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Molecular genetic researches of the *Börner* (*Vitis* sp.) *Phylloxera* resistance by means of microarray technology

Phylloxera infestation at sensitive vine

Phylloxera vitifoliae, probably the most important pest in viticulture, was introduced with American grape vines to Europe in the middle of the 19th century. Its infestation results on sensitive vines in tissue proliferations in the form of leave **galls** (Pic.1) and root **nodosities** respectively **tuberosities** (Pic.2). The latter are negligible for the vine production, when younger roots are affected. On the other hand tuberosities on older, woody roots can entail a substantial loss of vitality. In the worst case the grape vine dies.


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Phylloxera infestation at the resistant root stock *Börner*


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In contrast to sensitive *Vitis* sp. the root stock *Börner* responds to *Phylloxera* infestation with a **hypersensitivity reaction (HR)** in terms of **necrosis** on leaves (Pic.3) and roots (Pic.4). Hence the pathogen cannot feed on the roots. Because of its *Phylloxera* resistance, *Börner* is used in viticulture as a root stock. Because of it cannot be equally used in all locations, the development of new *Phylloxera* resistant root stock varieties is of great importance. Traditional breeding in this field takes up to fifty years. Therefore genetically modified vines are required.

Indol-3-acetic-acid (IAA)- triggering element of the HR in *Börner*?


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Phylloxera saliva contains **cecidogene** substances. They are responsible for the formation of galls and nodosities respectively tuberosities and necrosis. It is assumed that the plant hormone **indol-3-acetic-acid** (IAA), has such a triggering cecidogene effect. A chemical application of IAA on leaves and roots of sensitive grape vines causes the typical symptoms. While **untreated roots** (Pic.5) have no impacts, an external application of IAA at the resistant root stock *Börner* results in the described **necrosis** (Pic.6).


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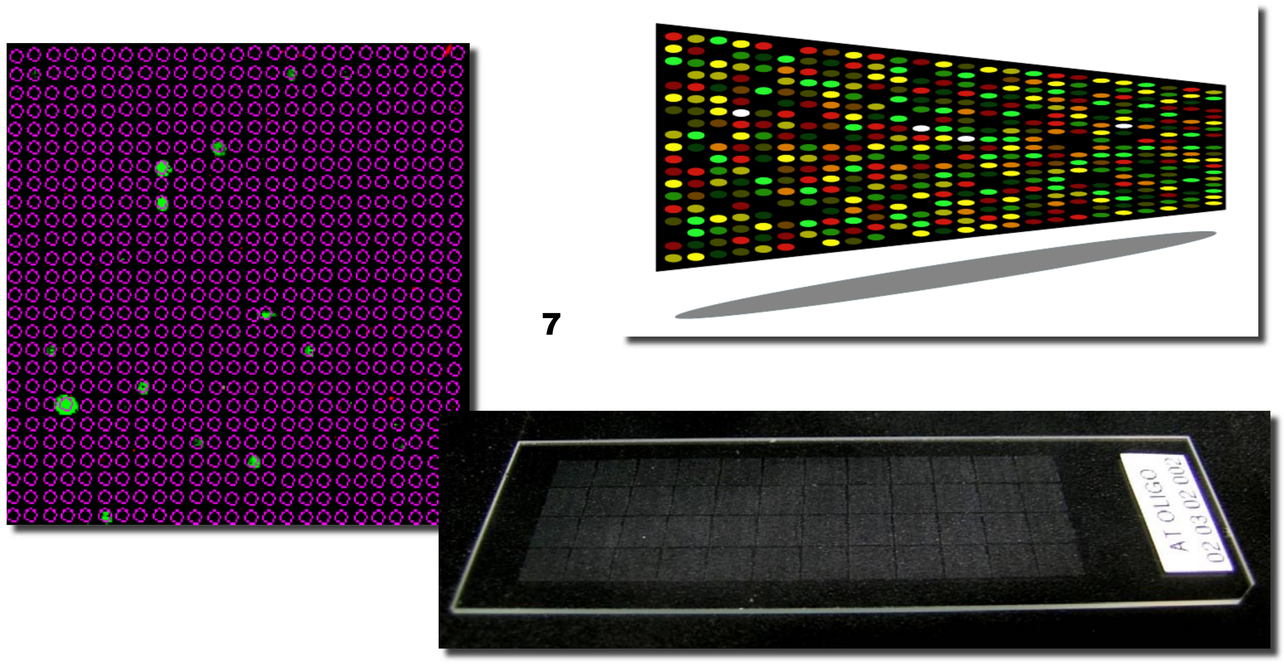
Which genes are involved in the HR?

To produce transgene *Phylloxera* resistant root stocks, the processes of the HR must be cleared up in detail. The involved and for the resistance responsible genes have to be identified. Molecular genetic methods can help to achieve this research aim. In particular the microarray technique is eligible to scan a large number of genes.

Gene identification with DNA-Microarrays

For gene identification by means of the microarray technique several steps have to be carried out. First *Börner* root tip tissues must be treated with IAA in defined incubation times. Subsequently the so treated material has to be collected over a period of time. In the next step untreated root material was collected as control tissue. Finally total-RNA was extracted to create cDNA, which was hybridised in a *flip-dye* experimental design on *Arabidopsis thaliana* whole genome microarrays (Pic.7).

Two different experiments were carried out. In the first one two different IAA treated samples were hybridised in different fluorescence labelling on one chip. In the second one IAA treated sample and the untreated control sample were hybridised in the same procedural method on one chip.



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Results of Microarray analysis

Assumed IAA function confirmed

The microarray analysis confirm the assumed IAA function. Therefore with the utmost probability IAA is the HR triggering substance in *Phylloxera* saliva. The results gave valuable information containing references to the transduction of the IAA signal. Evidences were found that signal substances like **ethylene**, **salicylic acid**, **jasmonic acid**, **calcium** and **reactive oxygen species** are activated by IAA. This substances are generally associated with the activation of resistance mechanisms.

HR associated genes identified

Numerous HR associated genes could be identified by the microarray analysis. Thus indices about a transcriptional activation of **phytolaexine**, **resistance- and pathogen-related-proteins** as well as representatives of the **hypersensitive-induced response family**, could be found in the IAA treated samples. Furthermore evidences for an activation of the auxin induced **ubiquitin/26S proteolysis pathway** and other signal components, like **kinase** and **transcription factors** could be determined.

Conclusion

In the context of the undertaken experiments potential transductional mechanisms of the *Phylloxera* resistance in *Börner* were found by means of the microarray technique. Many genes which are activated after the induction with IAA at *Börner* roots could be pointed out. They are associated with HR and resistance mechanisms. A decisive step for a transgene, *Phylloxera* resistant, root stock is done.