Kirsikoiden geenivarakokoelman valinta fenotyyppisten havaintojen ja DNA-markkerien avulla

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Tiivistelmä

MTT:lle sijoitettu kansallinen kirsikkakokoelma on syntynyt 1980- luvun luumu- ja kirsikkakeräysten tuloksena. Hapankirsikat istutettiin alun perin Hämeen tutkimusaseman koekentälle Pälkäneelle ja Ahvenanmaan tutkimusasemalle Jomalaan, jonne sijoitettiin myös makeita kirsikoita. Kaikki Hämeen kokoelman kirsikkakloonit on kuvattu 1992 kansainvälisesti hyväksytyillä UPOV:in määrittelemillä havainnointikriteereillä (deskriptorit), jotka liittyvät kasvin ulkomuotoon, kasvutapaan, kestävyyteen ja satoon. Vuonna 2003 osa havainnoista uusittiin niillä puilla, jotka olivat säilyneet siihen asti.

Kesällä 2005 kaikista jäljellä olevista klooneista kerättiin lehtinäytteet mikrosatelliitti-DNA –analyysiin, jossa näytteitä vertailtiin tunnettuihin suomalaisiin hapankirsikkalajikkeisiin ja paikalliskantoihin. Lisäksi puista tehtiin satohavaintoja, ja valittiin kasvutavan ja sadon puolesta lupaavimmalta vaikuttavat kannat. Hedelmän ja sen mehun värin sekä yleisen kasvutavan perusteella kokoelman puut jaettiin alun perin kolmeen ryhmään: 1) Amarelli –tyyppi, 2) Morelli –tyyppi ja 3) Rymättylä –tyyppi.

DNA-markkerianalyysin perusteella Amerellit ja Morellit erottuivat hyvin selkeästi omiksi ryhmikseen. Molemmille ryhmille löytyi useita ainoastaan sille ominaisia DNA-merkkejä. Rymättylä –tyyppiä edustavia näytteitä oli jäljellä enää kaksi, ja ne ryhmittyivät molemmat yhteen Amarellien kanssa, joka tulos oli yhdenmukainen v.1993 havaintojen kanssa. DNA –merkkien perusteella 72 tutkittavasta näytteestä löydettiin yhteensä 28 erilaista genotyyppiä eli erillistä kantaa. Sekä Amarellien että Morellien joukossa oli yksi suuri klooniryhmä, johon kuului 19 tai 20 täysin identtistä näytettä. Näiden voidaan olettaa kuuluvan samaan lajikkeeseen tai paikalliskantaan, jota hyvin menestyneenä on levitetty kasvullisesti lisäämällä alan harrastajien keskuudessa. Näiden, kuten myös pienempien klooniryhmien, keräyspaikat olivat yleensä melko lähellä toisiaan.

Geenivarakokoelmaan valittiin DNA –analyysin tulosten perusteelle mahdollisimman suurta muuntelua edustavat kirsikkanäytteet. Näiden joukosta tarkistettiin, että mukaan tulivat kenttähavaintojen perusteella kaikkein lupaavimmat näytteet. Valintaperusteissa tärkeimpinä tekijöinä olivat sadon laatu ja määrä sekä puun koristearvo. Valitut kannat otettiin MTT Laukaalle lisäykseen, ja uudet taimet istutettiin v.2003-2004 osaksi Piikkiön Tuorlan kansallista geenipankkikokoelmaa.

Results

In the data clusteranalysis Amarelle and Morello types were separated into two distinct groups 1-2 (Figure 2 in the poster). There was two large clone groups (one in the amarelle and one in the morello –group). The internal variation of the characteristics gave proofs of the similiarity of the clones inside the two groups . The morello group can be described with certain characteristics typical to these mostly from eastern and south-eastern Finland collected clones (Figure 1 MAP in the poster).

The cluster analyses made in this examination showed that the earlier in 1993 done research work to classify cherry clones according to phenological observations, fit very well together with this exam. The total amount of the clones were grouped generally in the same way in the cluster analyses than in 1993, when the grouping had its background in all the observations already made since that time. There was only one clone that seemed to not be easily categorized to those main groups, into the morellos or the amarelles. These clones represented mainly some kind of intermediates in the clone population . Prunus 010 (from Rymättylä) seemed to be a clear amarelle. Earlier it was considered as an own Rymättylä type together with Prunus clone 37 (from Turku) (PALONEN et al. 1998).

In both amarelle and morelle clusters there were two large internal groups which were very uniform. For example the fruit colour varied only quite little inside the largest amarelle and morello groups (Figure 2 Cluster tree in the poster). In the morello group A the fruit colour mean was 8,86 (Std Dev 0,351, N=15). If all morellos were included the mean value was 8,45. In the largest amarelle group the colour was lighter red 3,80 (Std Dev 0,918 N=10) and the colour of all amarelles was 4,17. (Table 2 and 3 and the DNA cluster tree in the poster).

