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Putative role of proteins involved in detoxification of reactive oxygen species in the early response to gravitropic stimulation of poplar stems

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Gravity perception and gravitropic response are essential for plant development. In herbaceous species it is widely accepted that one of the primary events in gravity perception involves the displacement of amyloplasts within specialized cells. However the signaling cascade leading to stem reorientation is not fully known especially in woody species in which primary and secondary growth occur. Several different second messengers and proteins have been suggested to be involved in signal transduction of gravitropism. Reactive oxygen species (ROS) have been implicated as second messengers in several plant hormone responses. It has been shown that ROS are asymmetrically generated in roots by gravistimulation to regions of reduced growth. Proteins involved in detoxification of ROS and defense were identified by mass spectrometry: i.e., Thioredoxin ***h*** (Trx ***h***), CuZn superoxide dismutase (CuZn SOD), ascorbate peroxidase (APX2), oxygen evolving enhancer 1 (OEE1), oxygen evolving enhancer 2 (OEE2), and ATP synthase. These differentially accumulated proteins that correspond to detoxification of ROS were analyzed at the mRNA level. The mRNA levels showed different expression patterns than those of the corresponding proteins, and revealed that transcription levels were not completely concomitant with translation. Our data showed that these proteins may play a role in the early response to gravitropic stimulation.

Introduction

Plants can reorient their growth direction by sensing organ tilt relative to the direction of gravity. With respect to gravity sensing in gravitropism, the classic starch statolith hypothesis, i.e., that starch-accumulating amyloplast movement along the gravity vector within gravity-sensing cells (statocytes) is the probable trigger of subsequent intracellular signaling, is widely accepted.

In trees, sedimentable amyloplasts in the endodermal cells may play a role in gravity perception leading to secondary xylem formation, eccentric growth and reaction wood formation in gravi-stimulated tree stems.[1] How the displacement of amyloplasts might trigger a signaling cascade is still a matter of debate.[2] Several different second messengers and proteins have been suggested to be involved in signal transduction of gravitropism.[2-4]

Although ROS such as superoxide anions and H_2O_2 are generally considered to be toxic by products of respiration, recent evidence suggests that the production of ROS might be an integral component of intracellular signaling.[5-7] Production of ROS occurs in response to many physiological stimuli such as during stomatal closure, adventitious root development and root gravitropism.[8] The excess production of ROS under biotic and abiotic stresses causes oxidative damage to cellular compartments.[9] Plants combat oxidative stress by inducing various protective enzymes and anti-oxidants. In mammalian cells, a variety of extracellular stimuli have been shown to induce a transient increase in the intracellular concentration of ROS, and specific inhibition of the ROS generation results in a complete blockage of stimulus-dependent signaling.[10,11]

ROS have been implicated as second messengers in several plant hormone responses. Joo et al.[12] showed that ROS are asymmetrically generated in roots by gravistimulation to regions of reduced growth. A function for ROS in root curvature was reported by inhibiting cell growth, thus contributing to tropisms. Auxin also induced ROS production in roots and the auxin transport inhibitor *N*-1-naphthylphthlamic acid did not inhibit hydrogen peroxide (H_2O_2)-induced root curvature, leading to the suggestion that ROS play a role downstream of transport in auxin signaling and gravitropism.[12,13]

However, the potential of ROS as a second messenger in gravitropism is still unclear. The implication of ROS in response to 45 min of inclination is consistent with the fact that we

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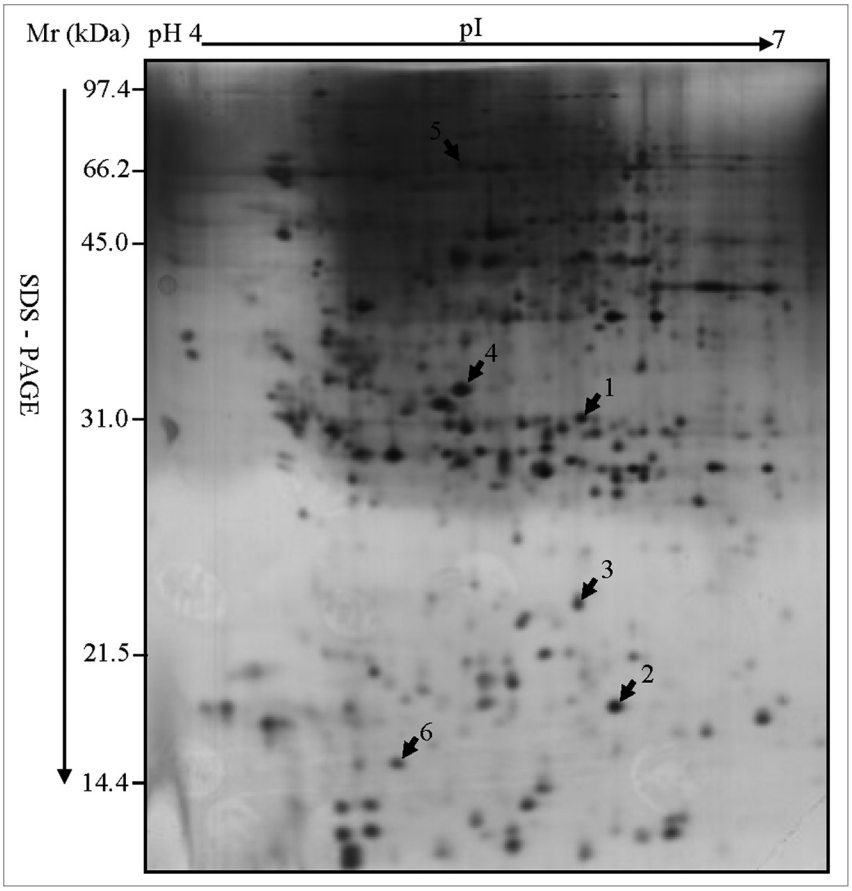

