

The effect of smoke on maize kernel germination and seedling vigour

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Smoke released from burning vegetation contains a chemical signal triggers germination of fire climax, non-fire climax species and crops like maize, also. The recent identification of the active compound, a butenolide derivative gave burst to determine the mechanism/s of action. However, despite these efforts the possible mode of action by which smoke affects germination remained unclear. It became increasingly clear that smoke has dual effect. On the one side smoke stimulate seed germination (Fig. 1), can break dormancy (Fig. 1 A and B) and improve germination characteristics of crops (Fig 2.). On the other side smoke increase 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 (drought, heat stress, etc.) are predominant. In these areas, improved germination characteristics and seedling vigour is an important agronomic trait for the establishment of seedlings which can help young plants to overcome such adverse effects usually led to low yields. The aims of the project are to investigate the physiological effect and mode, through which the active compound affects maize germination and seedling vigour, using microarray techniques, in silico promoter extraction and reporter - based genetic screens.

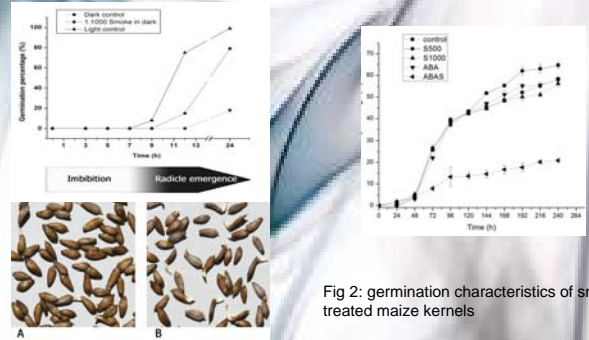


Fig 2: germination characteristics of smoke treated maize kernels

Fig 1: effect of smoke on the germination of photoblastic lettuce seeds. A: control after 34h, B: smoke treated after 24h

Smoke acts as a germination cue

-smoke induces the rapid decay of mRNAs present in mature and desiccated embryos (Fig. 3)

-transcript abundance of seed maturation specific genes are low (LOB, TonB, senescence associated protein, etc.) (Fig. 4)

-the most highly affected genes are involved in the 26S proteasome regulation, RING-domain containing E3 ligases and LRR-receptor like kinase mediated signal transduction (Fig. 4)

-tetratricopeptide repeat containing transcript is downregulated respectively

-the expression of a phytochrome P450 (ABA metabolism?) is upregulated during the whole course of the experiment

-other ABA and stress responsive genes are also upregulated

3h	
Annotation	LogFC
Ubiquitin-activating enzyme E1 2	5.220121
AAA ATPase domain, zinc finger domain	4.94518
G-protein coupled receptor, aspartyl aminotransferase	-3.87618
NADPH-cytochrome P450 reductase	-3.72046
Putative cytochrome P450	3.57205
SWAP putative splicing factor	-3.41693
sulfurfoxidase	3.305128
auxin-regulated protein	-3.3033
ATPase, histidine kinase	3.236698
receptor-like protein kinase homolog	-3.10262
calineurin B-like protein 9	-2.846
RING-type E3 ubiquitin ligase	-2.91149

6h	
Annotation	LogFC
Ubiquitin-activating enzyme E1 2	4.76261
lateral organ boundaries (LOB) domain family	-3.96516
putative senescence-associated protein 12	-3.43491
putative cytochrome P450	-3.15293
putative DMHC-type zinc finger protein	3.128584
putative glucanase	3.107974
Proteasome subunit alpha type 3	3.087519
protein kinase; transcription factor	3.085608
zinc transporter	2.946894
PIP1, NADPH oxidase; hypothetical protein	2.899835
putative LRR receptor-like kinase 2	2.896316

9h	
Annotation	LogFC
tetratricopeptide containing protein	-4.06876
TonB family protein (receptor?)	-3.74368
putative splicing factor, arginine/serine-rich 7	-3.74338
phosphatase putative NUI interacting factor	3.691469
lipid transfer protein-like protein	3.608196
similar to high affinity nitrate transporter protein	-3.40882
expressed protein	-3.30494
unknown protein	-3.1067
RING3-type ubiquitin ligase	-2.9941
40S ribosomal protein S24	2.986932
calineurin B-like protein 9	-2.92751

12h	
Annotation	LogFC
arsenical pump driving ATPase putative (IAINA4)	3.624659
inositol phosphate amidotransferase	3.383607
Mo 25 like, HEAT domain, involved in polarised growth	3.228118
prefoldin chaperone, c-myc	3.192058
chitinase	3.135215
transcription factor MADS32	3.106849
putative chitinase	2.894369
sgp1 protein	2.877145
denin stator phosphoprotein precursor-like protein	2.783518
putative 6-4 photolyase (LVR3)	2.757961
bZIP transcription factor	2.677371