As earlier told the most common qualities were the red- or dark-red colour of the fruit of morellos compared to amarelles (Pr >0,0001***, Table 2). Fruit flesh was in addition significantly darker in the morello group (Pr> 0,0001***, Table 2). Also morello fruit stalk was significantly longer than the stalk of amarelle berries (Pr >0,0001***, Table 3), but dealing with the diameter of fruit the significant difference between the two groups was quite small (Pr >0,0374*, Table 3). The amarelle and morello cherries had equal fruit weight, however (Table 3). Visually both the morello fruit stalk and the tree habitus itself was pendular or drooping. The size of flowers was bigger in amarelle group (Pr>0,0001***, Table 2) and the colour of leaf upsurface was stronger green and the leaf size was larger in the amarelle group (Pr>0,0001***, Table 2). The majority of these measurements were done in 1992 and 1993.

The healthy of the trees and the stem diameters were estimated and measured in 2005, when the trees had achieved their final size, as earlier told. The two cherry clone groups differed from each other concearning with the healthy of the trees but not in the stem diameters, however (Pr>0,0001*** and Pr > 0,075 Tables 2 and 3).

Table1. The scale of non parametric observations in the Prunus cerasus UPOV descriptions. The parametric observations were fruit diameter(mm), stem diameter (cm) , length of stalk (mm) and fruit weight (g).

Parametric	characters				
Fruit colour	1=yellow	2=orange-yellow	3=red on pale yellow ground	4=red	5=purple
Taste	1=very acid	2=acid	3=intermediate	4=sweet	5=very sweet
Flesh colour	1=whitish	2=pink	3=red	4=purple	
Healthy	1=badly damaged	10= healthy			
Flower size	3=small	5=intermediate	7=extra large		
Leaf size	3=small	5=intermediate	7=large		
Colour of leaf upsurface	3=pale green	5=intermediate green	7=dark green		

Table 2 . The non parametric characters observed and the statistically significant differences between the morello and amarelle groups of the cherries with t-test and P values together with median, minimum, maximum and df values.

Group	**Median**	**Min**	**Max**	**df**	**t value**	**P**
	Fruit colour					
Morellos	9	4	9	7	11,6	0,0001
Amarelles	4	1	9	17	8,7	0,0001
	Fruit flesh					
Morellos	4	2	4	7	13	0,0001
Amarelles	1	1	4	17	6,82	0,0001
	Healthy					
Morellos	6	2	9	7	10,21	0,001
Amarelles	7	5	9	18	12,27	0,001
	Flower size					
Morellos	3	3	3	2	.	.
Amarelles	2	5	4,5	8	10,36	0,0001
	Leaf size					
Morellos	1,5	1	3	6	5	0,0041
Amarelles	1	1	5	16	5,96	0,0001
	Leaf upsurface colour					
Morellos	6	5	7	6	20,07	0,0001
Amarelles	5	2	7	15	14,7	0,0001

Table 3. The parametric characters observed and the statistically significant differences between the morello and amarelle groups of the cherries.

Characters	Morellos Mean		Amarelles Mean		Morellos Std Dev	Amarelles Std Dev	Morellos DF	Amarelles DF	F-arvo	Pr>(t)
Fruit diameter, mm	12,8	a	13,5	b	1,507	1,225	49	25	1,8	0,0374*
Stem diameter, cm	12,1	a	10,4	a	4,098	3,5105	55	25	1,36	0,0775
Lenght of stalk, mm	38,4	a	32,4	b	4,1334	4,9149	30	25	1,76	0,0001***
Fruit weight, g	2,3	a	2,42	a	0,3847	0,4003	34	24	1,08	0,458

Microsatellite markers revealed 28 genotypes among the 72 studied accessions. In the data analysis Amarelle and Morello types were clearly separated into two distinct groups (Figure in the poster). Only one accession, PR301 a cherry of unknown origin, was placed distinctly outside these two groups. Two large clones, consisting of 19 and 20 accessions respectively, were detected, one in the Amarelle and one in the Morello –group. Some other duplications and smaller clones were also found. These clones were giver code numbers in order to recognize them in further comparisons with the phonological observations. Few accessions were nearly similar to another accession, separated by only one marker.

The examined nine microsatellite primer pairs produced 72 alleles altogether. Four of these markers were almost monomophic, i.e. present in all except one genotype. 42 markers (58%) had frequency >0,20, and 24 of these (33% of all) frequency ≥0,50, i.e. 2-4 alleles for each microsatellite were present in at least half of the genotypes. 21 markers (29%) were rare, occurring only in one genotype, with frequency of <0,05. Some markers were very tightly specific to either the Amarelle or the Morello –group, existing in all or nearly all accessions of the group, but in none of the accession of the other group. Numbers of this kind of group specific markers were 14 and 13 respectively. Summary of the results from different SSR loci is presented in table YY.

Table 4. Summary of SSR marker results

	Size range	No. of allels	No. of Amarelle spesific	No. of Morello specific
BPPCT002	168-182	3	0	1
BPPCT005	129-164	6	1	0
BPPCT007	114-185	7	1	0
BPPCT026	137-189	9	3	3
BPPCT034	207-241	7	1	2
BPPCT037	129-165	16	2	1
BPPCT038	98-132	7	1	1
BPPCT039	128-150	10	2	2
BPPCT040	121-145	7	3	3
Total		72	14	13