozygous markers on average. Based on so far analyzed samples, statistics of the kit are as follows: the highest number of alleles (23) detected in markers CH02c06 (chromosome 2), CH03d07 (chromosome 6), and CH04e05 (chromosome 7); the lowest number of alleles (12) detected in markers CH01f03b (chromosome 9), and CH02c09 (chromosome 15); the highest observed heterozygosity (0.908) found in marker CH01f02 (chromosome 12); the lowest observed heterozygosity (0.646) found in marker GD12 (chromosome 3); twelve markers (71%) had observed heterozygosity greater than 0.8; overall observed heterozygosity and expected heterozygosity were 0.823, and 0.814, respectively.

Keywords:
apple, germplasm, SSR marker, genotyping

Self-incompatibility characterization in segregating populations of apple trees with DNA markers for S-alleles

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Abstract:

Gametophytic self-incompatibility is a physiological mechanism that controls the sexual reproduction in species of the genus *Malus*. This mechanism is controlled by a single multiallelic locus called ‘S’. At least genetically semi-compatible genotypes should be used as pollinators and for apple breeding crosses. The objective of this work was to characterize the parents and respective populations of apple trees regarding the S-alleles to confirm their genealogy and to evaluate the competence of the markers used. These genotypes are part of the Epagri’s Apple Breeding Program germplasm, in Brazil. Specific sets of primers were used for the identification of 16 apple S-alleles by PCR. The S-alleles was confirmed previously in standard cultivars. Were evaluated two segregating populations resulting from the crossings between Fred Hough vs. Monalisa (54 individuals) and M-11/01 vs. M-13/91 (120 individuals). The expected segregations are 1:1:1:1 for full compatibility and 1:1 for semi-compatibility, that may be confirmed by the X2 test. The cross Fred Hough (S5S19) vs. Monalisa (S2S10) showed to be full-compatible. It was confirmed the expected proportion of 1:1:1:1 segregation for the S-alleles identified by PCR at its progeny, besides were identified two triploids among the hybrids. The cross M-11/01 (S3S5) vs. M-13/91 (S3S5) was identified as incompatible, thus no offspring were expected. However, the segregation of the S-alleles was 1:1, characterizing semi-compatibility. It is suggested that these descendants were originated from another cross performed in the same year (M-11/01, S3S5 vs. Joaquina, S5S9), whose S-alleles segregation in the progeny is coincident. In this way, the characterization of the S-alleles besides permit identify the compatibility between the parents did serve to find paternity divergences in segregating populations. Considering the results, the segregation of the DNA markers for the S2, S3, S5, S10, and S19 occurred together with their respective S-alleles, being efficient to their identification.

Keywords:
Malus domestica Borkh., S genotype, S-RNase, allele-specific PCR, segregation, apple breeding

Classification of Asian pears using SNPs derived from GBS

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Abstract:

For the genetic characterization of Asian pears, phylogenetic relationship and population structure were analyzed in 43 pear accessions using single nucleotide polymorphisms (SNPs) derived from genotyping-by-sequencing (GBS). Phylogenetic tree showed that the Asian pear accessions divided into 5 groups and each group represent *P. betulifolia, P. ussuriensis, P. bretschneideri, P. pyrifolia,* and unclassified accessions, respectively. Among 5 unclassified accessions, 'Najuseji' showed close genetic relationship with *P. betulifolia.* In case of 'Gamiri', it was included in *P. pyrifolia* group. These results correspond with principal component analysis and population structure. In the population structure, most of Korean native pears have mixed genome structure with *P. ussuriensis* and *P. pyrifolia*. Although, 'Sunchangingyeri' and 'Hangangbae' showed mixed ancestry, they could be classified as *P. pyrifolia*. Using SNPs derived from GBS, we classified Asian pear germplasms in detail. These results could help the management of Asian pear germplasms.

Keywords:
phylogenetic tree, population structure, principal component analysis, *Pyrus* spp.

An integrated genetic linkage map of apple (*Malus × domestica*) using GBS-based SNPs and SSRs

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Abstract:

Construction of genetic linkage map is essential preceding process for trait association analysis in apples (*Malus × domestica*). As a groundwork, genetic linkage map was constructed using anthracnose (*Colletotrichum gloeosporioides*) resistant cultivar 'Gala', susceptible cultivar 'Jonathan', and their F1 individuals. Using 'Gala', 'Jonathan', and 94 F1 individuals, genotyping-by-sequencing (GBS) was performed and 237,187 single nucleotide polymorphisms (SNPs) were detected. Apple and pear-derived 248 SSR markers were analyzed in 'Gala' and 'Jonathan'. Finally, 27,151 GBS-SNPs and 71 SSR markers were used to construct the genetic linkage map. The total length of integrated genetic linkage map of 'Gala' × 'Jonathan' was 1,706.5 cM and the average of marker interval was 2.09 cM. This map consists of 17 linkage groups (LGs) and each LG has 1 to 8 SSR markers. The order of SSR markers corresponded to previous apple genetic linkage maps. Our GBS-SNPs and SSR marker based genetic linkage map could be a useful tool for QTL analysis related to anthracnose resistance.