24h	
Annotation	LogFC
putative adenylate translocator (Brittle-1) protein	4.162883
Abscisic stress ripening protein 1	3.586831
unknown protein	3.251138
putative cytochrome P450	-3.1195
transcriptional factor B3 protein	3.061288
actin	2.844403
putative RNA pol II accessory factor	-2.80549
Unknown protein	2.738784
zinc finger	2.733769
auxin regulated protein	-2.65523
putative tubby-like protein (F-box domain, ABA signalling)	2.638667

27h	
Annotation	LogFC
photosystem II OE17 protein	5.212061
putative glycosyltransferase	4.7447
ATG9a3560/K907_6	4.644452
unknown protein	4.442983
putative nifU-like protein	4.41415
unknown protein	4.376412
ZnO/RN1	4.355252
putative CC-NBS-LRR resistance protein	4.2195
putative tubby-like protein (F-box domain, ABA signalling)	4.137392
fatty acid binding protein	4.103129
putative ABC transporter (AC004411)	4.085361

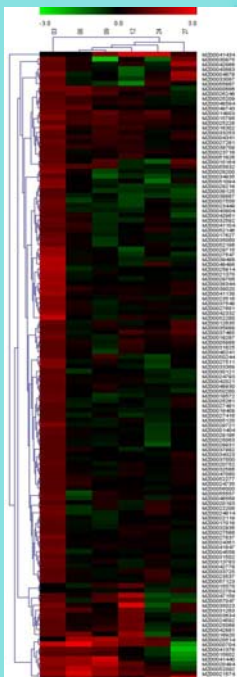


Fig 3:

Fig 4:

Smoke improves seedling vigour

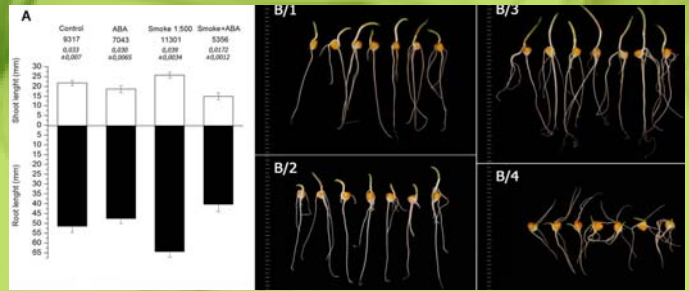


Fig 5: the effect of smoke and ABA on maize seedling vigour

Smoke greatly improved the seedling vigour of maize seedlings (Fig. 5A), and could be applied as a growth stimulant. Smoke treated seedlings (Fig. 5 B/3) grew more vigorously than controls (Fig. 5 B/1). The joint application of smoke and ABA resulted in decreased growth (Fig 5 B/4) compared to ABA controls (Fig 5 B/2) water controls and smoke treated seedlings (Fig. 5 B/3).

The microarray study revealed that smoke induces a stress-related changes in the global transcriptome of young seedlings. As Fig 6 A and B show, stress and ABA-related genes are overrepresented 24 and 48h after smoke treatment. The expression of the most pronounced smoke and ABA related transcripts were validated using Real-time PCR (Fig. 8). It appears that the 'hardening' effect of smoke is highly similar that of caused by ABA. These findings is supported by the facts that stress and ABA related messages and promoter motifs (Fig. 7; ABA related motifs are highlighted in green) are overrepresented after smoke treatment. In addition, the joint application of smoke and ABA decreased the seedling vigour (Fig. 5 B/4) which further suggest a possible interaction between the two compounds.

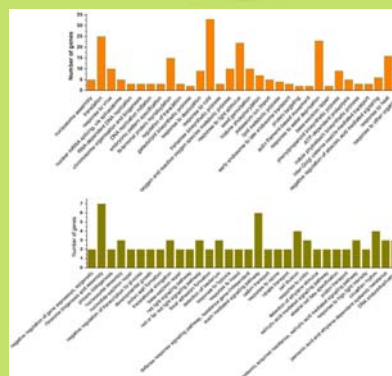


Fig 6 A and B: Gene Ontology terms 24 and 48h after smoke treatment



Fig 7: promoter motifs of smoke responsive genes

Fig. 3. Expression analysis of some selected genes in response to smoke treatment after 24 and 48h. RQ, relative quantification number Operon oligo identifiers are shown. MZ00051069 Unknown; MZ00044608 Unknown, similar to DIE/ALG protein; MZ00026223 NA, similar to basic leucine zipper family protein; MZ00000704 putative LRR receptor-like kinase 2; MZ00022704 cytochrome P450; MZ00020514 sulfiredoxin-like protein; MZ00030105 unknown protein, contains tetratricopeptide repeat; MZ00041434 Ubiquitin-activating enzyme E1 1.; MZ00019209 putative zinc finger protein; MZ00004101 putative MYB transcription factor; MZ00024064 calineurin B-like protein 9; MZ00047075 putative protein kinase