Figure 1. 2-D gel analysis of proteins extracted from poplar stem (basal part) gravistimulated for 45 min. Arrows and numbers indicate to identify proteins involved in detoxification of ROS. The number of each protein spot corresponds to its listing in Table 2.

identified previously a Thioredoxin *h*, cytosolic CuZn SOD, APX2, OEE1, OEE2, and ATPase, proteins involved in detoxification of ROS.[4] These proteins were previously identified as being differentially represented in lower internodes of inclined stems relative to vertical stems. In this report, these proteins were selected in order to investigate their expression patterns, in the early response to gravitropic stimulation at the mRNA level.

Results and Discussion

ROS have been implicated as second messengers in several plant hormone responses.[14] However, the excess production of ROS under biotic and abiotic stresses causes oxidative damage to cellular compartments.[9] Plants combat oxidative stress by inducing various protective enzymes and anti-oxidants. In Poplar stem, a number of protein spots involved in detoxification of ROS and defense were identified previously:[4] Thioredoxin *h* (Trx *h*) (spot n° 6), i.e., superoxide dismutase (CuZn SOD) (spot n° 2), ascorbate peroxidase (APX2) (spot n° 1), OEE1 (spot n° 4), OEE2 (spot n° 3), and ATPase (spot n° 5) (**Table 2**). These proteins were selected in order to investigate their expression patterns, in response to gravistimulation at the mRNA level for kinetics 0, 10, 20, 30, 45 min, 1h, 3h and 6h (**Fig. 2**).

The mRNA levels of *Trx h*, *ATP synthase*, *OEE1*, *OEE2*, *CuZn SOD*, *APX2* showed different expression patterns than those of the corresponding proteins. The result of the present investigation is supported by the previously established concept that transcription patterns are not directly concomitant with protein expression levels.[15]

Trx h expression was induced by gravitropic stimulation. Indeed, the real-time quantitative RT-PCR analysis showed two significant increases of *Trx h* mRNAs: from 20 to 30–45 min, and 3 h after gravistimulation, in basal internodes (**Fig. 2A**). Increase in *Trx h* mRNA expression levels has also been observed following salt treatment of rice seedlings.[16] Such regulation of Trxs suggests that redox balance is affected in the early step of gravi-stimulation. We previously observed that Trx *h* was also upregulated one week after stem inclination.[4] This is in agreement with the finding that production of ROS is essential for auxin-induced gravitropic signaling in maize roots.[12,17]

Interestingly, the energetic pathway seems to be affected by gravitational stimulation. The real-time quantitative RT-PCR analysis showed two significant increases of *ATPase* mRNAs: from 30 min and 3h after gravistimulation, in basal internodes (**Fig. 2C**). After 45 min of inclination, among the few genes that were downregulated at the base of the stem, we have found mitochondrial *ATP synthase* β chain. Indeed, genes coding for subunits of *ATP synthase* increased in their transcript abundance following gravitational stimulation of Arabidopsis root.[18] Alternatively to the energetic hypothesis, it has been postulated that the *ATP synthase* might be a response related to the detrimental effect caused by an oxidative stress in the synthesis of ATP, because ATP synthase catalyzes key phosphorylation reactions associated with aerobic catabolism.[19] In these past years, the involvement of oxidative burst plant defense genes in the gravitropic response has been evoked.[12,20] The implication of ROS in response at the inclination is consistent with the fact that we identified a cytosolic CuZn SOD and APX at the base of the stem. These ROS serve as substrates in metabolism and act as signals during development.[21] However, other evidence suggests that ROS play significant roles in intracellular signaling in gravitropism in maize.[12,13] CuZn SOD plays a central role in protecting against oxidative stress. It is interesting that it showed a downregulation pattern in response to gravistimulation. Consistent with our results, CuZn SOD also has been shown to be downregulated at the protein level by several oxidative stresses.[22] RT-PCR analysis showed significant increases of *CuZn SOD* mRNAs: from 20, 30, 45, 1h and 3h after gravistimulation, in basal internodes (**Fig. 2E**). This upregulation began 20 min after inclination and peaked at 30 min. It has