Keywords:
Genetic linkage map, Genotyping-by-sequencing, Single nucleotide polymorphism, Anthracnose resistance

Development of a SNP-Derived CAPS marker set associated with pollen fertility in *Pyrus* spp

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Abstract:

Pollen fertility representing the presence and absence of pollen grains is of agricultural importance because it leads to labor-intensive artificial pollination or requirement of pollinizers in commercial orchards. A genomic region associated with pollen fertility has been identified in our previous study. Newly three single nucleotide polymorphisms (SNPs) involved in pollen fertility were converted to cleaved amplified polymorphic sequence (CAPS) for use of marker-assisted selection (MAS) of the traits in the pear breeding program. A set of primers were designed to amplify a number of bands when the PCR amplicon is digested by endonuclease restriction enzymes. A total of 95 F1 individuals and their parents were used for validation of polymorphism. Most pollenless F1 individuals followed their maternal CAPS patterns, whereas F1 individuals with abundant pollen grains followed their paternal CAPS patterns. The set of CAPS markers developed in the present study would enable us more accurately to implement MAS of pollen fertility.

Keywords:
SNP-dCAPS, Pollen fertility, *Pyrus*, MAS

Genetic relationship of major apple cultivars and bud sports using SNPs detected by GBS

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Abstract:

Identification of parent-offspring relationships among cultivar is necessary for accurate analysis of genetic resources. To detect single nucleotide polymorphisms (SNPs), genotyping-by-sequencing (GBS) was performed in 95 apple cultivars. In total, 12,014 GBS-based SNPs were used to estimate genetic distance among 32 major apple cultivars. The phylogenetic tree showed high genetic similarity between their parent cultivars and offsprings. Seventeen cultivars including apple bud sports were closely grouped together with one of their parent cultivars. 'Jahong', which is sports of 'Hongro', was grouped with 'Hongro' and its bud sport cultivar ('Gumi Hongro'). 'Jonagold' and 'Kougetsu' are the F1 cultivars of 'Golden Delicious' × 'Jonathan'. 'Jonagold' was grouped the same cluster with maternal parent 'Golden Delicious' and 'Kougetsu' with paternal parent 'Jonathan'. However, 'Sobaek No. 2' (sport of 'Hongro') and 'Sobaek No. 3' (sport of 'Fuji') did not belong to same group of 'Hongro' and 'Fuji', respectively. Based on the results of phylogenetic tree, we were able to infer that 'Sobaek No. 2' and 'Sobaek No. 3' are not bud sports. GBS-based SNPs could be used for accurate phylogenetic analysis and for increasing breeding efficiency of apples.

Keywords:
Genotyping-by-sequencing, Single nucleotide polymorphism, Genetic relationship

Development of insertion and deletion markers associated with pollen fertility in Pears (*Pyrus* spp.)

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Abstract:

Insertion and Deletion (InDel) marker is a valuable complement to another sequence-based molecular marker such as single nucleotide polymorphism and simple sequence repeat for genetic analysis in plants. InDel marker shows co-dominant inheritance and wide genomic distribution. In our previous study, a genomic region related to pollen fertility was identified on the high-density genetic linkage map. Re-sequencing data of the parents were re-analyzed by customized bioinformatic pipeline to discover in silico InDel structures in the proximity to the genomic region. Candidate polymorphic InDel variations between the parents were used to design a set of primers generating 200-400 bp of PCR amplicon. InDel markers were applied to pollenless cultivar ‘Whangkeumbae’ (Pyrus Pyrifolia), normal cultivar ‘Minibae’ (P. hybrid), and their 88 F1 individuals. Predicting pollen fertility using an InDel marker (CPID154) showed 80.6% of accuracy in F1 individuals. The developed InDel markers in the present study should be validated in another mapping population.

Keywords:
InDel, Pollen fertility, *Pyrus*, MAS (CPID154) showed 80.6% of accuracy in F1 individuals. The developed InDel markers in the present study should be validated in another mapping population

Genetic relationship of pears (*Pyrus* spp.) using GBS-SNPs aligned to pseudo-chromosome of pear reference genome

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Abstract:

Pear (*Pyrus* spp.) is one of the most important fruit trees. However, classification of pear germplasm is often very difficult, due to long-term natural selection and gene flow, modern cross-breeding between different species, and self-incompatibility. We classified pear accessions using genotyping-by-sequencing (GBS) based SNPs aligned to pseudo-chromosome of pear reference genome. SNPs were filtered with (i) bi-allele (ii) missing data < 10%, (iii) minor allele frequency > 5%, and (iv) polymorphism information contents (PIC) > 0.3. Total of 6,239 SNPs were detected by filtering criteria and used to analyze genetic relationship. Cluster analysis was performed by MEGA7 with unweighted paired group method using arithmetic averages (UPGMA) and TASSEL 5. The eighty-eight pear accessions were divided into five groups. Group I contained *P. pyrifolia* and Group II included P. ussuriensis and 'Ganghwatongbae A'. Group III composed *P. bretschneideri* and 8 Korean native pears. Group IV consisted of *P. betulifolia. P. communis* was included group V with interspecies hybrid cultivars between *P. pyrifolia* and *P. communis*. Our findings provide important information for management of pear germplasm and selection of breeding materials.

