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WG1 From genome to berry fruit

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CONTENT

AN ATTEMPT OF BERRY FRUIT GERMPLASM RESOURCES IN EUROPE IN EARLY 2006

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Keywords: berry species, accession

Following a draft questionnaire sent, only few countries partially answered to it: Germany, Hungary, Denmark, Lithuania, Bulgaria, Portugal, Scotland and Romania.

After this first attempt one can conclude:

Major species:	No. of accessions
- currant	303 (black, red, white)
- raspberry	187
- blackberry	92
- blueberry	99
- gooseberry	103

Minor species: (for the moment)

- seabuckthorn	63
- chokeberry	9
- cluehoneysuckle	36
- elderberry	90
- cornelion	10

Our further concern in this field is to get data from all the European countries including former Soviet Union countries.

MULBERRY GENETIC RESOURCES IN EAST PART OF TURKEY

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Keywords: mulberry, Morus spp., genetic resources, biodiversity

The white, black and red mulberry (Turkish name ‘Dut’) is widely grown in Turkey. With its temperate climatic conditions, Eastern part of the country is rich in genetic resources of native mulberry. In this region, Erzurum district in particular has notable populations of black, red and white mulberry, which have been cultivated in gardens for their delicious edible fruits. In addition to fresh consumption, another important use of mulberry fruit in this region is for various confectionery products such as jam, marmalade, ‘mulberry pestil’, ‘mulberry pekmez’ and ‘mulberry kome’, and also for medicinal purposes, e.g. to treat mouth lesions. This study was carried out in 2004 year in Eastern part of Turkey aimed to determine genetic resources of mulberries which natively grown in Erzurum district of Turkey. Phenological properties, including harvest period (beginning and end of harvest for each village) was observed according to Lale (1992). For chemical characteristics of fruits (Total soluble solids, vitamine C and fruit color as represented L, a and b value were determined with digital refractometer (Kyoto Electronics Manufacturing Co., Ltd, Japan Model RA-250HE), titration (AOAC, 1995) and chromometer (Minolta Chromometer model CR-300), respectively. With this study, it was determined that the region has very important mulberry genetic resources. There were huge differences within mulberry populations in terms of fruit weight, yield and TSS. The altitude of mulberry growing areas in this region varied from 500 m to 1600 m. Harvest period started middle of June and lasted to first week of August. According to altitude, fruits can be picked 6-10 times during harvest period. 99% percent of trees were belonging to *Morus alba*. Fruit weight and yield per tree varied from 1.5 g to 4 g and 20 to 105 kg.

CHARACTERIZATION OF *FRAGARIA* GENUS WILD SPECIES AND PIONEERS CULTIVATED SPECIES (*FRAGARIA X ANANASSA* DUCH.) ACCESSIONS, IN THE SOUTH COST OF SPAIN

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Keywords: genetic resources, Fragaria, morphological characterization.

The strawberry germplasm collection at IFAPA-C.I.F.A. Málaga, comprising 218 accessions of *Fragaria* x *ananassa* Duch., 200 breeding lines, and 100 accessions of other related species. This strawberry germplasm collection was included in the European Strawberry database and includes some of the core collection varieties of special interest. This collection is the source of the Spanish National strawberry breeding program to obtain new varieties well-adapted to the agronomic and environmental conditions of Huelva and other Mediterranean production areas. All the new varieties of *F. x ananassa* of this collection are already well agro-morphologically characterized. However, most of the accessions belonging to related species and old varieties, named pioneer varieties, are still no characterized. During the last three years (2002-2005) a morphological characterization of these accessions has been developed. Altogether 97 accessions were characterized: 27 of *Fragaria vesca*, 24 of *F. chiloensis*, 8 of *F. viridis*, 8 of *F. moschata*, 7 of *F. virginiana*, 1 of *F. gracilis*, 1 of *F. nipponica*, 1 of *F. ananassa* cuneifolia, 17 pioneer varieties of *F. x ananassa*, and 3 of related species such as *Potentilla spp*. and *Duchesnea indica*. A big intra and inter-specific variability has been observed. On the other hand, the resistance/susceptibility to *Colletrotrichum acutatum* of 3 accessions of *Fragaria vesca*, 10 of *F. chiloensis*, 1 of *F. viridis*, 1 of *F. moschata*, 7 of *F. virginiana*, and 1 of *F. gracilis*, has been tested. The results of this work have contributed to improve the use of the strawberry collection for breeding programs.

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ASSESSMENT OF GENETIC DIVERSITY IN BULGARIA RASPBERRY GERMPLASM COLLECTION BY MICROSATELLITE AND RAPD MARKERS

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Keywords: raspberry, genetic markers, SSR, RAPD, locus

In the last decade a heightened interest has been focused on the soft fruits /strawberry and raspberry/ as major sources of health - associated compounds such as antioxidants. As a traditionally important producer of soft fruits Bulgaria has a rich germplasm collection of both Bulgarian and foreign varieties with valuable agronomic characters of commercial value.

