# EVALUATION OF SOMACLONAL VARIATION IN HOPS BY AFLPs

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## INTRODUCTION

Hops (*Humulus lupulus*) are mainly vegetatively propagated and each individual within a cultivar can be considered to have a identical genotype making them a suitable target for DNA identification technology. Information obtained from this technology has many applications including quality control purposes, breeding selection strategies, cultivar identification and detection of somaclonal variants.

A number of different molecular markers types are available but current interest centers on those amenable to analysis by the polymerase chain reaction (PCR). A new high-multiplex PCR-based method for DNA profiling known as AFLP™ or amplified fragment length polymorphism (AFLP) has the potential to generate a large number of polymorphic genetic loci to analyse genetic diversity without depending on the availability of sequences information.

In a previous work AFLPs analysis has been assayed to analyse genetic variation and identification of 27 hop cultivars. On average 67% polymorphisms per combination were displayed. Ten groups of hop cultivars could clearly be separated by this technique (submitted for publication).

In this study AFLP analysis are applied to detect the presence of somaclonal variants produced along the development of the procedure of plant regeneration from callus and to evaluate the influence of the longevity of the callus on somaclonal variation.

## PLANT REGENERATION FROM CALLUS

## PLANT REGENERATION FROM CALLUS CULTURES

Complete plants have been regenerated from callus induced on internodal segments of three cultivars: Brewers Gold, Nugget and Chinock.

Zeatin at 4.56 µm produced caulogenic calli. Subculture of these calli on the same culture medium supplemented with benzylaminopurine (4.4 µm) and indolebutyric acid (0.49 µm) promoted shoot regeneration which gradually increased up to the third subculture. Results published by Gurriarán et al. (1999) Plant Cell Reports 18: 1007 - 1011.

## DNA EXTRACTION AND AFLP PROTOCOL

DNA was extracted from freeze-dried young leave material using the kit Dneasy ™ (Quiagen). AFLP analysis was performed according to Vos et al, (1995) with the modifications described by Cervera et al., (1998). DNA was digested with *Mse*I (New England Biolabs) and *Eco*RI (Pharmacia). After adaptors ligation, preamplification of prepared template was performed with primers complementary to the core of the adaptor sequences with an additional selective 3´nucleotide. The PCR reactions were performed in 20µl vol of 10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 50mM KCl, 0.2mM of each dNTP, 30ng of each primer (GENSET) *Eco*RI+A (E11), *Mse*I+C (M20) and *Mse*I+T (M21), 0.4U of Taq DNA polymerase (Boehringer) and 5µl of diluted digested-ligated DNA fragments.

Selective restriction fragment amplification was performed using a ($^{33}P$) labelled Eco RI primers with two combinations 1-E36/M31 ($E_{ACC}M_{CTT}$) and 2-E36/M39 ($E_{ACC}M_{TCC}$) (Table). We used 5µl of the preamplification template for each PCR reaction.

Samples amplified with different primer combinations were loaded into 4.5 % denaturing polyacrilamide gels and electrophoresed for 2 h. Gels were later dried onto chromatography paper, and exposed to autoradiographic film.

| Primer combination EcoRI/MseI | Nº of bands |
|---|---|
| ACC/CTT | 62 |
| ACC/CAC | 58 |
| ACC/CCA | 55 |
| ACC/CGT | 63 |
| ACC/TCC | 48 |
| ACC/TAC | 50 |
| ACC/TAA | 40 |
| ACC/TGG | 43 |
| AAC/TCC | 55 |
| ACA/TCC | 61 |
| ACG/TCC | 57 |

**EACH PRIMER COMBINATION WAS ASSAYED ON EIGHT SAMPLES:**

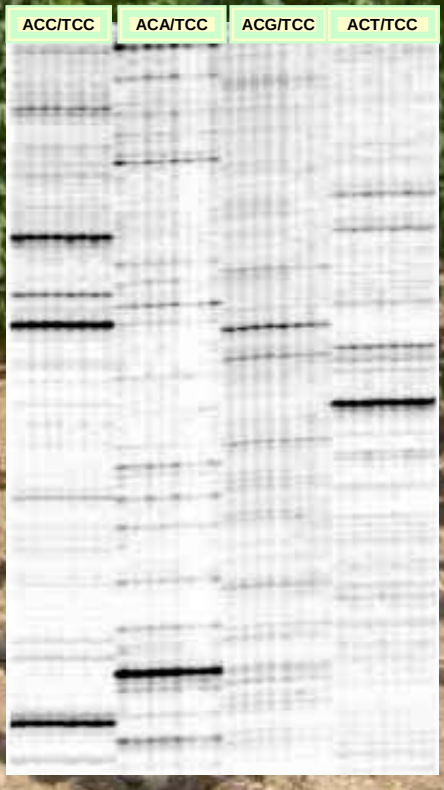
**1. Plant in field conditions**

**2. Plant growing in vitro**

**3. Plant regenerated from callus cultured 60 days**

**4 and 5. Plants regenerated from callus of 120 days**

**6, 7 and 8. Plants regenerated fron callus of 180 days**




This Figure shows typical fingerprint of the samples after PCR amplification with 16 different primers combinations and autoradiography. The score of the presence or absence of every amplified fragment in every sample allowed to obtain identical profiles. Therefore, no somaclonal variation is induced by the in vitro culture or the callus phase.The longevity of the callus showed no influence on the induction of somaclonal variation.

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