Keywords:
genetic diversity, germplasm, *Pyrus* spp., phylogenetic tree

A comparative assessment of GBS-SNPs aligned to scaffold and pseudo-chromosome level of pear reference genome

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Abstract:

For comparative mapping and genome-wide association study (GWAS) analysis, it is important to confirm accurate loci of genes and markers in pear. Recently, pear reference genome based on pseudo-chromosome level was published, thereby genetic analyses such as genetic relationship, quantitative trait loci mapping, and GWAS in pear have been facilitated. We compared genetic information of GBS-SNPs produced by aligning sequencing reads to scaffold and pseudo-chromosome level of reference genome to identify more suitable SNPs information according to levels of reference genome. Two SNP matrixes were obtained by aligning raw sequencing reads to scaffold and pseudo-chromosome levels of reference genome, respectively. Four criteria were used for SNP filtering: (1) bi-allele; (2) missing data value < 10%; (3) minor allele frequency > 5%; and (4) polymorphism information content (PIC) value ≥ 0.3. As a result, the number of filtered SNPs was 4,452 and 6,379 in the scaffold and pseudo-chromosome level, respectively. The average of PIC value in scaffold and pseudo-chromosome level 0.347 and 0.348, respectively. The heterozygosity in pseudo-chromosome level was 0.357, which larger than 0.298 in scaffold level. Development of genetic markers based on a pseudo-chromosome level will be appropriate for genetic analysis, since the number of SNPs and values of PIC and heterozygosity were higher than scaffold level. Our finding could be used for GWAS, quantitative trait loci mapping, and marker-assisted selection.

Keywords:

Pyrus spp., genetic analysis, heterozygosity, polymorphism information content

Improved *Prunus persica* reference genome by single-molecule sequencing and chromosome conformation capture technology

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Abstract:
A diploid Pnuns species include the peach, plum, apricot and cherries and produce drupe fruits in agriculture important crops worldwide. These genome have very small genomes of 200-300Mb and chromosome number (2n=2x-16) is a close relative number with rosacea ancestor (2n=2x=18). Whole-genome shotgun assembly of a doubled haploid genotype of the peach cv. Lovell with an estimated genome size of 265 Mb was reported in 2013 and updated in 2017. This genome coverage is 227.3 Mb (86%) and 227.4 Mb (86%), respectively. Here, we report an improved assembly of the peach genome (P. persica, Korean cultivar cv 'Mi-Hong') using single-molecule sequencing, and chromosome conformation capture technology (Hi-C). Our assembly features a contig N50 size of 25.8 Mb, representing a ~ 100 fold improvements and assembly genome size of 241 Mb (93%). About chromosome matching by genome sequences, making on pseudomolecule of peach, a total 217.5 Mb allot the eight chromosomes in this study. There are several gabs and inverted sequences in comparison with previous peach reference sequences.

Keywords:
peach; genome sequencing; pseudomolecule; Pacbio sequencing; Hi-C

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Monitoring fruit quality traits during ripening process in apricot through gene expression analysis by using qPCR

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Abstract:

The achievement of high fruit quality in one of the main objectives proposed in apricot (*Prunus armeniaca* L.) breeding programmes. Ripening process is an essential step during fruit development which will give rise to ripe fruit with the acquisition of quality traits, responsible for the final phenotype. Apricot fruit displays several phenotypes with a great diversity in quality traits as a result to its wide genetic background. The transcriptomes of two contrasted apricot genotypes from the same segregating progeny were sequenced at three fruit developmental stages during fruit ripening at CEBAS-CSIC apricot breeding programme. These RNA-seq results allowed the identification of key candidate genes involved in the main differential expressed pathways. Monitoring the expression of these key genes using qPCR in nine reference apricot varieties that differ in quality traits like colour, ripening date, soluble solids, firmness and acid content allowed the establishment of a correlation between expression patterns and its specific phenotypes during the ripening process. This valuable information will be applied in the development of specific molecular markers to be used in MAS strategies in apricot breeding programmes.

Keywords:
Apricot, monitoring, quality traits, fruit ripening, candidate gene, RT-qPCR

Comparative gene expression analysis related to striped skin of `Fuji´ apple and its color enhanced somatic mutant

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Abstract:

The ‘Fuji’ apple developed in Japan remains popular cultivar to date due to its inner high quality, and ‘Mishima’, its enhanced color somatic mutant, was cultivated together. They also have attractive striped skin determined first by the ground color and second by the superimposed color (If present) resulted from a blend of carotenoid and anthocyanin. The accumulation of anthocyanin in peel enable to form stripes. In this study, ‘Fuji’ and ‘Mishima’ were characterized at five developmental stages. The expression level of pigment biosynthesis genes related to the accumulation of carotenoid and anthocyanin was evaluated. Our L* and a/b values data indicate that ‘Mishima’ fruit has more redness than ‘Fuji’ fruit. In case of carotenoid genes, MdZISO and MdCRTISO and about anthocyanin genes, most of structural genes, MdMYB10, and MdGST were expressed higher in ‘Mishima’. These observations may have enhanced the redness in ‘Mishima’ inducing diverse apple skin phenotype.