Table 1. List of primers used for RT-qPCR experiments

Gene	Primer for	Primer rev	Size (bp)	Tm (°C)
APX2	GGA CGA TCA GAC ACC CAG AT	CCT TCT GGA GGT GGA TCA GA	214	58
Cu/Zn SOD	TGG CAC CAT CTT CTT TAC CC	TGA CAT TTC CCA GAT CAC CA	215	59
OEE2	GCA GGC AGT ACA GGA AGA GG	TCA GAA CCT GAC CAG GGA AC	248	61
OEE1	GGT GTG CCT TCT AGG ACC AG	TCG GAA CTC CTT CAG CAC TT	179	61
ATPase	CAC TCA ATC CGG TTG GTT CT	TGG AGC CTC CCT ATG AAT TG	243	59
Trx *h*	AGG GAA AGG GGT CTC AGA AA	ATT GCC TCC ACA TTC CAC TC	178	55

been shown that APX is highly sensitive to inactivation by ROS and is often insufficient to protect the photosynthetic apparatus from photoinhibition during severe drought stress.[23] The absence of APX2 results in reduced photosynthetic activity, during light stress and altered stomatal responses.[24] In our study the APX2 was present in the control but not detected after inclination 45 min. No upregulation of the transcripts *APX2* occurred after inclination, as demonstrated by comparison with the control (**Fig. 2B**). *APX2* expression significantly decreased at 20 min, 30 min, 3h and 6h after inclination. The downregulation of the transcripts *OEE1* (which is bound to photosystem II) might also be interpreted as a response to oxidative stress caused by inclination. It has been shown that deviation from regular redox homeostasis can be sensed in chloroplasts and that specific chloroplast signals control nuclear gene expression.[25] The decrease in *OEE1* transcript (**Fig. 2D**) could be a consequence of such signaling and thus would impair oxygen-evolving activity and photosystem II stability.[26] No upregulation or a slight decrease in *OEE2* expression was observed at 1h and 6h after inclination (**Fig. 2F**). OEE2 was identified as the molecule inducibly phosphorylated by AtGRP-3 and WAK1. In addition, the phosphorylation of OEE2 was enhanced in Arabidopsis infected with avirulent *Pseudomonas syringae*. These data suggest that OEE2 is a molecule downstream of AtGRP-3/ WAK1, possibly in defense signaling.[27] It is currently investigating whether AtGRP-3/ WAK1 signaling is related to the production of ROS such as H_2O_2 and $O^{\bullet}_2$ from chloroplasts under stress conditions.[27]

Involvement of oxidative stress proteins in the gravitropic response was to some extent unexpected, although rapid non-pathogen- related induction of the oxidative stress is known to occur in response to wounding, extreme temperatures, UV irradiation, salt, and osmotic and mechanical stimulation.[28,29] The oxidative stress proteins have not yet been considered to play a role in gravitropism, except for the recent study on role of auxin-induced ROS in root gravitropism.[12] Joo et al.[12] demonstrated that gravity-induced asymmetric ROS generation in roots of maize (*Zea mays*), unilateral application of ROS to vertical roots stimulated root bending, and scavenging of ROS by antioxidants inhibited root gravitropism. Theses results support our findings of involvement of oxidative stress genes in the gravitropic response. Our data showed an implication of ROS signaling in the decurving process via Trx *h*, CuZn SOD, APX2, OEE1, OEE2, and ATPase. It was however not surprising that ROS could participate in gravitational stress signal integration, because ROS in plants act as regular signal transducers in various processes. How molecular mechanisms involving ROS was integrated into a physiological signal that leads to the gravitropic response remains to be elucidated.

