Methods of *in vitro* storage of hops.

VÚRV PIEŠŤANY

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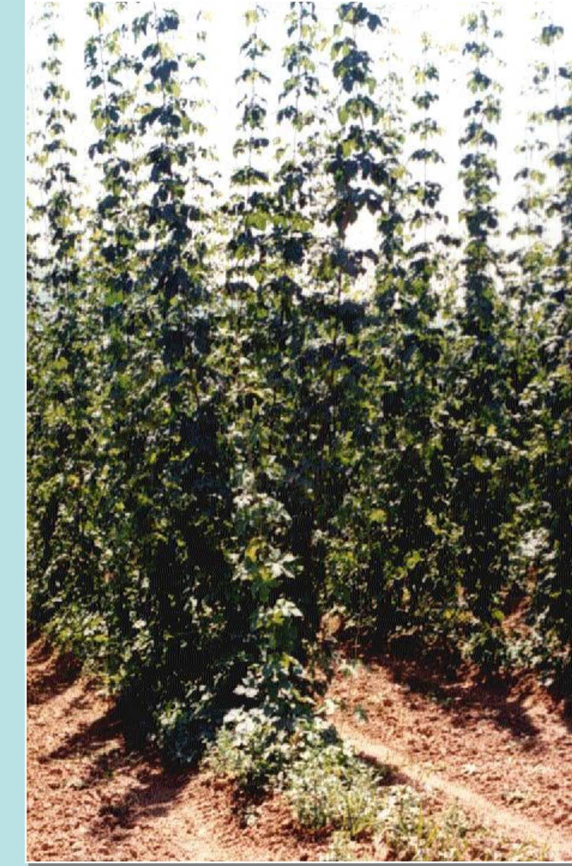
Hop (*Humulus lupulus* L.)

Kingdom:
Plants (*Plantae*)
Subkingdom:
Higher plants (*Telomophytae*)
Phyllum:
Vascular plants (*Tracheophyta)*
Class:
Angiosperms (*Magnoliopsida*)
Order:
Nettles (*Urticales*)
Family:
Hemp (*Cannabaceae*)
Genus:
hop (*Humulus* Linnaeus, sp.)
Species:
Common hop (*Humulus lupulus* L.)
Japanese hop (*Humulus japonicus*)

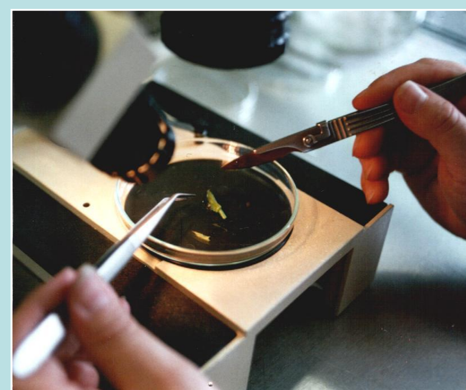
- Perennial herbaceous climbing plant
- stems annual, slender, climbing, up to 9 m in length
- Dioecious with unisexual flowers (male and female flowers develop on different individuals)
- Chromosome number: 2n = 2x = 20
- In hop gardens only female plants are grown
- Important technical crop: the main harvested products are female inflorescences (cones)
- Grown on one stand up to 10-15 years (wild hops can live up to 100 years)
- Commercial propagation exclusively vegetatively (shoot-cuttings, root-cuttings, rootstocks)
- Cones used in brewing industry – α- and β -bitter acids

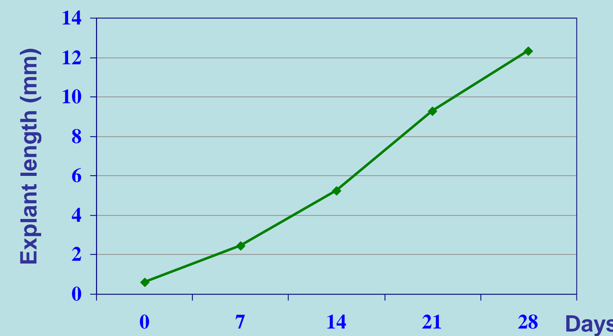


Meristem culture

Meristem-derived explant:
Size: 0,2 – 0,6 mm
Morphology: apical dome + 1-2 leaf primordia