Keywords:
Anthocyanin, Apple skin, Carotenoid, Quantitative real-time PCR, Somatic variation

Phenylalanine ammonia-lyase gene expression in apricot cultivars grafted onto different Prunus rootstocks

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Abstract:

The incorporation of new apricot cultivars for Spanish growing fruit growers introduced by different public and private breeding programs need the study of the environmental adaptation and graft compatibility behaviour of these new varieties in the new regions. Grafting compatibility with new scion cultivars has a direct impact on the different tree characteristics (yield, fruit quality, efficiency…) and premature mortality of trees. Hence, the fast evaluation for the relationship between scion and rootstock before releasing them on the market is very important to increase the efficiency. In this study, the determination of graft compatibility was carried out in new apricot cultivars (13 varieties) grafted onto two different ’Prunus’ rootstocks, ‘Marianna2624’ (Prunus cerasifera x Prunus musoniana) and ‘Miragreen’ (Prunus cerasifera x Prunus davidiana). Vegetative parameters (graft length, number of leaves) and the analysis of the phenylalanine ammonia-lyase (PAL) gene expression were performed in the different grafted combinations three months after grafting. It is known that PAL genes (PAL1 and PAL2) were differentially regulated under the stock-scion interactions between graft partners of different degree of compatibility. The results obtained showed a lower graft growth and higher expression level of PAL1 for the cultivars grafted on the rootstock ‘Marianna2624’ in comparison to ‘Miragreen’ which indicated a higher level of enzymatic activity and a higher level of incompatibility. The results will provide knowledge about the state of the unions at early stages of development providing information about the most suitable combinations to establish in the field.

Keywords:
scion-rootstock interaction, incompatibility, PAL, *Prunus armeniaca* L

Transcriptome Sequencing to Detect Genes Related to Secondary Metabolites in Schizandra chinensis

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Abstract:

The Schizandra chinensis (Turcz.) Baill. (Korean name: Omija) is a plant species which fruits have been used as a traditional herbal medicine for a long time in East Asia. Schisandra fruits have beneficial effects including detoxificant, antioxidant, anticarcinogenic and anti-inflammatory activity. However, the underlying molecular mechanisms of medical potential are largely unknown.

To understand and engineer genes involved in secondary metabolic pathways related to lignans including schizandrin and gomisin, we performed transcriptome sequencing by using long-read isoform sequencing (Iso-Seq) and short-read RNA-sequencing (RNA-Seq).

A total of 132,856 assembled transcripts were generated with an average length of 1.9 kb and high assembly completeness. Of those unigenes, 71.6% were predicted to be complete full-length (FL) ORFs and exhibited a high gene annotation rate. These genes were among 71 functional groups in three classes of biological processes, molecular functions and cellular components by gene ontology (GO) annotation, and involved in KEGG pathways related to lignan biosynthesis, flavonoid and phenylpropanoid biosynthesis. Furthermore, we successfully identified unique full-length genes involved in phenylpropanoid biosynthesis an extrapolated their regulatory network. In conclusion, our results suggest that long-read, full-length or partial-unigene data with high-quality assemblies are invaluable resources as transcriptomic references in S. chinensis and will provide a valuable resource to understanding of the huge potential of pharmacological relevance of Omija plant.

Keywords:
Schizandra chinensis, herbal medicine, transcriptome

Molecular characterization of an extensive set of European pear accessions with SSR markers

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Abstract:

Pear species (Pyrus spp.) are among the most commercially important fruit trees. P. communis (European pear) and P. nivalis (perry pear) are commonly cultivated in Europe and temperate regions, while P. pyrifolia or P. × bretschneideri are the main cultivated pear species in Asia. Since pear varieties and landraces play a key role in both heritage and sustainable agricultural production, numerous accessions are preserved in germplasm collections of research institutes or private and amateur conservatories across Europe. Appropriate characterization and evaluation of agronomic traits in such collections are crucial for improved management and valorization of these genetic resources. Current characterization also involves genotyping using molecular markers such as SSR (microsatellite) markers. In comparison with reference cultivars, genotyping enables tracking errors in varietal identification and helps germplasm curation by detecting duplicates both within and between collections.

One of the major objectives of the ECPGR-funded project “PomeFruit_C&E” is to promote the molecular characterization of European pear collections and proceed to the standardization of the SSR data across collections. Such standardization is required to compare the SSR datasets at the European scale and beyond.

A new set of 19 SSR was defined at INRA-Angers, including 11 out of the 12 previously defined ECPGR SSR set, supplemented by 8 SSR chosen according to the literature. For this project, SSR data were standardized and analyzed for 4,967 accessions collected from 27 Europeans countries:

- 2,593 accessions originating from 20 European countries were newly genotyped with the 19 SSR,
- previously generated SSR datasets for 2,374 accessions originating from 7 other European countries were standardized through the development of dedicated allelic correspondence tables.

After removal of missing data, we obtained a dataset with at least 9 standardized SSR for 4,912 accessions, which is sufficient for duplicate detection with limited risk of genotype misidentification. Among these 4,912 accessions, only 2,206 distinct genotypes were identified. We assigned a PUNQ code (Pyrus UNiQue genotype code) for each genotype in order to better characterize any new accession according to this standardized dataset.

Keywords:
Pyrus, genetic resources, microsatellite markers

Transcriptome Sequencing to Detect Genes Related to Secondary Metabolites in Schisandra chinensis

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Abstract:

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However, the underlying molecular mechanisms of medical potential are largely unknown.

