CRYOPRESERVATION OF HOP GERMPLASM IN THE CZECH REPUBLIC

Petr Svoboda[1], Miloš Faltus[2], Ivana Malířová[1]

[1]Hop Research Institute, Kadanska 2525, CZ43846 Zatec, Czech Republic

[2]Crop Research Institute, Drnovska 507, CZ16106 Prague, Czech Republic

INTRODUCTION

Hops breeding, growing and utilization has been traditional and important in the Czech Republic from the middle age. Nowadays hop collection includes 391 accessions placed at the Hop Research Institute (HRI) in Zatec in field conditions but they are endangered by biotic and abiotic stressors.

OBJECTIVE

The objective of this study was to verify the possibility to use simple method for cryopreservation of hop germplasm.

MATERIALS AND METHODS

Plant material
- 42 cultivars of species *Humulus lupulus* L., and one clone of *Humulus japonicus* Sieb. et Zucc.

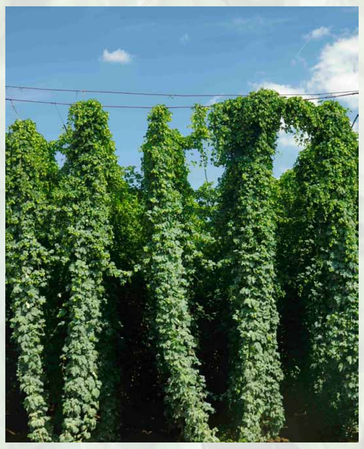
Virus detection and eradication
- in vitro cultures were **heat-treated** to eliminate viral pathogens (Svoboda 2002)
- **virus determination** was carried out by DAS - ELISA method according to Clark and Adams (1977)

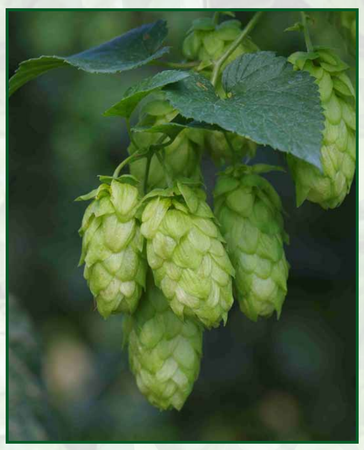
Cultivation conditions
- in vitro cultures were grown at 25 ± 2°C and photoperiod 16/8 h (80 µmol m^{-2} s^{-1}) (Svoboda, 1992) on **MS medium** without phytohormones with sequestrene iron 138 (EDDTA chelated) (Aynalem et al., 2006, Reed and Aynalem, 2003)

Explants acclimation
- nodal cuttings were transferred on modified MS medium (Faltus et al., 2007) for 7-10 days
- cultivation temperature was decreased to 2 ± 1 °C for **cold acclimation** for next 7-10 days
- 0.7 M sucrose solution was added into containers with explants for **osmotic treatment** for next 7-10 days at low temperature

Cryopreservation
- shoot tips (1-2 mm) were dissected and transferred into Petri dishes with filter paper and **loaded** by 0.7 M sucrose and phytohormones for overnight
- shoot tips were transferred onto aluminium foils and **dehydrated** above silicagel for 1.5 – 1.75 h
- shoot tips were **cryopreserved** by plunging aluminium foils into liquid nitrogen

Explant recovery
- shoot tips were **thawed** in water bath at 40 °C
- shoot tip r**egeneration** was performed on recovery medium (Faltus et al., 2007)
- survival and recovery were evaluated 2 and 8 weeks after thawing, respectively
- the minimal number of plants to recover from the cryobank sample was calculated by method described by Dussert et al. (2003)





RESULTS

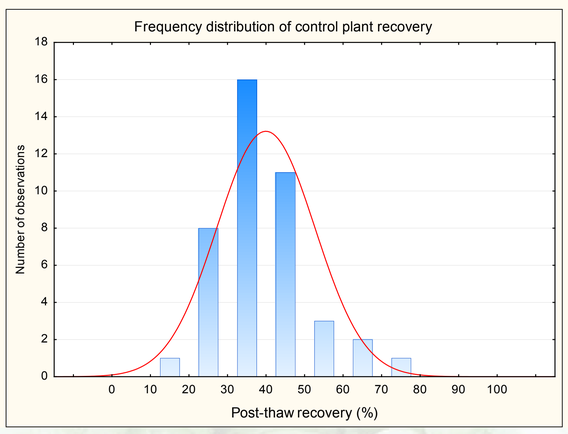
Explants recovery after cryopreservation
- the cryopreservation procedure was tested for cryopreservation of 42 hop genotypes (Fig. 1)
- average plant recovery after cryopreservation was 40%
- 79% of accession showed higher plant recovery than 30%
- the minimal number of plants to recover for each cultivar

(Table 1) was calculated as a sum of minimal numbers of viable plants in particular cryopreservation procedures
- altogether of 7720 apical shoot tip meristems were stored in cryobank

Table 1. Cryopreserved cultivars of Humulus lupulus and a clone of Humulus. japonicus from the Czech hop germplasm collection including Unique Accession Number within national inventory, number of stored shoot tips, average post-thaw recovery and minimal number of viable plants stored in cryobank.