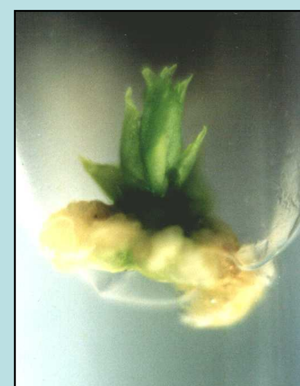
Isolation of apical meristem and establishment of meristem culture





Growth of meristem explants of hop in *in vitro* culture during the cultivation on initiation medium MSC

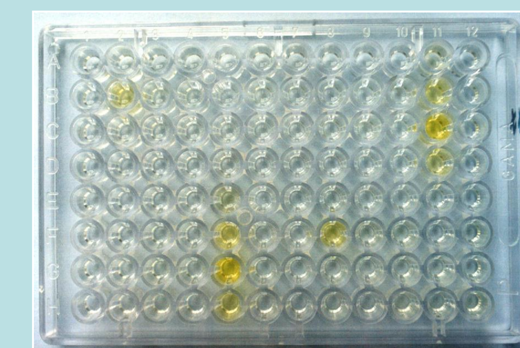
week 0



week 2

week 5

ELISA testing and micropropagation of virus free plants

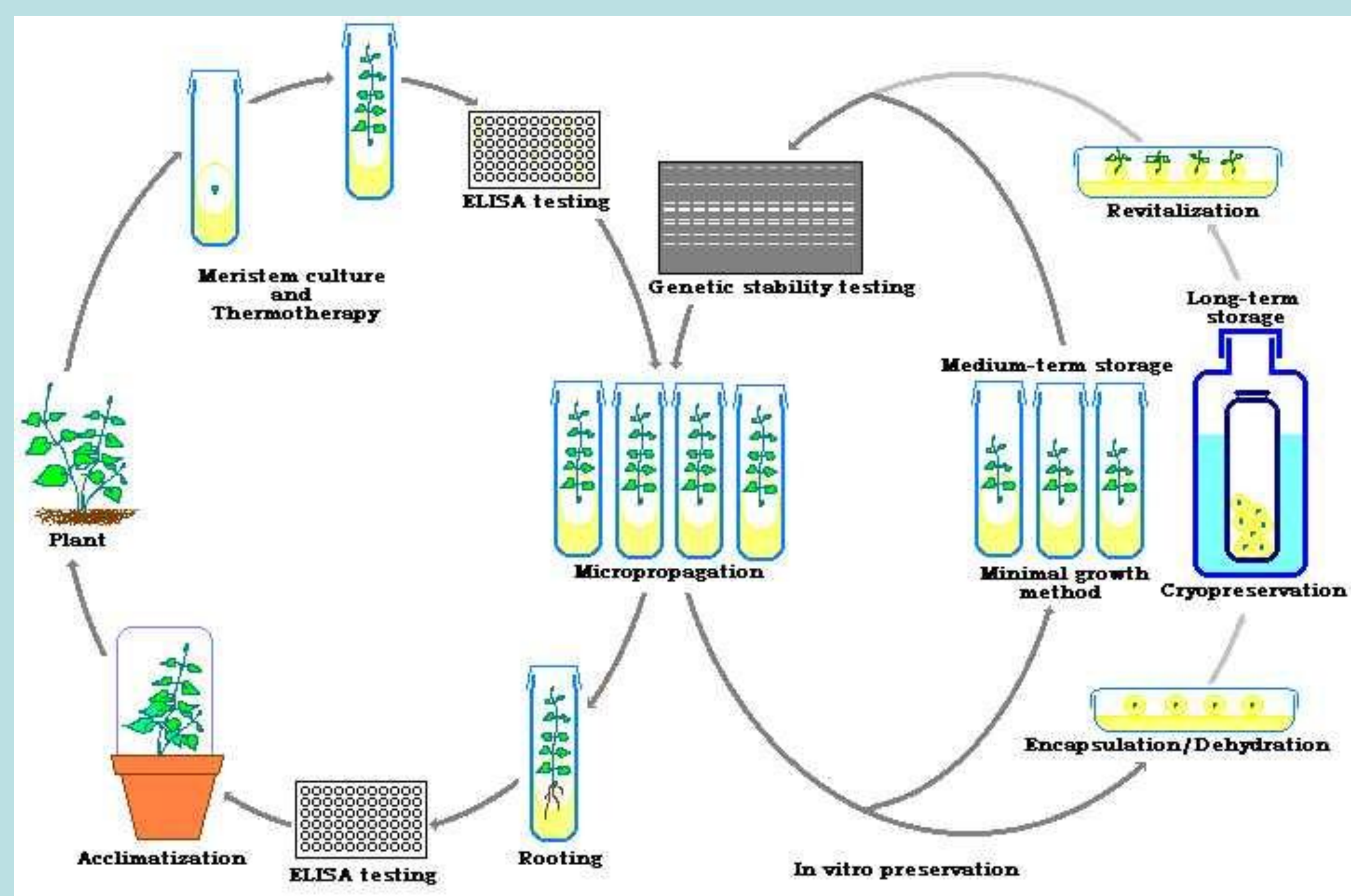


ELISA testing of meristem culture-derived shoots of hop for presence of viruses

Micropropagation of ELISA tested virus-free plants by culture of nodal explants

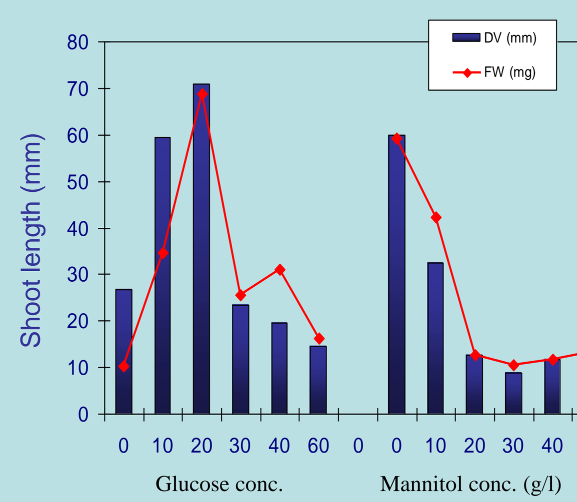
In vitro storage

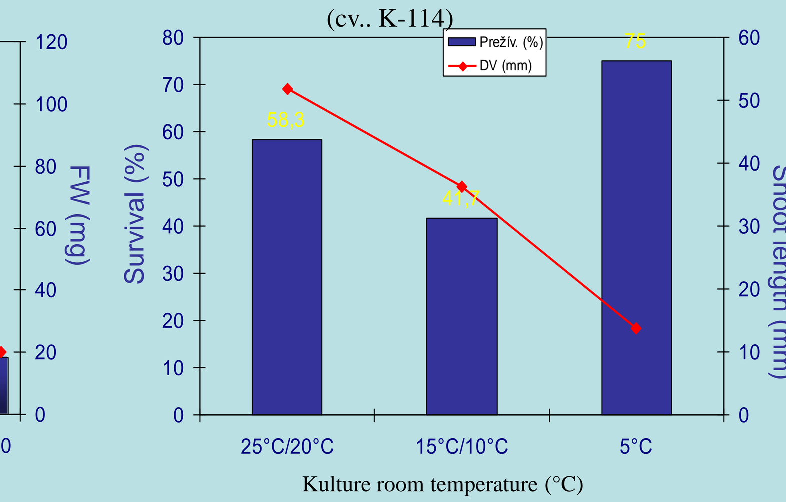
The system of *in vitro* storage of hops at RIPP Piešťany