To understand and engineer genes involved in secondary metabolic pathways related to lignans including schizandrin and gomisin, we performed transcriptome sequencing by using long-read isoform sequencing (Iso-Seq) and short-read RNA-sequencing (RNA-Seq).

A total of 132,856 assembled transcripts were generated with an average length of 1.9 kb and high assembly completeness. Of those unigenes, 71.6% were predicted to be complete full-length (FL) ORFs and exhibited a high gene annotation rate.

These genes were among 71 functional groups in three classes of biological processes, molecular functions and cellular components by gene ontology (GO) annotation, and involved in KEGG pathways related to lignan biosynthesis, flavonoid and phenylpropanoid biosynthesis. Furthermore, we successfully identified unique full-length genes involved in phenylpropanoid biosynthesis an extrapolated their regulatory network.

In conclusion, our results suggest that long-read, full-length or partial-unigene data with high-quality assemblies are invaluable resources as transcriptomic references in S. chinensis and will provide a valuable resource to understanding of the huge potential of pharmacological relevance of Omija plant.

Keywords:

S. chinensis, herbal medicine, transcriptome,

Optimizing whole-genome prediction for blueberry breeding

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Abstract:

Blueberry (*Vaccinium* spp.) is an important polyploid crop with significant beneficial effects for human's health. To supply market demand, the blueberry breeding cycle takes from nine to twelve years. Apart of its genetic complexity, the feasibility of genome prediction has been proven for blueberry, enabling a reduction in the time for the breeding cycle and increasing the genetic gain. However, genotyping costs still hinder the implementation of genome-based breeding methods for this and other crops. In order to optimize the relationship between genotyping cost and model accuracy, we evaluate the effect of number of markers and sequencing depth on phenotype prediction. For this we used high-density marker data and phenotypic information of yield and quality-related traits from a real blueberry breeding population of 1847 individuals. We evaluated ten filters of marker data (ranging from 500 to 72K) and compared five simulated Poisson distributions for sequencing depth (2X, 6X, 12X, 24X, and 48X) and real data (60X). Our results show that we can substantially reduce the number of markers (from 72K to 10K) and sequencing depth (from 60X to 10X) with no significant loss in prediction accuracy. Therefore, we can significantly reduce genotyping costs to accurately perform genome prediction in blueberry, allowing faster release of varieties with an economically feasible application of genome prediction for blueberry. The benefits and pipeline described in this study can be applied to optimizing genome prediction for other polyploid species.

Keywords:

autopolyploid, genome selection, breeding, *Vaccinium*

EUCARPIA

Epigenetic mechanisms controlling peach bud dormancy and its release: preliminary results

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Abstract:

To cope with critical environmental conditions during winter, perennial deciduous fruit trees have evolved adaptive mechanisms such as bud dormancy (a cease of bud growth). Bud dormancy is overcome by a period of chilling temperatures (Chilling requirement, CR). Recently, global warming makes always more often the environment unable to satisfy the CR of fruit trees. Consequently, fruit trees show irregular phenologies with detrimental effect on their productivity. A deletion in the tandemly arrayed family of DORMANCY-ASSOCIATED MADS-box (DAM) genes has been proposed to be the cause of the non-dormant phenotype of the evergrowing (evg) mutant of peach (Prunus persica). Subsequently, it has been demonstrated that the expression of DAMs is epigenetically controlled, mainly through chromatin modifications. However, the knowledge on the genetic factors involved in the regulation of bud dormancy events is still scarce and fragmentary. The main goal of this work is to improve our knowledge on the epigenetic mechanisms involved in the control of bud dormancy and its release. To reach this goal we used a spontaneous nectarine mutant, named slow ripening (SR), which phenotype seems to be due to an alteration of epigenetic mechanism regulating buds and fruit development, possibly inherited by its female parent (cv Fantasia). Preliminary results indicate that, at the same CR, DAM genes are differentially expressed in SR and Fantasia. Here we present data integration of genome-wide analyses (RNA-Seq and DNA methylation) during bud dormancy, for depicting a more complete picture of bud dormancy epigenetic control.

Keywords:

Prunus persica, spontaneous mutant, DNA methylation, chilling requirement, RNA-seq

EUCARPIA

Structural and functional analysis of endodormancy-regulator genes in apricot (*Prunus armeniaca* L.)

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Abstract:

