

DEVELOPMENT OF A METHOD SUITABLE FOR CHROMATIN IMMUNOPRECIPITATION IN GRAPEVINE ROOTSTOCKS

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Vitis vinifera L. is the most important world fruit crop, it has thousand of variety and it is unmatched in term of wine quality however it has not the genetic resistance for diseases and parasites characteristic of American and Asiatic species.

To protect *V. vinifera* varieties of two main root pests, phylloxera and parasitic nematodes, viticulture is based of grafting them to rootstocks derived from either North America *Vitis* species or interspecific *Vitis* hybrids.

Many rootstocks show scarce resistance to environmental stresses that's why the selection of resistant rootstocks is a crucial factor for the development of sustainable agricultural models. The selection of genotypes able to cope with stress conditions requires a though knowledge of the molecular, biochemical and physiological bases stress resistance.

A lot of different pathways showed to be involved in grapevine response to abiotic stresses. In particular genes of hormonal pathways and secondary metabolites seem to be the most interesting in this way; that's why we need to study their transcriptional profiles and the mechanisms of genetic regulation.

The aim of this work is the study of the responses to abiotic stresses in terms of chromatin modifications. *V. vinifera* has a genome fully sequenced and annotated so now we can think to explore the contribution of chromatin to the regulation of the genome activity in response to environmental stresses (*). The newly established rootstocks chosen for this work are a sensible one: the 101.14 and a resistant one: the M4 (*V. vinifera* x *V. berlandieri*) x *V. berlandieri*.

Nowadays there is not any protocol for chromatin immunoprecipitation (ChIP) designed for grapevine, that's why we need to develop an our own method. In the first step we set the protocol on leafs and later we will use the method developed, with appropriate changes, on roots.

Since a lot of material is necessary for set the protocol and for the seasonality of the plants, we need to propagate them *in vitro* so as we have enough leaf material to test each step of the protocol and then use the knowledge gained for *in vivo* material.

The chromatin state of candidate marker genes previously selected by mRNAseq under stress conditions is evaluated to identify those with a putative epigenetic control. Chromatin is immunoprecipitated using commercial antibody designed for the detection of histonic modifications; for instance H3K4me3 that is detected in actively transcribed genes.

Once the method will be fine, 101.14 and M4 rootstocks will be grown under controlled conditions of drought and salt stress at different intensity levels. In order to characterize the plants response, tissues from roots and leaves will be collected and then the chromatin state will be studied.

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