# Assessment of somaclonal variability in hop *in vitro* culture by molecular methods

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## INTRODUCTION:

Meristem *in vitro* culture belongs to wide used technologies of plant tissue cultures. But utilization of plant tissue culture is associated with the occurrence of somaclonal variability, which was first defined by Larkin and Scowcroft (1981). Use of meristem tip culture is considered as method with less prone to the risk of genetic instability (Nehra and Kartha, 1994). In spite of this fact, some phenotypical differences were found in clonal population of virus-free hop meristem derived plant in hop gardens. Molecular analysis of somaclonal variation provides an opportunity to eliminate an influence of environmental factors and to provide a quantitative measure of somaclonal variability (Veilleux and Johnson, 1998). Different molecular techniques can be employed to detect somaclonal variability (Henry, 1998).

The development of DNA technology has provided a number of methods to detect the differences at DNA sequence level. In my experiments, I used different molecular methods (RFLP, STS, RAPD, ISSR, AFLP) for assessment of somaclonal variability in hop meristem tip culture. I aimed to evaluate molecular genetic stability/variability of derived meristem *in vitro* clones from different hop genotypes and verificate their identity/diversity with original hop genotypes.

## MATERIAL AND METHODS:

*Plant material and DNA isolation*

For experiments were used 12 and 11 meristem derived in vitro clones from two plants of cv. Yeoman (UK), 5 meristem derived in vitro clones from one plant of cv. Southern Brewer (South Africa), 5 meristem derived in vitro clones from one plant of cv. Eroica (US), 5 meristem derived in vitro clones from one plant of cv. Premiant (CR), 3 meristem derived in vitro clones from one plant of cv. Galena (US) and 3 meristem derived in vitro clones from one plant of Osvald's clone 72. DNA was isolated from in vitro plants and young leaves of original plants by Saghai-Maroof et al. (1984) modified by Patzak et al. (1999).

*Restriction Fragment Lenght Polymorphism (RFLP)*

Samples of genomic DNAs were digested with HindIII, loaded to 0,8% agarose gels and alkaline blotted to positively charged nylon membranes after electrophoresis (Southern, 1975). Molecular hybridization was performed according to Matoušek et al. (2000) with radioactive labeled probes for hop chalcone synthase (Matoušek et al., 2000) and hop valerophenone synthase (Okada, 1998).

*Sequence-Tagged Sites (STS)*

STS analyses were performed according to Brady et al. (1996) for alleles 11a59 and 3a88, Tsuchiya et al. (1997) for loci B72WF2/R2 and 193F3/R3, Araki et al (1998) for primer combination I., and Murakami (1998) for primer combinations no. 1, 2, 3, 4 and 5.

*Random Amplified Polymorfic DNA (RAPD)*

RAPD analyses were performed according to Patzak et al. (1999). Thirty two RAPD decamer oligonucletide primers were used for RAPD analysis according to Patzak (2001).

*Inter-Simple Sequence Repeat (ISSR)*

ISSR analyses were performed according to Patzak (2001). ISSR reactions were based on seven 17 nt microsatellite sequences as PCR primers and their combinations without anchored sequence on 5' or 3'end.

*Amplified Fragment Length Polymorphism (AFLP)*

AFLP analyses were performed according to Patzak (2001). Five primer combinations, MseI+CAT + EcoRI+ACG, MseI+CTG + EcoRI+ACG, MseI+CTC + EcoRI+ACT, MseI+CAA + EcoRI+ACT and MseI+CTG + EcoRI+AAC, were used for AFLP analyses.

*Genetic diversity analysis*

The cluster analysis was revealed by NTSYS-pc v. 2.02 for WINDOWS (Exeter Software, US). Similarity was estimated using Jaccard's (1908) similarity coefficient (JCS), which ranges from 0 (all fragments between accessions were different) to 1 (all fragments between accessions were identical). The dendrograms were generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering procedure.

## RESULTS AND CONCLUSIONS:

Any differences were not found between mother plants and meristem derived in vitro clones by RFLP analysis of two specific genes, which produced characteristic polymorphism between used cultivars. There is very low chance to find somaclonal variability by RFLP analysis of gene coding regions. It is possible that RFLP analysis of some repetitive sequences could be more advantageous. This method needs considerable amount of DNA and it is also more laborious and time consuming than PCR methods.