Bud dormancy of temperate fruit trees is one of the most important characteristics affecting crop reliability. Dormancy can be divided into the endo- and ecodormancy phases. Endodormancy is a genetically controlled mechanism that is triggered in early autumn by external factors and trees require a certain amount of chill for endodormancy-release. We have identified and characterized the structure and function of apricot (*Prunus armeniaca* L.) homologs of three dormancy-regulator genes including the ParCBF1 (C-repeat binding factor), ParDAM5 (dormancy-associated MADS-BOX) and ParDAM6 genes. All highly conserved structural motifs and the 3D model of the DNA-binding domain indicate an unimpaired DNA-binding ability of ParCBF1 transcription factors. A phylogenetic analysis was carried out to confirm that ParCBF1 is most likely homologous to *Prunus mume* and *Prunus dulcis* CBF1. ParDAM5 also contained all characteristic domains of the type II (MIKCC) subfamily of MADS-box transcription factors. The unimpaired function of ParDAM5 is supported by the homology modelling of its protein domains. To check the function of candidate genes, their expression was studied in two subsequent dormant seasons with differences in chilling accumulation over the dormancy period. ParCBF1 showed an increased rate of expression in response to decreasing ambient temperatures in autumn and winter. The expression levels of ParDAM5 and ParDAM6 changed according to CBF1 expression rates and the fulfilment of cultivar chilling requirements, determined according to the Utah and Dynamic models. The Dynamic model showed lower variation in chill accumulation across years that the Utah model, which indicates that Dynamic model may also provide more accurate assessment in colder climates like Hungary. The concomitant decrease of gene expression with endodormancy release is consistent with a role of ParDAM5 and 6 genes in dormancy induction and maintenance. This pattern indicates a similar function of those genes in apricot as their homologs have in other closely related *Prunus* species. Cultivars with higher chilling requirements and delayed flowering time (Zard and Stella) showed higher expression levels of ParDAM5 and ParDAM6 toward the end of endodormancy as compared to those of early-flowering cultivars (Aurora and Goldrich). These results support that the newly identified apricot gene homologs have a crucial role in dormancy-associated physiological mechanisms.

Keywords:

apricot, CBF, DAM, dormancy, flower development, gene expression, microsporogenesis

Genetic and Genome study of Pyrus pyrifolia

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Abstract:

Pears (*Pyrsus* spp.) are one of the important fruit crops in temperate regions. The Rosaceae family contains antioxidant fruits, such as apples, plum, berry's and cherries. Pyrus pyrifolia cultivar 'Wonwhang (WH)' (BioSample SAMN05196235) genome sequencing were conducted a nuclear, chloroplast (KX450876), mitochondria (KY563276). The nuclear genome size is estimated to be 535 Mb by K-mer analysis. The nucleotide genome assembly characteristics included high detection of 97% with 2,221 scaffolds, 649kb of N50 and 517Mbp. Three genetic maps in inter and intra cross populations were constructed with SNPs polymorphisms by genotype by sequencing (GBS). Owing to recent WGD in Pyreae, there are more than two loci in different homologous chromosomes in genetic maps. Follows on Hi-C mapping mainly, we could decide to pseudomolecule on 17 chromosomes with 440.3Mb and 964 scaffolds with 85.23% coverage to the assemble genome in WH. SSR-containing genome sequences were subjected to categories of function putative genes with homology search, 510 SSR markers selected in this study. Of these, 70 markers were distributed across 17 chromosomes with more than one locus. We conducted QTL analysis with several breeding traits of fruit ripening with sweetness, acid, and texture. Catching four QTLs in one population (P. prifoilia and P. communis, inter cross) in sweetness, two SSR markers were decided in this population. Expected benefits of the pear genome sequencing will include identifying genes and utilizing them to develop new varieties to increase the market value of the fruit.

Keywords:
genome sequencing; pseudomolecule; GBS; QTL

Scion cultivar breeding at the Faculty of Agriculture, Novi Sad

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Abstract:

Genetic improvement of pome and stone fruit species at the Faculty of Agriculture, Novi Sad, covers different aspects of intraspecies and interspecies hibridization, selection from the natural populations, breeding for resistance against diseases and pests, utilization of natural mutations, development of early ripening cultivars using embryo culture, development of columnar, dwarf and decorative forms. In apple breeding objectives have mainly focused on meeting aesthetic standards, with good eating quality and durable disease resistance combining vertical (Vf gene) and horizontal mechanisms where gene donor was autochthonous apple variety ‘Bihorka’. Two red overcoloured cultivars with mid and late picking time, ‘Ivana’ and ‘Iskra’, were released in 2018. Both selections are resistant to scab and highly tolerant to powdery mildew. Significant genetic diversity was obtained from interspecific cross Nashy pear (cv ‘Nijiseiki’) × *Pyrus communis* (cv ‘Williams’). ‘Ivanino zlato’, released in 2018, is pear cultivar completely resistant to all economically important diseases and pests. Quince genotypes tolerant to fireblight has been selected as on farm spontaneous seedlings. A new cultivars as a red sports of apple varieties ‘Idared’ (cv ‘Gordana’, low vigor - require M26 or MM106 as rootstock in high density orchards, do not need support), ‘Moren’s jonagored’ (cv ‘Jerina’) and ‘Elstar’ (cv ‘Semendria’) were released in 2018. Peach variety ‘Ivana’ (mutation of ‘Springcrest’) is a firm yellow flesh, early ripening peach well exceeding fruit attractiveness of ‘Spring lady’. Red flesh and pillar peach and nectarine breeding activities are in progress. The in vitro embryo culture of immature embryos from early-maturing sweet cherry and apricot cultivars, used both as seed and pollen parents, resulted in large number of bipolar plants without implementing micropropagation. Development of very late maturing apricot cultivars, ripening in September, is based on Hindukush germplasm. Ornamental apple breeding has been extended from columnar tree habit, six varieties released in 2007, to nearly all main fruit species combining many variations in growth habit and size of tree, leaf color and fruit characteristics. Two selections of dwarf apples are submitted to release – ‘Sofia’ and ‘Mia’. Unique decorative forms were achieved by on farm selection, utilization of specific genetic potential of varieties and selections, rootstock/scion interactions, and application of traditional horticultural skills. That gives fruit species wider opportunities for use in home landscape design, as sidewalk plants or container plants.