In addition to morphological descriptions used for classification of species and cultivars and characterization of the Bulgarian raspberry germplasm collection a programme for assessment the genetic diversity by both microsatellite and RAPD markers was recently initiated. The application of molecular markers should allowed more efficiently understanding of the crop plant genetic architecture and the identification of species and cultivars by means other than morphological characters. Molecular markers data should also give an unable of breeders to expand genetic diversity in breeding material and allow selection of new perspective forms in future breeding programmers.

In this study we present our initial results on the assessment of the genetic variation in Bulgarian raspberry accessions using SSR **(**Simple Sequence Repeat**)** and RAPD markers. Microsatellites (SSRs) were chosen as one of the most suitable molecular marker system for genotyping of raspberry germplasm collection. The proven advantages of SSR markers are their high information content (PIC), co dominant inheritance, locus specificity, extensive genome coverage and simple detection using labeled primers that flank the microsatellite and hence define the microsatellite locus (Graham *et al*., 2002; 2004). In addition to SSR markers, the information from RAPD analysis will allow more precise estimation of genetic diversity in Bulgarian raspberry collection.

ANALYZING STRAWBERRY VARIETY TRIALS USING RANDOMIZATION BASED AND MODEL BASED APPROACHES

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Keywords: mixed model, multi-environment trials, spatial modelling

In the Norwegian strawberry breeding programme we have an established routine testing of second stage advanced selections together with the current market cultivars. These trials are conducted at 4 NCRI outstations and managed and harvested according to the routines of the local industry. The data sets from these trials have previously been analysed statistically using classical analysis of variance, i.e. a traditional linear model. The common lack of orthogonality in multi-environment trials (METs) has introduced the linear mixed model approaches in the analysis of these types of data. The advantages of the mixed linear models include the ease with which incomplete data (not all varieties/clones in all environments) can be handled, the ability to use more realistic within-trial models for the error variation (e.g. incomplete blocks, spatial correlation models) and the ability to assume some sets of effects (e.g. variety and/or environment effects) to be random rather than fixed.

I have analysed the commercial yield output of 5 strawberry METs from the period of 2001 to 2005 from the Norwegian strawberry breeding programme. Each MET consisted of 4 individual trials. Comparison of the models, starting with the basic randomization based model and adding extensions to control spatial variation, was done using the Akaike Information Criterion (AIC) supplemented with graphical devices like the variograms. The trials were laid out in a twice replicated α-lattice design and the additional modelling of the trials included incorporation of both trends and extraneous variation.

In five of the twenty trials analysed I observed no improvement by extending the model further than the base (randomized complete block) model. Three of the trials were judged best when correlated errors between plots was added to the base model, and four trials were best analysed using the α-lattice analysis. Two were judged best when using a combination between a α-lattice analysis and correlated plot errors, while I in the six remaining cases had to include linear and cubic trends across the fields. In four of these six an additional improvement was observed when correlated errors were included.

Although rank shifts between the entries were observed when moving from the base approach to the best approach, the huge benefits of using these relatively laborious and complex modelling approaches are not apparent to me as far as these strawberry clonal trials concerns. The ranking in single trials with and without modelling was fairly stable. The lowest correlation coefficient was 0.95, while the lowest rank correlation was 0.94. In a comparison within each of the 5 METs these correlations were even higher. So, even though we observed a significant improvement in the AICs, this did not have substantial influence on the ranking of the clones. One possible explantion could be that the trials were all established with drip fertigation systems which might remove much of the global and local variation otherwise found in even small sized trials. Still, a reduction in the pairwise standard errors resulted in significant higher probabilities when comparing a given selection with the best check cultivar.

STRAWBERRY VARIETAL EVALUATION FOR AUTUMN PRODUCTION

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Keywords: Fragaria x ananassa Duch, varieties, out-of-season production, plastic tunnels

Among the small fruit crops, strawberry is the main and the most popular fruit in Portugal with about 15,000 t produced annually and 500 ha of acreage. Using different growing systems and cultivars it is possible to produce strawberries all year-round in this country.

Nowadays, the demand for autumn strawberry fruit is increasing mainly for exportation market. Due to its mild autumn and winter temperatures, Portuguese southwest coastal areas are suitable for autumn production and growers in this region are very much interested in this off-season strawberry production mostly due to the economic advantage.

The extension of the strawberry crop season to autumn has been achieved in Portugal by using day-neutral Californian cultivars and under crop protection (polyethylene tunnels). These cultivars are planted in June-July as frigo plants (bare-rooted plants) and the harvest period occurs during October, November and beginning of December.

The use of waiting bed or tray plants of European cultivars has been reported successfully for strawberry autumn production. It reduces the time between planting and harvest and could facilitate autumn production with fruits of good quality in our mild autumn and winter areas.