Any differences were not also found between mother plants and meristem derived in vitro clones by STS analysis. A chance, that some change occurs in short specific sequence, is very improbable. Even though, this method is very appropriate for DNA fingerprinting of cultivars, it is unsuitable for monitoring of somaclonal variability.

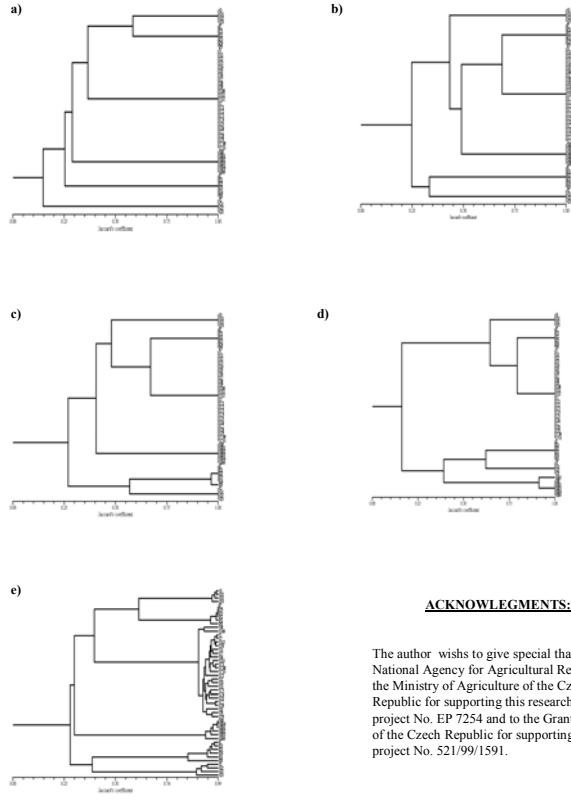
One different meristem derived in vitro clone (E5) was found by RAPD analysis. OPE-03 and OPG-04 primers produced the different RAPD patterns. The frequency of sequence changes of DNA was low and this variability (0.965) did not exceed genetic dissimilarity between mother plant and other cultivars. This method randomly goes through all genome and it has a chance to find some changes of DNA sequence, but the most of them are only point mutations without any effect on phenotype. The short primer sequence, poor interpretation of results and low reproducibility are some disadvantages of this method. Its advantages are speed, lower cost and laboriousness, no previous sequence information.

One different meristem derived in vitro clone (SB2) was also found by ISSR analysis. $(CGT)_5C$ and $(CGT)_5C+(TGTC)_4T$ primers produced the different ISSR patterns. The frequency of sequence changes of DNA was low and this variability (0.913) did not exceed genetic dissimilarity between mother plant and other cultivars. This method goes through microsatellite sequences in genome and it has a chance to find some changes of number of repetitions. But the most of microsatellite sequences are in heterochromatin, which have not high influence on phenotype. This method is very similar to RAPD, but it is interesting, that RAPD and ISSR found different meristem derived in vitro clones. Even though, this method does not go through all genome, it is suitable for monitoring of somaclonal variability.

All meristem derived in vitro clones were different from mother plants by AFLP analysis. But their variability (0.993 - 0.824) did not exceed genetic dissimilarity between mother plant and other cultivars. It is evident, there are many sequence differences on DNAs of meristem derived in vitro clones. The combination of restriction digestion and PCR can detect these changes and successfully monitor somaclonal variability. But many steps can increase some nonspecific reactions in AFLP procedure. Poor interpretation also can distort results. Disadvantages of this method are time, cost and working exactingness, high quality of chemicals and genomic DNA. Its advantages are effectiveness, high level of polymorphism and no previous sequence information.

All these differences may be due to mutation in DNA priming sites or deletion/insertion between priming sites (DeVerno et al., 1999), mainly in hypervariable "hot spots" sequences (Linacero et al., 2000), which have not significantly influenced phenotype of hop varieties. Mechanisms, inducing variability in in vitro conditions, are same as mechanisms in nature and in vitro stress only increases rate of mutations (Linacero et al., 2000). Detected molecular differences were similar to natural variability within clonal multipropagation of hop in field conditions by genetic diversity analysis.

Dendrograms of individual hop varieties and regenerated *in vitro* mericlones revealed by UPGMA cluster analysis based on Jaccard's similarity coefficient determined using **a)** 27 RFLP markers, **b)** 66 STS markers, **c)** 188 RAPD markers, **d)** 61 ISSR markers and **e)** 366 AFLP markers.

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