No.	Accession name	Plant ID	„No. of cryopreserved meristems„	„Average recovery in control sample„	„Minimal number of plants to recover with 95 % propability„
1	Osvalduv klon 31	08X9000005	540	31	88
2	Osvalduv klon 72	08X9000008	380	28	40
3	Osvalduv klon 136b	08X9000014	120	28	12
4	Saladin	08X9000031	120	33	16
5	Opolsky	08X9000032	120	27	13
6	Volynsky	08X9000033	120	23	8
7	28-30 Polsko	08X9000041	120	33	16
8	CZ-K26 Polsko	08X9000043	120	45	27
9	Cocnean	08X9000046	120	45	27
10	Petrovacky polorany cervenak	08X9000048	60	33	16
11	Spalt	08X9000052	120	63	46
12	Hersbruck	08X9000053	120	18	5
13	Kent	08X9000069	120	75	52
14	Jakima	08X9000096	240	44	70
15	Malling	08X9000146	120	25	6
16	Willamette	08X9000181	120	35	21
17	Sladek	08X9000206	320	38	39
18	Podlesak	08X9000208	120	45	35
19	Bor	08X9000211	100	36	14
20	Galena	08X9000223	600	43	155
21	Comet	08X9000227	140	35	28
22	Spalter Select	08X9000228	120	50	32
23	Sara	08X9000236	160	23	16
24	Blsanka	08X9000245	120	54	44
25	Premiant	08X9000246	240	33	32
26	White Bine	08X9000260	180	39	27
27	Avans	08X9000266	120	38	24
28	Harmonie	08X9000295	280	34	49
29	Merkur	08X9000304	120	45	30
30	Columbus	08X9000306	120	49	39
31	Outeniqua	08X9000307	120	36	25
32	First Gold	08X9000313	120	25	12
33	Southern Star	08X9000314	340	58	90
34	Southern Promise	08X9000314	180	47	44
35	clone 12269 (H. japonicus)	-	120	38	20
36	Petham Golding	08X9000133	320	22	30
37	Taurus	08X9000274	120	70	55
38	Agnus	08X9000299	220	55	73
39	Hallertauer Gold	08X9000185	260	37	53
40	Strieselspalt	08X9000055	180	50	51
41	Centential	08X9000272	120	36	19
42	Rubín	08X9000297	220	45	54
	Total		**7720**	**40**	**1553**

Fig. 1. Frequency distribution of mean post-thaw recovery of 42 cryopreserved hop accessions. Each observation represents average recovery of one unique genotype. Red line means normal distribution.



CONCLUSIONS
- simple method for cryopreservation of hop germplasm was verified
- average plant recovery after cryopreservation was 40%
- 79% of accessions showed higher plant recovery than 30%
- 42 hop genotypes of hop was successfully stored in the Czech Plant Cryobank at the Crop Research Institute in Prague and they represent backup collection for the base collection located in Hop Research Institute in Zatec

HOP EXPLANTS REGENERATION, CV. COLUMBUS

AFTER DEHYDRATION

AFTER CRYOPRESERVATION

ACKNOWLEDGEMENT

This study was supported by the research project QJ1630301 of the Ministry of Agriculture of the Czech Republic.

LITERATURE CITED:

Aynalem, H., Righetti, T., Reed, B.M. 2006. In Vitro Cellular and Developmental Biology - Plants. 42:405-410.
Clarck, M. F. and Adams, A. N. 1977. In: J. genet. Virol., vol. 34:474–483.
Dussert, S., Engelmann, F. and Noirot, M. 2003. CryoLetters., 24:149-60.
Faltus, M., Bilavcik, A., Zamecnik, J. and Svoboda, P. 2007. Advances in Horticultural Science, 21:219-224.
Jakse, J., Bandelj, D. and Javornik, B. 2002. Mol Ecol Notes 2:544-546.
Reed, B.M. and Aynalem, H.A. 2005. Acta Horticulturae. 668: 257-262.
Svoboda, P.: Pl. Prod. 37, 1991 (8):643 - 648.
Svoboda, P.: Pl. Prod., 38, 1992 (6):523 – 528.
Stajner, N., Jakse J., Kozjak, P. and Javornik B. 2005. Plant Sci 168: 213-221.