To evaluate the performance of Elsanta and Flamenco cultivars grown in southwest region, a trial was established in a soilless culture and under a polythene tunnel with different plant type and planting date. Different types of plants were used, frigo (A and A^+), waiting bed and tray plants, that were planted on the 28th September, 10th October and 17th October. During the growing cycle some vegetative growth, flower and fruit production were recorded. Results of vegetative growth, flowering and yield responses of those two cultivars are presented and discussed.

Further trials need to be programmed to evaluate other European varieties that could be well adapted to our climatic conditions.

INHERITANCE OF FRUIT TRAITS IN A MODEL POPULATION OF *FRAGARIA* x *ANANASSA*

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Keywords: inheritance analysis, aroma, dry matter, SPME, gas chromatography

The inheritance analysis of aroma compounds, dry matter and soluble solids are demonstrated by a model population. Two cultivars were chosen, cv. Mieze Schindler and cv. Elsanta, which are very distinctive from each other in pedigree, phenotype and fruit traits, including aroma and dry matter. Nineteen flavour impact compounds for the strawberry aroma are defined by human sensory, gas chromatography/mass spectrometry and /olfactometry. A reliable method was developed, using an effective sample preparation (automated headspace solid phase micro extraction) and gas chromatographic separation coincides with a pattern recognition for data processing (virtual electronic nose). This method allows to assist breeding programs by preparation of hundreds of samples by a simple procedure.

Two hundred seedlings of the cross combination cv. Mieze Schindler x cv. Elsanta were randomised selected from a population of 438 seedlings and propagated with three plants per each. Fruit samples were taken into the analysis in 2005.

BREEDING AND BIOTECH FOR IMPROVING STRAWBEERY NUTRITIONAL QUALITY

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Keywords: strawberry, breeding, GMO, nutritional value

Fruit possess different antioxidant properties depending on their content in antioxidant molecules. As a consequence of the complexity of fruit matrix, the AA can be interpret as reaction carried on by a single compound and/or by a complex mixture. The final result in Antioxidant Activity (AA) and the determination of Total Phenolic Content (TPH) of a fruit, is strictly reported to the fruit sampling, procedure of extraction of antioxidant compounds from fruit and also depends on which target, free radical generator or oxidant, is used in the measurement.

For different strawberry genotypes (wild and cultivated) a deep analyses of nutritional attributes (NA) was made for cultivar and selection that have been included in breeding programs aimed to obtain improved fruit patterns and contents. To develop new genotypes, the availability of new sources of NA is an important feature. The introduction of the genetic material from the wild germplasm (*F. virginiana glauca*) allowed a significant improvement of the fruit nutritional quality.

Progenies from strawberry crosses having *F. virginiana glauca* as a common parent showed a significant increase in their fruit quality and nutritional features, thus confirming the interest of the wild species to improve cultivated strawberry for several characters. On the contrary, most of the *R. parvifolius* offspring resulted with a low AA content, thus evidencing the low contribution of this wild species in increasing the NA of commercial raspberry fruit, as also already found by other authors.

The genetic background in NA modification, was also studied in strawberry fruit from genetically modified plants for two different genes involved in auxin and cytokinin metabolisms and plant development (*DefH9-iaaM* and *rolC* respectively). The increase of auxin endogenous metabolism induced in strawberry fruit - achens by the expression of the *DefH9-iaaM* resulted with a positive effect in increasing plant productivity without altering berry quality and nutritional value. The increased plant cytokinin metabolism induced by the expression of *rolC* gene had an effect on plant development (increased vigour and adaptability), but also lead to an improvement of fruit nutraceutical background.

EVALUATION OF STRAWBERRY AND BLACKCURRANT CULTIVARS IN LITHUANIA

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Keywords: Strawberry, blackcurrant, yield, fruit size, cultivars, pest and disease resistance

Evaluation of ten strawberry (*Saulenė, Honeoye, Kent, Elkat, Polka, Dangė, Senga Sengana, Pegasus, Bogota* and *Pandora*) and five new blackcurrant (*Gojai, Svajai*, *Dailia, Senjorai* and *Salviai)* cultivars were studied at the Lithuanian Institute of Horticulture.

Among strawberry cultivars only *Dangė* and *Elkat* remained in excellent condition during all the period of three-year growing. Strawberry cvs. *Saulenė, Honeoye* and *Kent* start flowering early (05.15-17), *Elkat, Polka, Dangė, Senga Sengana, Pegasus* – averagely late (05.19-20), and *Bogota* and *Pandora* – very late (05.26-31). Strawberries of cvs. *Saulenė, Pandora, Senga Sengana, Honeoye, Polka, Elkat* and *Dangė* were very productive (11-17 t ha^{-1}), *Pegasus* – productive (9 t ha^{-1}), *Bogota* and *Kent* - averagely productive (6-7 t ha^{-1}).