Keywords:
germplasm, scion cultivar breeding, pome fruits, stone fruits

Stone fruit rootstock breeding at the Faculty of Agriculture, Novi Sad

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Abstract:

The Balkan Peninsula could be considered as secondary center of genetic diversity for many Prunus fruit species of interest for rootstock breeding: *Prunus cerasus, Prunus gondouinii, Prunus fruticosa, Prunus avium, Prunus domestica, Prunus cerasifera, Prunus spinosa, Prunus armeniaca, Prunus persica, Amigdalus nana, Prunus persica × Amigdalus communis*. Genetic diversity is recognized at the levels of genus, species, many local varieties and populations, and geographic variation as a result of ecologically determined differences (latitude, altitude, temperature, moisture availability and soil characteristics). Local *Prunus* varieties are often population varieties where genetic diversity is continuum of morphological characteristics as a result of propagation by suckers and chance seedlings, heterozygosity, self-compatibility and cross-pollination with related germplasm. Valuable asset of numerous observed on farm specific rootstock/scion interactions represent inexhaustible source of breeding ideas. Collected germplasm was the basis for fruit plant improvement program through rootstock breeding in *Prunus*. The ultimate goal was to propose pre-selection simulation model of the rootstock and scion vigor and rootstock/scion interaction based on the investigation of their morphological and anatomical characteristics and calculation of theoretical hydraulic conductance. Radial water uptake is influenced by the root system branching pattern and the spatial distribution while axial water pathway is determined by the root, rootstock and scion stem xylem characteristics. Hydraulic conductivity of the rootstock and scion varieties was in agreement with their hereditary controlled plant growth, irrespective of the presence of multiple abiotic stresses. Among parameters potentially useful in tree vigor reduction, vessel frequency, vessel lumen area, and percentage of vessels on wood cross section are proved to be most reliable. These xylem properties is effective way to estimate the vigor and dwarfing capacity of specific genotype as rootstock. Water movement model through fruit trees as preselecting method for prediction of tree vigor was determined by functional hydraulic conductance of root system, rootstock stem and scion stem of different cherry rootstocks and sweet cherry varieties. Deductive pre-selection simulation model could be applied in the rootstock and scion breeding programs and as a guide for rootstock/variety specific spacingtrees in high density planting.

Keywords:
Prunus, germplasm, rootstock breeding

Effect of different iron sources and cytokinins on in vitro shoot multiplication of Juglans spp

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Abstract:

In micropropagation of *Juglans* spp. chlorosis symptoms can negatively affect multiplication of the shoots in some genotypes also reducing the subsequent rooting of the explants. The type of cytokinin supplied to the basal medium can also have a critical effect on the shoot regeneration and quality response. Considering the increasing interest in Italy for micropropagation of Juglans spp., the present study was addressed to evaluate the effect of the application of different chelated forms of iron, in combination with a partial substitution of agar with pectins, on in vitro shoot cultures of *Juglans regia*, cv. Chandler and of a genotype of *J. microcarpa*, to determine the effect on shoot development, chlorosis and multiplication. The effect of the natural cytokinin, meta - Topolin (mT) (from 0.5 to 3 mg/L), in comparison with N6 - benzyladenine (BA), was also evaluated in *J. microcarpa* to further optimise the multiplication phase in this species. The use of ethylenediaminetetraacetic acid ferric sodium salt, in combination with pectins, significantly increased the number of shoots, shoot height and reduced the chlorosis in cv. Chandler, while it did not significantly affect shoot multiplication and quality in J. microcarpa which were, on the other hand, positively influenced in this species by the substitution of BA with mT. The present study was performed in the framework of a project (PORT.NOC), financially supported by the Italian Ministry of Agriculture and Forestry (MiPAAF) to perform studies focused on evaluation of rootstocks tolerant to *Phytophthora* and black-line and on *J. regia* cultivars.

Keywords:
iron chelates, *Juglans regia, Juglans microcarpa*, meta - Topolin, micropropagation, pectins

Qualitative and nutraceutical characterization of ancient and commercial pear varieties

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Abstract:

Pear (*Pyrus communis* L.) fruit is rich in health promoting antioxidant compounds such as phenolic compounds. In Italy, pear is an important crop and its production is the third producer in the world (FAOSTAT, 2016). However, in the last years the use of a small number of commercial varieties has resulted in a loss of agrobiodiversity, thus there is a growing interest for the agrobiodiversity recovery and valorization of local varieties also through the studies of nutraceutical fruit properties. This study was aimed to characterize the quality traits and nutraceutical properties of peel and pulp of 2 Italian local pear varieties currently conserved at the National Fruit Tree Germplasm of CREA-OFA, Rome, in comparison with three commercial varieties, “Conference”, “Coscia” and “Decana”. Total soluble solids (TSS) were measured by digital refractometer and titratable acidity (TA) by volumetric titration. The antioxidant activity (AA) was measured by the DPPH and determination of total phenolic compounds (TPC) and antocianins (ANT) was performed by spectrophotometric analyses. As in the other fruit species, TSS and TA values were found to be related to the genotype and both AA and TPC were significantly higher in the peel than in the flesh in all the cultivars and also vary in relation to the genotype. The cv. Angelica, showed the highest AA and TPC and ANT content in the peel, while the highest values of these parameters in the flesh were found in “Decana” and “Mastantuono”. These preliminary results represent a contribution toward the re-valuation of ancient pear varieties as potential source of bioactive compounds and for their use in breeding programs