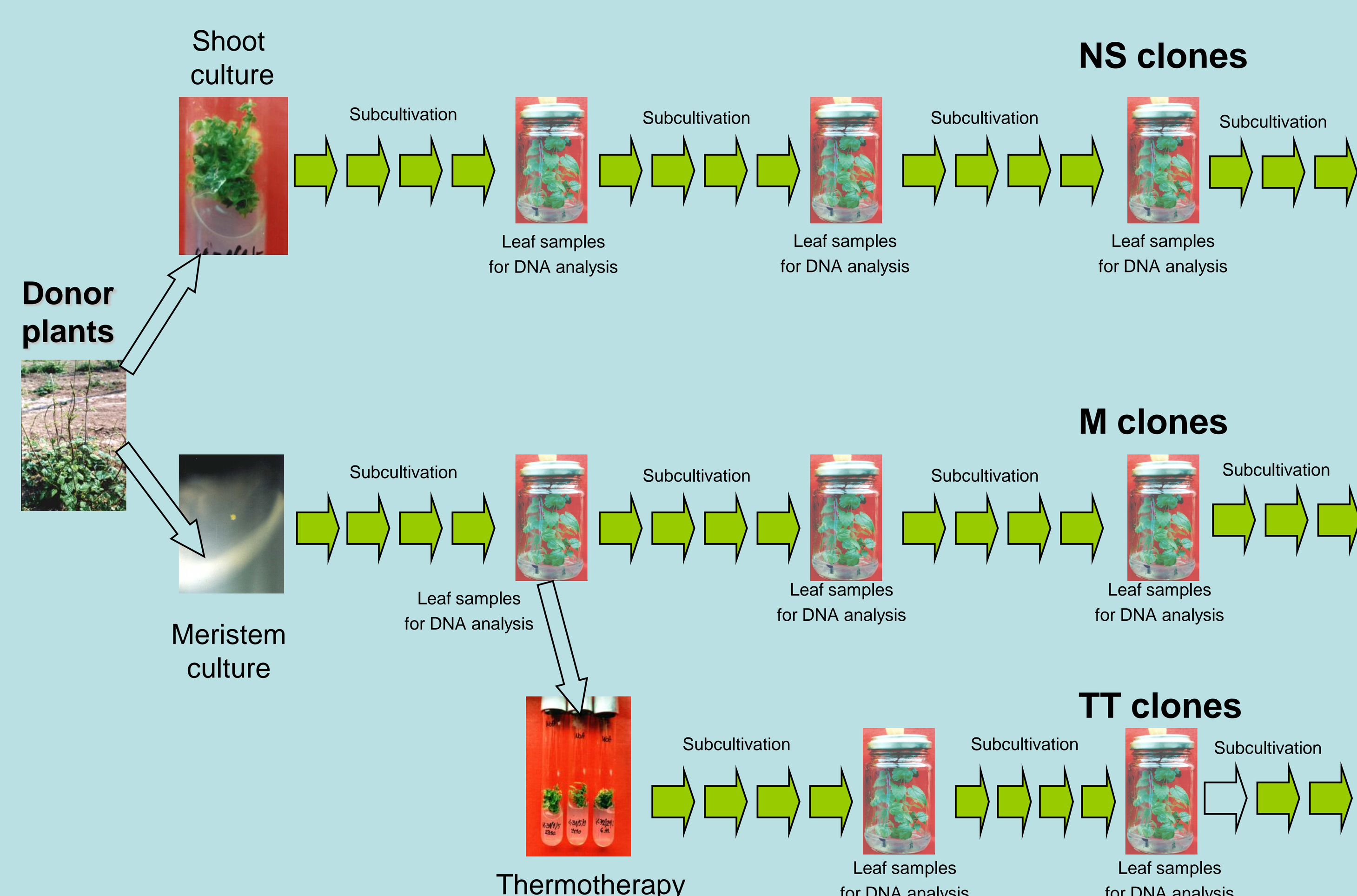
Optimization of *in vitro* storage system