The bushes of new blackcurrant cultivars were winterhardy, resistant to spring frosts, powdery mildew, sufficiently resistant to anthracnose, leaf spot and gall mite. The fruit of blackcurrant cv. *Gojai, Svajai* and *Senjorai* was very large (1.7-1.8 g), cv. *Dailiai* and *Salviai* – large (1.5 g) and standard cv. *Titania* – medium size (1.1 g). Average yield of fruits reached 5.8-10.3 t ha^{-1}.

The taste of blackcurrant cv. *Dailiai, Gojai* and *Svajai* was good (7.3-7.5 scores), while cv. *Titania* and new cv. *Senjorai* and *Salviai* ranged between 7.0-7.1 scores. Very high (230 and 212 mg 100g^{-1}) ascorbic acid contents were established in cvs. *Salviai* and *Svajai*. The fruits of these new blackcurrant cultivars can be used fresh and for processing.

DOMESTICATION AND BREEDING OF CLOUDBERRY

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Keywords: Rubus chamaemorus, breeding, hermaphroditic genotype

Many wild berries have commercial potential in Northern Europe but cloudberry (*Rubus chamaemorus* L.) has up till now the highest value among *Rubus* species. The trade of cloudberries in Finland varies from 100 to 1000 tons per year, the total value being 1-6 million euros. In addition, households pick cloudberries five times that amount. The biggest problems for cloudberry in nature are spring frosts, poor pollination and a disproportion of male and female flowers. Cloudberry grows regularly on peatland in the circumpolar regions and spreads mainly by means of an extensive rhizome system. It is a dioecious perennial plant and the flowers have normally either fully developed male or female organs. We have in our collection a rare hermaphroditic cloudberry clone from Pyhäntä, Finland, with both fully developed male and female organs. This clone has produced berries through self-pollination and the seedlings have later shown to be male, female or hermaphroditic genotype. For the breeding purposes and cultivation tests we have collected large cloudberry collection into the University of Kuopio. In summer 2000, a contest “Biggest cloudberry of Finland” was organised. Altogether 265 pickers around Finland sent a total of 765 cloudberries. The biggest "giant" size cloudberry from the cloudberry contest weighing 6.52 g was from Nurmes, Finland. Of all berries, 23 were over 6 g, 43 were between 5-6 g, 113 between 4-5g, 79 between 3-4 g and only 7 were under 3 g. Seeds were separated, calculated and weighed from the most interesting berries. The average seed number correlated with berry weight. However, the seed number of the berries over 6 g varied widely, from 20 to 38. Rhizomes isolated from the origin of the berry samples of over 6 g are presently growing in the greenhouse. Also several breeding lines and seedlings from this contest are in our collection. The first Finnish cloudberries have been named from this collection, the hermaphroditic "Cloudy", and the female "Ruby" with red berries. The growth rate and flowering of these clones are under observation. The clones have been crossed with the hermaphroditic cloudberry in order to obtain new superior cloudberry clones.

POLYPLOIDY AND GENOMICS

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Keywords: polyploids, mapping, genomics

Polyploidy, which is the occurrence of more than two copies of the basic set of chromosomes in a somatic cell, is prevalent in plants, where at least 70% of angiosperms have experienced one or more events of genome duplication (complete or partial) in their evolutionary history. The prominence of polyploidy in higher plants suggests that it is a key factor in plant evolution leading to the formation of new species.

Rounds of polyploidization, by addition of several genomes, are followed by rapid genetic and epigenetic changes, which affect genome structure for regulating chromosome pairing as well as gene expression for proper development.

Construction of polyploidy linkage maps will depend on the origins of genome redundancy created by polyploidy, and therefore will depends on the chromosome pairing behavior at meiosis. According to their origin, autopolyploids and allopolyploids are distinguished. In general, autopolyploids showed random pairing at meiosis (polysomy) while allopolyploids showed preferential pairing (disomy).

For construction of polyploidy linkage maps when the behavior at meiosis is not known, first the construction of the linkage groups in coupling phase is performed following by the study of the coupling/ repulsion phase in disomic behavior. This approach has been developed in the auto-allopolyploid sugar cane.

In this presentation, I will present our genetic works conducted on the linkage map of the octoploid strawberry and its use in the research of molecular markers linked to quantitative traits (Quantative Traits Loci, QTLs).

THE USE OF SSR MARKERS AS A HELP FOR STRAWBERRY CULTIVAR IDENTIFICATION

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Keywords: genetic fingerprinting, Fragaria x ananassa, microsatellites, genetic diversity

Molecular markers are powerful tools for cultivar identification in many species. Accurate and unequivocal cultivar identification is important for breeders' right protection, especially for vegetatively propagated plants, and to prevent unregulated propagation and distribution of high-value strawberry cultivars. Problems of misidentification of cultivars have arisen with morphological characters, that the reason why molecular markers are playing an increasingly important role in cultivar identification. The aim of this work was to characterize molecularly the strawberry cultivars cultivated in Europe. Thirty of the major cultivars (*F. x ananassa*) grown in Europe were screened for polymorphism using seven SSR markers. Fifteen primers pairs of SSR were tested and seven were selected since they gave informative pattern. Two SSR, EMFvi018 and BFACT26, produced respectively 17 and 13 polymorphic fragments which were sufficient to distinguish among all tested cultivars, including closely related genotypes. Specific fingerprints were generated from the molecular marker data. In addition, the relationships between the cultivars were examined from both the molecular and the pedigree data. Genetic similarity between varieties was estimated using Jaccard's coefficient based on SSR marker data and a dendrogram was built using the unweighted pair group method of arithmetic averages (UPGMA). These results confirm that SSR analyses are useful as a mean of check the identity of cultivars because it is simple, fast, cost effective and highly discriminant. Moreover, many studies showed that SSR markers very useful for studying the genetic diversity among *Fragaria* species.