Keywords:
Antioxidant compounds, *Pyrus communis*, spectrophotometric analyses

In vitro adventitious regeneration in peach: the effect of the natural cytokinin meta-Topolin

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Abstract:

The potential for the improvement through the application of gene transfer techniques is still difficult in peach [*Prunus persica* (L.) Batsch.] since this species is highly recalcitrant to adventitious shoot regeneration from mature tissues. Peach shoots have been previously regenerated from in vitro propagated leaves, however the protocols still need to be defined in most of the cultivars. In the present study the effect of different cytokinins on adventitious shoot regeneration in 2 peach cultivars, Rich Lady and Independence, was evaluated. In vitro shoots were grown for one subculture on a multiplication medium containing 5, 10 or 15 mg/L meta - Topolin (mT), an aromatic natural cytokinin, or N6 - benzyladenine (BA), then apical leaves were excised and transferred on a culture medium supplied with α-naphthaleneacetic acid (NAA) and BA, thidiazuron, mT or zeatin to induce adventitious regeneration. Leaves were cultured 21 days in darkness on these media and then transferred to the same auxin-free media. Adventitious regeneration was obtained in leaves from shoots previously grown both on mT and BA, but with different extent. In Rich Lady, the highest regeneration response (32%) was obtained either with the pretreatment with 10 mg/L BA and applying BA during the regeneration phase and with 5 mg/L mT and with mT in the regeneration phase. The highest regeneration responses in cv. Independence were till 19% using 10 or 15 mg/L mT in multiplication phase, regardless the type of cytokinin applied during regeneration. These results appear promising for the application of cisgenesis and genome editing by CRISPR/Cas approach, aimed to genetic improvement of productivity and resistance of peach cultivars in the frame of the project “BIOTECH, sub-project “Biotechnology for sustainable fruit crops” (BIOSOSFRU), financed by the Italian Agriculture Ministry.

Keywords:

benzyladenine, leaf explant, *Prunus persica (*L.) Batsch., shoot pretreatment.

Cryopreservation of Italian cultivars of hazelnut (*Corylus avellana* L.): further steps

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Abstract:

The use of a limited number of cultivars for intensive cultivation has negatively affected agrobiodiversity of fruit species. Application of cryopreservation can be a valuable complementary tool for long term germplasm conservation since can reduce costs and risks of loss of genotypes in respect to conservation in field. The present study was performed to optimise the cryopreservation protocol in *Corylus avellana* L. Italian cultivars: lateral buds, excised from in vitro grown plantlets, of “Tonda Gentile Romana” (TGR) and “Montebello” (MB) were cryopreserved by the encapsulation-dehydration technique evaluating the effect of two methods of dehydration of beads (silica gel or laminary flow) and of the application of 2 cytokinins (1.5 mg/L N6 - benzyladenine or 2 mg/L meta - Topolin) in the regrowth phase. The highest survival was obtained dehydrating alginate beads for 8 h with silica gel in both cultivars. On the other hand, the regrowth response to the type of cytokinins in the medium was different in the 2 cultivars: in TGR higher regrowth (45%) was reached without significant differences between the two cytokinins, while in MB, meta - Topolin induced significant higher regrowth (40%) than N6 - benzyladenine.

Keywords:

agrobiodiversity preservation, cytokinins, encapsulation-dehydration, *in vitro* culture.

Fruit characteristics of sweet cherry cultivars bred at Czech Republic

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Abstract:

Sweet cherry cultivars bred at Research and breeding station Holovousy Ltd. (Czech Republic) were evaluated for their fruit characteristics. Fruit firmness (index 1 -100), sugar content (° Brix), fruit size (width in mm) and fruit weight (g) of sweet cherry cultivars were recorded in the years 2017 and 2018 in experimental plantings at location Holovousy. The highest firmness was recorded for cultivar ´Tamara´ in 2017 (82.7). The highest sugar content was recorded in 2017 for the variety ´Vanda´ (23.5 ° Brix). The largest fruits were recorded in 2017 for cultivar ´Tamara´ (29.7 mm). The highest fruit weight was recorded for ´Horka´ in 2017 (12.9 g). Fruits were characterized by lower firmness (60.0) in 2018 than in 2017 (63.9). Fruit were significantly sweeter (19.5 ° Brix) in year 2017 than in year 2018 (16.5 ° Brix). Size of the fruit was higher in the year 2017 - 27.0 mm than in the year 2018 - 26.3 mm. Higher fruit weight was recorded in the year 2017 (average 10.7 g) than in the year 2018 (average 9.0 g). Year-on-year differences can be explained by unusually warm and dry weather in 2018 and by the frost damage during tree flowering in 2017.

Keywords:
P. avium; fruit size; fruit firmness; fruit weight; sugar content