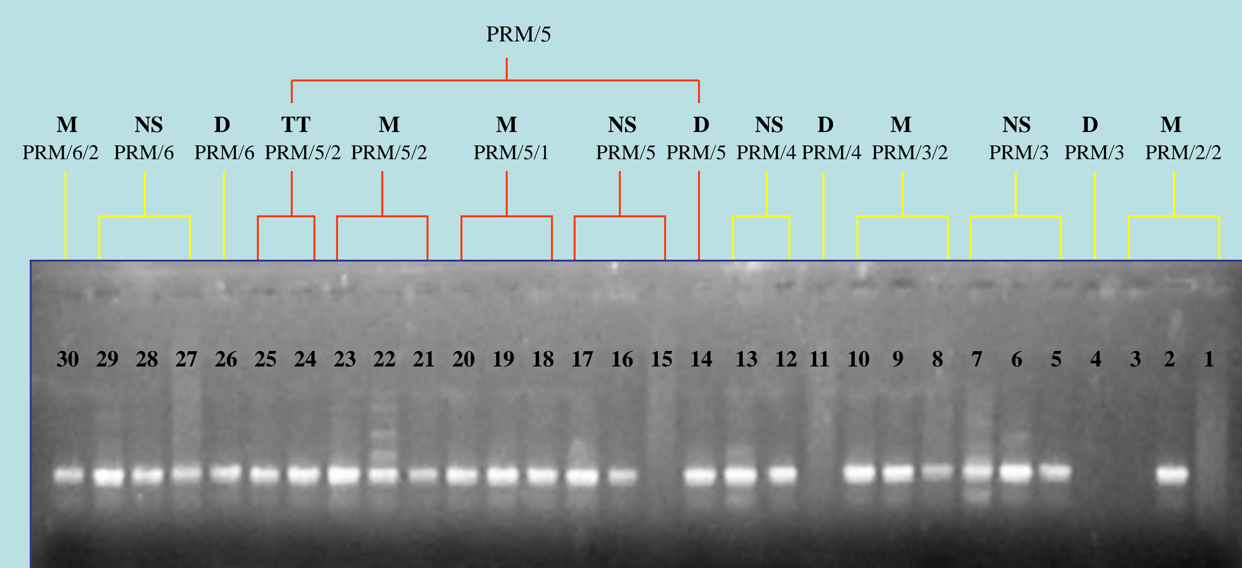
Minimal growth method (MGM)
- appropriate pre-culture
- appropriate explant preparation
- modification of culture medium composition (minerals, glucose, solidifying agent)
- modification of culture conditions (culture vessel, temperature, illumination)
- addition of growth retardants (mannitol, sorbitol, ancymidol, paclobutrazol)





Genetic stability of *in vitro* stored material





PCR analysis of microsatellite sequences of hop cv. Premiant using the primer HBV5. For amplification of DNA regions containing microsatellite sequences the primer HBV5 was used and the PCR reaction was performed in 20 µl volumes containing 10mM Tris-HCl (pH = 8.3), 50mM KCl, 1.5 mM $MgCl_2$, 0,25 mM dNTP, 1mM primer, 0,75 U *Taq*-DNA polymerase and 25 ng DNA. The initial denaturation was done for 2 minutes at 94 C followed by 40 cycles of 1 min. at 94 C, 1 min. at 48 C and 3 min. at temperature 72 C. The last step was 7 min. at temperature 72 C. Amplified products were separated electrophoretically in 1.5% agaróse gel in 0.5 x TBE buffer and stained with ethidium bromide. We analyzed 30 clones: 1 = PRM/2/2 (M,V4); 2 = PRM/2/2 (M, V8); 3 = PRM/2/2 (M, V12); 4 = PRM/3 (D, V0); 5 = PRM/3 (NS, V4); 6 = PRM/3 (NS, V8); 7 = PRM/3 (NS, V12); 8 = PRM/3/2 (M, V4); 9 = PRM/3/2 (M, V8); 10 = PRM/3/2 (M, V12); 11 = PRM/4 (D, V0); 12 = PRM/4 (NS, V4); 13 = PRM/4 (NS, V12); 14 = PRM/5 (D, V0); 15 = PRM/5 (NS, V4); 16 = PRM/5 (NS, V8); 17 = PRM/5 (NS, V12); 18 = PRM/5/1 (M, V4); 19 = PRM/5/1 (M, V8); 20 = PRM/5/1 (M, V12); 21 = PRM/5/2 (M, V4); 22 = PRM/5/2 (M, V8); 23 = PRM/5/2 (M, V12); 24 = PRM/5/2 (TT, $V_5$0); 25 = PRM/5/2 (TT, V4); 26 = PRM/6 (D, V0); 27 = PRM/6 (NS, V4); 28 = PRM/6 (NS, V8); 29 = PRM/6 (NS, V12); 30 = PRM/6/2 (M, V4). PRM = cv. Premiant; D = donor (field grown) plants; NS = clones derived from nodal segments, M = mericlones; TT = mericlones subjected to *in vitro* thermotherapy; V0 = leaf sample from field-grown plants (control 1); V4, V8, V12 = leaf samples from *in vitro* plants after 4, 8 and 12 subcultivations onto medium MSW_0Ph; $V_5$0 = leaf sample from M plants before *in vitro* thermotherapy. In the columns 1, 3, 4, 11 a 15 there are no amplified products, probably due to lower quality of isolated DNA.

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