ANALYSIS OF THE *FRAGARIA X ANANASSA* GENOME: GENETIC MAP CONSTRUCTION, AND QTL DETECTION INVOLVED IN MATURATION FRUIT PROCESS

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Keywords: genetic map, SSRs., QTLs, quality fruit

The complexity of the octoploid genome of strawberry (*Fragaria* x *ananassa*) is one of the main reasons for its limited genetic knowledge in spite of the economic importance of this crop.

A genetic map was constructed with codominant and transferable markers (SSRs and RFLPs) in a population generated with parents currently used in variety selection. At the moment our genetic linkage map of *F.x ananassa* encompasses 60 RFLPs obtained from *Prunus/Malus* probes and 187 strawberry SSRs that provide 312 loci grouped on 28 linkage groups. The genetic distance of this map is 1315 cM with 1 marker every 4.08 cM in average. The linkage group size is around 47 cM, and in relation to linkage group size in *Prunus* map we suspect that we have 80% of coverage map in strawberry genome.

This map is a necessary resource for the genetic analysis of quantitative characters involved in strawberry fruit quality (QTLs). Segregation data of 90 individuals of map population during 4 or 6 years was evaluated. We are analyzed 28 traits related to fruit quality aspects as size, weight, length, diameter, colour, firmness, sugar content, precocity and others. The data analysis by MapQTL software using interval mapping function detects 19 QTLs that are significant in all years, explain between 10 to 20 % of phenotypic variance. Some of these QTLs were validated in 5 different populations by segregation analysis of associated marker in front of traits data of 3 consecutive years.

PROGRESS ON THE DEVELOPMENT OF A TRANSFERABLE LINKAGE MAP FOR *FRAGARIA*

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Keywords: fragaria, genetic mapping, microsatellites, EST, functional genomics

Our first linkage map for diploid *Fragaria*, published in 2004, comprised 68 microsatellite (SSR) markers arranged in seven linkage groups that covered the *Fragaria* genome, but marker density was low. Research teams in Europe and the USA have recently developed a large number of novel *Fragaria* SSR markers from both coding and non-coding genome sequences. Working in collaboration with partners from Spain and France, East Malling Research has now extended the diploid *Fragaria* linkage map and provided near saturation of the genome with SSR markers. The map is composed of 175 SSR markers and spans 424 cM over seven linkage groups. The average marker spacing is 2.3 cM / marker and the map contains just eight of gaps of over 10 cM. This linkage map is now being used as the reference map for the genus and has provided a framework for synteny studies with the octoploid strawberry (*F.* x *ananassa*), the results of which have provided evidence for four homeologous diploid genomes in the cultivated strawberry. Also, the placement of 25 genes of known function is facilitating synteny studies with other economically-important *Rosaceous* genera, giving an insight into genome evolution within the *Rosaceae*.

TOWARDS THE PRODUCTION OF GENETICALLY MODIFIED STRAWBERRIES WITH INCREASED CONSUMER ACCEPTABILITY

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Keywords: strawberry, genetic modification, 'clean vector' technology, strawberry-own gene/promoter sequences

Breeding for improvement of strawberry cultivars is difficult. The complicated genetic constitution of the strawberry genome has kept most researchers from investing in the development of methods that can assist in the breeding of strawberry. Only recently, the first results towards the production of a genetic map for strawberry have been published. Next to classical breeding, the last decade genetic modification of strawberry has gained increasing interest. In principle, genetic modification allows a relatively quick improvement of existing important strawberry cultivars, for example, by the introduction of disease resistance genes. However, the availability of suitable genes and regulatory sequences that will result in desired improvements is limited at the moment, and identification of such information still requires large investments. Furthermore, the public attitude toward genetically modified crops in general is, at least in Europe, still rather sceptic and EU regulations are still rather restrictive. This is presently blocking the introduction of genetically modified strawberries on the EU market.

In order to increase acceptance of genetically modified strawberries by consumers, it would help when the transgenic strawberry plants are produced according to the 'clean gene' approach, which means that transgenic plants are free of foreign, undesired coding and regulatory DNA sequences. In addition to this criterion, the strict use of strawberry's own DNA sequences as genes- and promoters-of-interest is considered to be additionally beneficial in this concept.

