# Dynamics of DNA Methylation in Chestnut Trees Development

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**Keywords:** *Castanea sativa*, ageing, maturation, reinvigoration, epicormic shoot, dormancy, epigenetic, methylation, HPCE

**Abstract**

**During life cycle trees undergoes physiological, biochemistry and morphological changes mainly controlled by the alteration of gen expression, process that are associated to change of the morphogenetic capacity and dynamics of the epigenetic factors, among which DNA methylation is one of the best know processes. Dynamics of changes in genomics DNA methylated 5-deoxicytidines during chestnut development and maturation stages was determined. Also, it was examined whether reinvigoration of chestnut trees causes variation in the methylation degree.**

## INTRODUCTION

Since 1989, research efforts of the Oviedo University Group EPIPHYSAGE (www.uniovi.es) have been focused on: a) woody plants morphogenesis manipulation (*Corylus avellana, Olea europaea*, *Juglans regia, Pinus radiata, Eucalyptus globulus, Castanea sativa)* and b) the definition of the molecular bases of differentiation- re-differentation processes. Results obtained allow validation of two molecular markers of aging, maturation and phase change referred to endogenous polyamine contents and global DNA methylation level.

In an individual, each of the cells contain the same set of genes but their phenotype can vary according to expressed and repressed ones. Alterations in gene-expression patterns, without changes in DNA sequences are referred to as epigenetic mechanisms. The most important and characterizing epigenetic marker associated with gene silencing is DNA methylation. Since DNA methylation is involved in gene transcription and chromatin conformation, methyl-cytosine has been considered a good candidate for the control of those genes whose expression is required in a particular cell type during development. It has been widely demonstrated that DNA methylation is essential for normal plant development and changes in DNA methylation patterns result in abnormal plant development (Finnegan et al., 1996; Richards, 1997; Finnegan et al., 1998). In a similar fashion, DNA methylation content can act as a molecular marker for specific plant development processes involved in aging, reinvigoration and phase-change in gymnosperms (Fraga et al., 2002a,b) and angiosperms (Zluvova et al., 2001; Bitonti et al., 2002; Baurens et al., 2004).

*Castanea sativa* is a multipurpose species in which both juvenile and mature phases may occur on the same adult tree. The upper parts of a tree exhibiting determinate growth are chronologically younger and often exhibit mature characteristics, whereas the lower, physiologically older parts may retain juvenile attributes. This it is a good experimental system to study epigenetic alterations like one regulatory mechanism during development and differentiation related to loss of morphogenic ability. We have recently reported differences of genomic DNA methylation by enzymatic hydrolysis and High Performance Capillary Electrophoresis (HPCE) in shoot apex from different developmental situations related with in vitro responses to axillaries buds stimulation (Hasbun et

al., 2005). Recent improvements in the quantification method allow us to validate these differences and to rectify our previous results about epigenetics changes in relation to development and maturation stages and reinvigoration processes.

## MATERIALS AND METHODS

Shoots from trunk base which present decreasing juvenile-like ability (juvenile phase) and inferior third level of the crown considered as adult (mature vegetative and reproductive phases) were collected from 50 years-old chestnut trees during May (2005) to study ontogenic development and reinvigoration effects. Dormancy shoot apexes (buds) of three different types of shoots were also included taken in December (2005) to study maturation state. Growth of basal shoot (epicormic shoots) was considerate like a natural reinvigoration process. To study its effect in genomic methylation one, two and growth seasons, which present decreasing juvenility tissues, were taken.

Genomic DNA isolation was performed with a plant genomic DNA extraction kit (DNeasy Plant Mini, Qiagen) from shoot apexes (3-5 mm) and buds with same modifications to assessment a RNA totally free-DNA. Genomic DNA methylation was carried out as reported Fraga et al. (2002) with same modifications. Genomic DNA was exposed to enzymatic hydrolysis with nuclease P1 and alkaline phosphatase and separations of nucleosides by high performance capillary electrophoresis. The instrument was programmed to run a voltage gradient of 10 kV over 20 min.

## RESULTS

Because the material analyzed were exactly the same genotype and were collected at the same time after the spring growth, the percentages of genomics DNA methylation depended exclusively on the ontogenic state of the caulinar meristematics zones (shoots apex). Chestnut aging implied a progressive increase of methylated 5-deoxycytidines content (Fig. 1). This allowed us to associate shoot apex of juvenile individuals (without reproductive ability) with a DNA methylation degree of 10 %, while mature tree zones show greater percentages depending of the reproductive ability. Mature vegetative shoots showed 12,5% methylation of the total 5-deoxycytidines. However, floral differentiation implied a gentle decrease of genomic DNA methylation (12%). During bud dormancy genomic DNA methylation reach a common value close to 20%, independently of the buds ontogenic state.

