Transcriptome analysis of germinating maize kernels reveals substantial differences between the effects of smoke-water and the active compound butenolide

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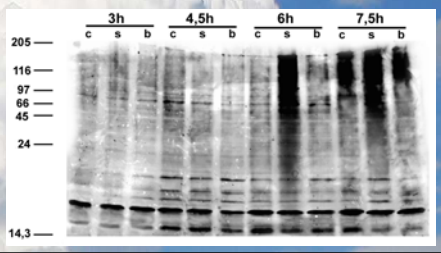
Smoke released from burning vegetation contains a chemical signal that triggers the germination of fire-climax, non-fire-climax species and crops such as maize. The recent identification of the active compound, a butenolide molecule, have given renewed momentum to determining the mechanism of action of smoke. Smoke-treatments can be effective in stimulating seed germination, improving germination characteristics of crops, as well as increasing seedling vigour. Different kind of stresses are the major constraints for maize production worldwide, since this crop is largely grown in areas in which unfavourable conditions, such as drought and heat stress, are predominant. In these areas, improved germination characteristics and seedling vigour is an important agronomic trait for the establishment of seedlings. This can help young plants to overcome such adverse effects which usually lead to lower yields.

The aim of the project is to investigate the molecular background of smoke and butenolide action through which smoke and the active constituents affects maize germination and seedling vigour. **GCP Project G4007.24: „Seed smoke treatment to favour germination under water stressed conditions"**

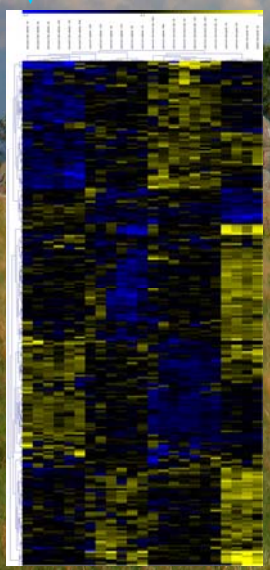
The total transcriptome of smoke and butenolide treated kernels is quite different. After smoke exposure, the sulfiredoxin-like protein gene and a LRR receptor kinase-like gene were upregulated. In the first 3 h, the transcript abundance of the ABA signaling negative regulator calcineurin 9 B like gene (CBL9) and an unknown gene with a tetratricopeptide repeat (TTR) sharply declined. In smoke-treated seeds, the most obvious changes were observed in the expression of the ubiquitin activating enzyme 1 (UBE1) which was upregulated at all time points.

After butenolide treatment, a senescence-associated protein-related gene was upregulated at all time points, except at 9 h, where a sharp decline in the expression was observed. A putative plastidic phosphate translocator-like protein 1 and a glycosyltransferase domain-containing gene was also constantly upregulated. The most notable gene however, which was upregulated during the whole course of the experiment is a tonoplast intrinsic protein (TIP3.1), a member of the aquaporin family. Analysis of the microarray data obtained from comparison of the butenolide- and smoke-treated samples showed that the master genes are the TIP3.1 aquaporin, a senescence-associated protein-related gene and the SAM-dependent methyltransferase.

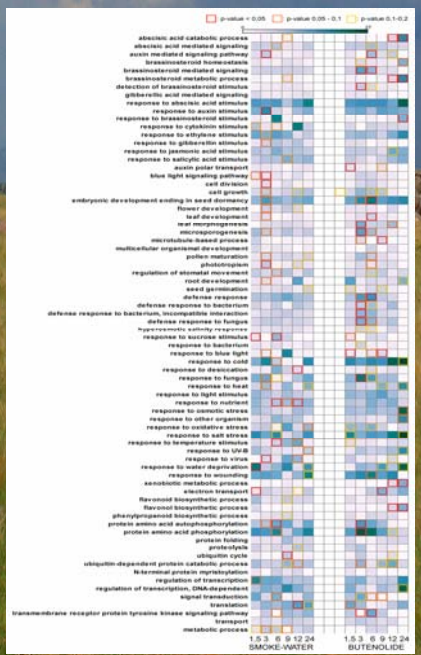


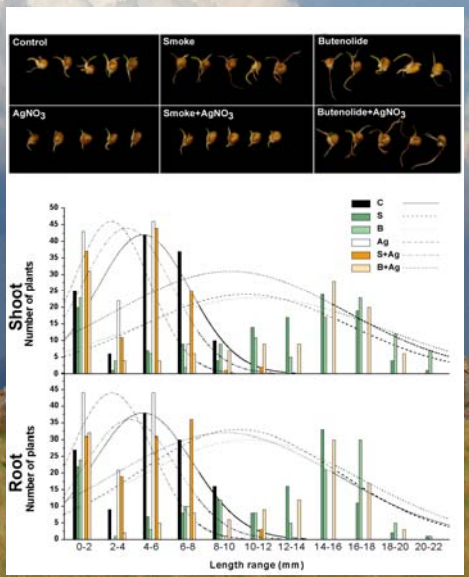
Smoke-treatment (s), and not butenolide (b), enhanced the ubiquitination of the proteins dramatically after 6 h of exposure. UBE-1 upregulation after smoke treatment is followed by increased ubiquitination. At 3 and 4.5 h, the level of ubiquitination was similarly low in both treatments, and at 7.5 h all the samples showed an increase in signal intensity - although in the smoke-treated samples the ubiquitinated proteins were more prevalent - suggesting that the smoke treatment resulted in accelerated ubiquitination.

The expression pattern of 21 selected genes which seemed to be most affected ones (expression changed in all experiments, fold change ≥ 2)



The expression patterns of genes which were affected in at least six different comparisons by either of the treatments with fold change ≥ 2.





TIP 3.1 aquaporin plays a crucial role in butenolide, but not in smoke action. Treatment of the seedlings with a combination of butenolide and silver nitrate (aquaporin inhibitor) showed an alleviation of the adverse effect of the silver nitrate, whereas simultaneous treatment with both smoke-water and silver nitrate show no such reduction in the effect of silver nitrate inhibition. This effect of the butenolide in combination with silver nitrate was demonstrated by the frequency distribution of the seedling shoot/root size which was not significantly different from the butenolide-treated plants.

Contrary to the obvious difference in the gene expression pattern, light signaling, phenylpropanoid pathway, brassinosteroid and stress-related GO terms are similarly overrepresented after smoke and butenolide exposure. Light colours indicate low representation; blue/green colours show overrepresentation. Red squares: raw p-value < 0,05; orange squares: raw p-value 0,05 - 0,1; yellow squares: raw p-value 0,1 - 0,2.

Conclusions Although smoke-water and butenolide treatment of maize kernels result in a similar physiological response, the gene expression and the protein ubiquitination patterns are quite different. Treatment with smoke-water enhanced the ubiquitination of proteins and activated protein-degradation-related genes. This effect was completely absent from butenolide-treated kernels, in which a specific aquaporin gene was distinctly upregulated. These findings indicate that the array of bioactive compounds present in smoke-water form an environmental signal and may act together in germination stimulation. As far as the nature of smoke and butenolide perception is concerned, it is highly possible that the smoke 'signal' is perceived by a receptor that is shared with the signal transduction system implied in perceiving light and stress factors or some kind of specialized receptor (F-box proteins) exists in fire-prone plant species' which diverged from a more general one present in a common ancestor, and also found in the non fire-prone plants allowing of a somewhat weaker but still significant response.