At Plant Research International we have developed a plant transformation system which allows the elimination of undesired DNA sequences, like selectable marker genes, after transgenic plants have been selected. Using this system we have produced genetically modified strawberry plants from which the kanamycin resistance gene (*nptII)* has been deleted. Next to this, we have isolated and characterised several strawberry genes with are related to fruit flavour, fruit colour, fruit firmness, allergenicity and disease resistance. Our current focus is on the isolation and characterisation of genes that regulate biosynthesis of proanthocyanidins, a class of flavonoids recognised for its health promoting properties. In addition we have isolated strawberry gene promoter sequences for tissue-specific expression of genes of interest.

The use of strawberry-derived promoter and gene sequences in strawberry itself and the possibility to remove undesired (non-host) gene sequences from the plant genome allows the production of genetically modified strawberry plants, of which the genome contains exclusively genes and regulatory sequences of strawberry origin. Sociological studies indicate that such modifications, involving genetic material only from the species itself, would enhance consumer acceptance.

SELECTION OF STRAWBERRY GENOTYPES FOR MOLECULAR ASSESSMENT OF PLANT RESISTANCE TO *VERTICILLIUM DAHLIAE*

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Keywords: strawberry, segregating progeny, phenotyping, Verticillium dahliae, resistance

Plant resistance to pathogenic fungi is usual a quantitative trait dependent on combined activity of many host genes with wide range of response-complex. Recognition of fundamentals of this process and generation of molecular markers correlated with tolerance to pathogens is the main aim of breeding for resistance. In our study three cultivars 'Honeoye', 'Kent' and 'Gerida' known as a susceptible (*s*), and three tolerant (*t*) cultivars 'Senga Sengana', 'Salut' and 'Filon', as well as the progenies derived from twelve crosses between these cultivars were applied to the study on molecular basis of resistance to *Verticillium dahliae*, causal agent of significant loss in strawberry production in Poland.

Preliminary result of microsatellite-based analysis of genomic DNA isolated from the plants belonging to susceptible cultivars and from the tolerant plants showed the presence of several fragments specific for each of these groups. In further studies on the differences on the level of single genotype over 2200 plants being progeny of three types of crosses: *s* x *s*, *t* x *t,* and *s* x *t* were tested. Plants were cultivated on the field with previously determined concentration of pathogen. Progeny of each family were planted in seven subfields with different *V. dahliae* concentrations (from 3.5 to 53.6 propagules/gram of soil). Observation of plant response confirmed strong dependence of distribution of differentially reacted genotypes (scoring scale 0-5) on family. According to expectations, the highest number of infected plants was found in *s* x *s* and *s* x *t* progenies. The percentage of plants without symptoms (class 0) varied from 92% to 36-38%, dependent on family. The percentage of completely destroyed plants (class 5) from 0.6 to 34%, dependent on cross. High level of resistance to the pathogen was noted for progenies of *t* x *t* crosses ('Salut' x 'Senga Sengana', 'Filon' x 'Senga Sengana'). However, the distribution of differentially reacted genotypes was not simply correlated with increase of *V. dahliae* concentration, what suggested high diversity within investigated populations. The results of observation and statistical assessment of symptoms development dynamics will allow to select the seedlings for bulked segregant analysis and further molecular tests.

COMBINING ABILITY ANALYSIS OF STRAWBERRY CULTIVARS FOR RESISTANCE TO *VERTICILLIUM DAHLIAE*

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Key words: strawberry, Verticillium wilt, combining ability, GCA, SCA

The experiment was carried out on the F1 progenies (full sib-families) obtained from twelve crosses between six cultivars using diallel cross design according Griffing's IV method. Following parental forms were crossed: 'Gerida', 'Honeoye' and 'Kent' – as the susceptible genotypes to *Verticillium dahliae*, and 'Filon', 'Salut' and 'Senga Sengana' – as the tolerant ones to this pathogen. Progeny families arranged in an incomplete diallel cross design (some crosses have not given their progenies) were tested in field using randomized complete block design with four replicates. In September of 2005 level of the infection by *V. dahliae* and the plant vigour of individual plants were evaluated, using the ranking scale. Level of the infection ranged from 0 (healthy plants) to 5 (the most heavy infected or dead plants); plant vigour ranged from 0 (dead plants) to 5 (the most vigorous plants).

Means of the observations of evaluated traits on plants at plots were subjected to the preliminary analyses of variance in accordance with the model for progenies in the randomized block design. Because the means for the traits among hybrid families differed significantly, they were subjected to the analyses of variance for an incomplete diallel design using Griffing's fixed model in the IV method. Then, the analyses of variance for an incomplete diallel cross design using Griffing's fixed model in the fourth method were done. Statistical analysis was performed using algorithms developed by Garretsen and Keuls (1978) and Mądry and Ubysz-Borucka (1982). The GCA and SCA effects for the traits were estimated and analyses of their significance were done using the analyses of variance. A detailed analysis of the significance of the GCA and SCA effects was carried out using a procedure based on the Bonferroni's inequality (Garretsen and Keuls, 1978).