For the reinvigoration process, there was a gradual increase in DNA methylation in the epicormic shoot apex (Fig. 2). This correlated to consecutive lost of the reinvigoration effect until reaching a value near the observed on mature vegetative shoots after 3 growth seasons.

## DISCUSSION

DNA methylation has a pivotal role in aging. Reinvigoration could be based as an on-off gene control mechanism. Results obtained from the study of genomic DNA methyaltion are important from academic point of view to applied fields (Rodríguez et al., 1999; Fraga et al., 2002a,b,c; Esteller et al., 2001, 2002). The use of more efficient DNA extraction methods and the optimization of methylation quantification protocol presents consistent results on epigenetic modifications of chestnut.

Chestnut development involves an increase of genomics DNA methylation. Reinvigoration has an opposite effect. Genomic DNA methylation could be considered as a epigenetic marker related to aging and re-invigoration. The process of de-differentiation and cellular reprogramming, like flowering, is associated with a decrease of methylation. This reflects the epigenetic plasticity of vegetal tissues.

Bud dormancy is accompanied with an increase of global methylation independently of aging. This data validates our results, because meristems remain inactive during the dormancy due to a changes in the transcriptional activity of DNA. This is partially governed by gene silencing induced by the methylation of cytosine residues to 5-methylcytosine (Law and Suttle, 2003).

**ACKNOWLEDGEMENTS**

The National Project MDCT-AGL2004-00810/FOR and the R. Hasbún fellowship of Chilean Government (CONICYT) supported this research.

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**Figures**

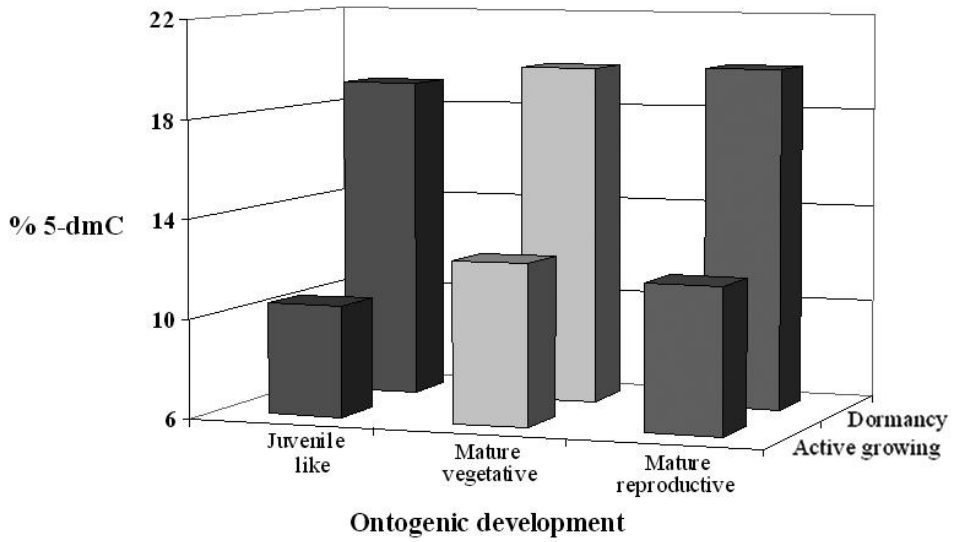


Fig. 1. Percentage of 5-deoxymethylcytidine (5-dmC) in shoot apex with different ontogenic development and maturation state taken of the same chestnut tree.

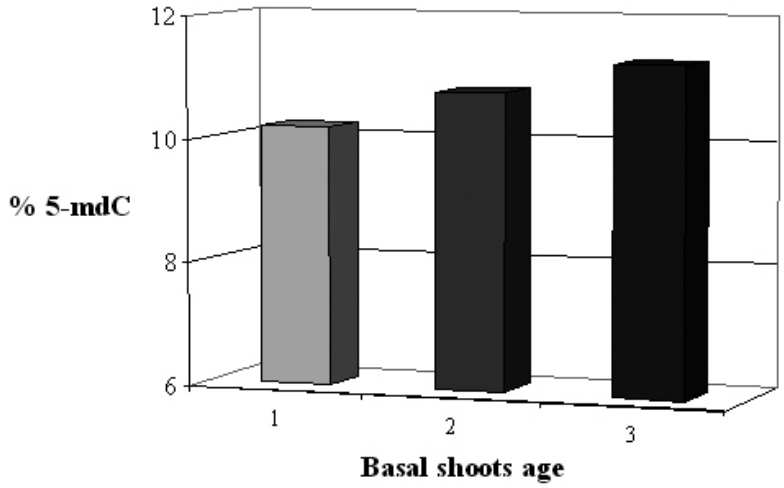


Fig. 2. Percentage of 5-deoxymethylcytidine in shoot apex in function of growth seasons of basal shoots taken of the same chestnut tree.