The analysis of variance showed, that GCA effects for both plant vigour and plant susceptibility to *V. dahliae* were highly significant (at the $\alpha<0,01$ level). SCA effects played an important role in affecting the plant vigour; they were significant at the $\alpha<0,05$ level. SCA effects for the plant susceptibility to *V. dahliae* were not significant. These results suggest that predominantly additive effects are involved in the inheritance of the susceptibility or resistance to *V. dahliae*. Both additive and non-additive effects played an important role in the inheritance of plant vigour.

The highest significantly positive GCA effects for the plant vigour and the lowest significantly negative GCA effects for the susceptibility to *V. dahliae* had 'Senga Sengana' and 'Salut'. In contrast, the lowest significantly negative GCA effect for the plant vigour and the highest significantly positive GCA effect for the susceptibility to V. dahliae were detected for 'Honeoye' cultivar.

Estimates of SCA effects for the parental pairs showed that SCA effects did not differ significantly from zero for both evaluated traits.

Results from the one-year studies revealed that two of the six evaluated cultivars - 'Senga Sengana' and 'Salut', as tolerant to *Verticillium* wilt, were the most valuable and promising for the strawberry breeding program aimed to obtain genotypes tolerant to *Verticillium* wilt caused by *Verticillium dahliae*. The least useful for this direction of breeding program was 'Honeoye'.

EFFECT OF ENDOGENOUS MICROBIAL CONTAMINATION ON GROWTH AND REGENERATION ABILITY OF *RUBUS IDAEUS* L.

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Keywords: Rubus idaeus, microbial contamination

An endogenous microbial contamination is a significantly damaging problem in plant cultivation and propagation *in vitro*. In our experiments *Rubus idaeus* cultures were established from apical and axillary buds of mature plants growing in orchard, with the purpose to optimize *in vitro* regeneration/multiplication system as a prerequisity for the successful application of biotechnological methods and for the large-scale superior quality plant production. After many months of *in vitro* cultivation the sudden bacterial contamination was detected in some culture dishes, when temperature of culture room accidentally increased to 32-34° C. After this event, the testing of all *in vitro* cultures was realised by cultivation on MS multiplication medium with addition of yeast extract (88 $mg.l^{-1}$) and bacto-peptone (265 $mg.l^{-1}$), with aim to detect undesirable latent endogenous contamination. The cultures were cultivated 10 days on the mentioned medium. After 10 days cultivation, visible bacterial contamination was observed on the media in contact with the shoots. During our previous experiments we have found phytophatogenic bacteria *Pseudomonas sp.* in plum explants, which were collected from mature trees at the Research Institute of Fruit and Decorative Trees in Bojnice, what is also a source of *Rubus* experimental material. From literature the data are available, that this pathogen has been isolated from the water source used for crop watering, but it may also exist in soil per se or as part of the rhizoplane flora. Microbiological testing of *Rubus* cultures confirmed occurrence of above mentioned bacteria and also bacteria *Erwinia sp*. For elimination of latent bacterial contamination antibiotic treatment was tested. The contaminated cultures were treated by cultivation on MS multiplication medium supplemented with a combination of antibiotics Timentin (125 $mg.l^{-1}$), Streptomycin sulfate (250 $mg.l^{-1}$) and Gentamycin (6.25 $mg.l^{-1}$) during 14 days of culture. After antibiotics treatment the cultures were again cultivated on medium with yeast extract and bacto-peptone to detect remaining bacterial contamination. The results of treatment showed that used antibiotics were effective in elimination of bacterial contamination. During the following cultivation of treated shoots on regular multiplication medium the vigorous, dark green shoots were formed, with high multiplication rate. On the other hand, the untreated shoots grew very slowly, had light green to yellowish colour and no multiplication ability.

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NEW STRAWBERRY VARIETIES FROM ITALIAN BREEDING ACTIVITY

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Keywords: breeding, new varietes, fruit quality, disease resistance

The Italian public strawberry breeding program started 35 years ago and now the major objective is to produce new and improved dessert cultivars that are well adapted to three wide Italian areas (southern regions, the Po Valley and northern mountain areas) characterised by different environmental conditions and management techniques. Breeding objectives reflect current strawberry industry trends and are specific for each growing area, but the improved of the quality of the fruit is a common objective to the all areas. A good quality fruit has to have a good taste, high sweetness and acidity, high firmness, a big size, with long conical regular shape, a light red skin colour, uniform and steady until the eating time. In the last ten years one major aim of the breeding programs has been to extende the season of production using both Short-Day and Day-Neutral types, expecially in relation to the fall culture in the northern areas, i.e. a double (autumn-spring) cropping system from a single planting.

Eight new cultivars have been released in 2003 and 2004: Dora, Eva, Irma and Queen Elisa (patented by CRPV-Cesena) for the Po Valley conditions; Adria and Sveva (patented by University of Marche - Ancona) for mid Adriatic conditions; Demetra and Rubea for the South.

The last results of this activities carried out in the North Italy will be reported and 2 new varieties (Record and Sugar Lia) will be patented in UE by CRPV in 2006.

Record

A late season, short-day cultivar adapted to the Northern environmental conditions. Origin: in Cesena (Italy) from the public breeding activity of the Italian National Project “Frutticoltura”, mainly financed by the Ministry of Agriculture; developed by W. Faedi and G. Baruzzi (CRA-Istituto Sperimentale per la Frutticoltura–Forlì Section), M. Baudino and R. Giordano (Consorzio di Ricerca Sperimentazione e Divulgazione per l’Ortofrutticoltura Piemontese - Cuneo), P. Lucchi (Centro Ricerche Produzioni Vegetali - Cesena). Idea x Marmolada; cross made in 1992 in Cesena; selected in Cesena in 1994; tested as 92.340.3; issued in 2005; assigned to CRPV in Europe. Fruit: large, conic regular shape; medium-firm skin, very bright orange-red color; medium firm flesh, medium good organoleptic characteristics, high ascorbic acid contents. Plant: vigorous, good yield, well adapted to organic colture and poor soils, also not fumigated; tolerant to *Colletotrichum acutatum, Sphaerotheca macularis* and soil-borne pathogens; susceptible to *Diplocarpon earliana* and *Xanthomonas fragariae*.

Sugar Lia

An early season, short-day cultivar adapted to the Northern environmental conditions. Origin: in Cesena (Italy) from the public breeding activity of the Italian National Project “Frutticoltura”, mainly financed by the Ministry of Agriculture; developed by W. Faedi and G. Baruzzi (CRA-Istituto Sperimentale per la Frutticoltura - Forlì Section), M. Baudino and R. Giordano (Consorzio di Ricerca Sperimentazione e Divulgazione per l’Ortofrutticoltura Piemontese – Cuneo), P. Lucchi (Centro Ricerche Produzioni Vegetali - Cesena). Sel. 91.143.5 (a complex pedigree including Cortina, Cardinal, Belrubi, Holiday, Addie) x Miss; cross made in 1996 in Cesena; selected in Cesena in 1998; tested as 96.62.10; issued in 2005; assigned to CRPV in Europe. Fruit: medium-large, conic regular shape; very firm skin, bright red colour; firm flesh, high sugar content, hardly aromatic; medium-high ascorbic acid contents. Plant: medium vigorous; productive but it needs an early planting date for high yield on fumigated fertile soils; medium susceptible to *Alternaria alternata* and *Sphaerotheca macularis*, susceptible to *Colletotrichum acutatum, Xanthomonas fragariae* and *Phytophthora cactorum*.

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR IDENTIFYING GENUS *FRAGARIA* CULTIVARS

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Keywords: SSR, fingerprinting, genetic relationship, genus Fragaria

Microsatellites, or simple sequence repeats (SSRs), are very useful markers for genetic analysis since they are co-dominant, multi-allelic and generally highly polymorphic. In this study, microsatellite markers were produced in strawberry to verify their usefulness in cultivar fingerprinting and linkage map development (James *et al*., 2003). A genomic library, enriched in AC microsatellite type, was developed for the species *Fragaria vesca* var. 'Ilaria'. A total of 54 primer pairs were synthesized and used in the amplification of genomic DNA from *F. vesca* 'Ilaria' and *F.* × *ananassa* 'Queen Elisa'. Twenty-three primers amplified both species, eight amplified only *F. vesc*a, seven gave faint bands in one or both species and 17 gave unexpected band sizes or no amplification (Cipriani and Testolin, 2004).

Eleven pairs of primers were selected and used for cultivar fingerprinting of 10 varieties of the cultivated species *Fragaria* x *ananassa* (Alba, Madeleine, Maya, Patty, Onda, Paros, Roxana, Idea, Miss and Queen Elisa). Amplified SSR products were separated using a MegaBace 500 automatic sequencer and allele sizes were determined using the Fragment Profiler 1.0 software package and the results showed a high level of polymorphism among the analyzed cultivars. The microsatellite UDF-005 was the most informative among those tested because it produced a different allelic profile for each variety. Miss and Queen Elisa showed similar plant morphology that could lead to incorrect variety identification. However, the microsatellite analysis easily discriminate the two varieties, with 10 of the 11 primers pairs revealing allelic differences in the two genotypes.

Twenty-five primers were used in *Fragaria vesca* fingerprinting to discriminate 23 samples among which there were some known relationships. Amplified products were separated in 5% poly-acrylamide gel by electrophoresis and visualized by silver staining. Genetic similarity analysis, by DICE coefficient, was carried out among the samples of *Fragaria vesca* to verify the relationship. Genetic similarity analysis represents a precise mirror of the accession's pedigree.

These results confirm the microsatellite's features that make them powerful tools for fingerprinting analysis.

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