Materials and Methods

Plant materials. Hybrid poplar (*Populus tremula x Populus alba*), clone INRA n° 717-1-B4 was multiplied clonally in vitro on Murashige and Skoog medium,[30] acclimatized in hydropony,[31] and grown in a controlled environment growth chamber (16h photoperiod at 60 µmol.m-2.s-1, 22°C/18°C (day/night) and 70% of relative humidity). At the 14 internodes stage, the poplars showing straight stems were transferred on a new device for tilting.[4] After one week on the device in a straight position, poplars showing 20 developed internodes were inclined at 35° from the vertical axis for 45 min, as described early by Azri et al.[4] Starting from the base, internodes 1 to 5 (showing preponderant secondary growth) were harvested from inclined and non inclined plants, frozen in liquid nitrogen and stored at -80°C until protein and RNA extraction.

Protein extraction and 2-DE analysis. Trees were leaned for 45 min. Upright trees were used as controls. Two independent biological replicates were performed using five leaned trees and five upright trees. For each replicate, base internodes from five leaned and five upright trees were sampled. The internodes from 10 trees representing each sample type were pooled and ground in liquid nitrogen. From each pooled sample three replicate protein preparations were isolated. Total protein were extracted and identified as described early by Azri et al.[4] The silver-stained gels were scanned using a densitometer GS-800 (Bio-Rad). Spot abundance was normalized as a relative volume to compensate for differences in sample loading and gel staining, using the PDQuest software. The spots whose quantity varied significantly after inclination were numbered (**Fig. 1**).[32] After automated detection and matching, manual editing was performed. Triplicate gels using independent protein preparations were analyzed for each condition. Statistical analyses were performed using the Student's *t* -test ($p < 0.05$).

RNA extraction and real-time RT-PCR experiments. After one week on the device in a straight position, poplars showing 20 developed internodes were inclined at 35° from the vertical axis for 0, 10, 20, 30, 45 min, 1h, 3h, and 6h as described earlier

Table 2. Differentially expressed proteins following gravistimulation of polar stems was presented by Azri et al.[4]

Spot number[a]	Protein name	Organism	Assigned name[b]	Method	Peptides matched[c]	% Coverage[d]	Exp. pI/*Mr*[e]	Theor. pI/*Mr*[f]	Score[g]	Ratio I/C
1	L-ascorbate peroxidase (EC 1.11.1.11)	*Populus trichocarpa v1.1*	>grail3.0028011501	MS/MS	1	4	5.8/33.0	5.4/26.9	50	abs
2	CuZn-superoxide dismutase (EC 1.15.1.1)	*Populus trichocarpa v1.1*	>eugene3.00700152	MS/MS	2	17	6.0/15.7	5.6/15.3	87	0.36
3	23 KDa Oxygen evolving enhancer protein 2	*Populus trichocarpa v1.1*	>estExt_fgenesh4_pg.C_LG_V1224	MS/MS *	4	39	5.8/21.2	8.6/28.1	301	0.02
4	33 KDa Oxygen evolving enhancer protein 1	*Populus trichocarpa v1.1*	>estExt_Genewise1_v1.C_LG_V3745	Maldi-Tof	8	28	5.4/33.8	5.8/35.1	62	2.68
5	ATP synthase beta chain mitochondrial (EC 3.6.3.14)	*Populus trichocarpa v1.1*	>estExt_fgenesh4_pg.C_LG_X1053	Maldi-Tof	17	42	5.5/60.3	6.0/59.8	132	1.63
6	Thioredoxin *h*	*Populus trichocarpa v1.1*	>estExt_fgenesh4_pg.C_LG_V1461	MS/MS	4	36	5.1/13.1	5.6/12.6	128	ns

These proteins involved in detoxification of ROS. [a]Assigned spot number as indicated in Figure 1. [b]Assigned name: Blastp. from the JGI database (http://genome.jgi.-psf.org) in *Populus trichocarpa* species. [c]Number of matched peptides. [d]Sequence coverage %: percentage of predicted protein sequence covered by matched peptides. [e]Exper pI/Mr, experimental pI and mass of protein in KDa. [f]Theor pI/Mr, theoretical pI and mass of protein in KDa. [g]Statistical probability of true positive identification of predicted proteins calculated by MASCOT (http://www.matrixscience.com). abs: the spot was present in the control but not detected after inclination. ns: indicates no significant difference was found between the means calculated for the spot in the control and inclined conditions. The means of normalized quantities was calculated from three replicas for spots from the control (C) and inclined (I) conditions, and statistical analyzes were performed ($p < 0.05$ student test). The ratio I/C indicate the significant difference between the means of the normalized quantities from the inclined and control conditions.

by Azri et al.[4] For each time of the kinetics, the basal portions of the tow stems were used. Total RNA was extracted according to the method of Chang et al.[33] and then treated with RNase-free RQ1 DNase (Promega, Charbonnières-les-Bains, France). RNA concentration and quality were determined at 260 and 280 nm using the NanoDrop1000TM spectrophotometer (Thermo Fisher Scientific) and checked by agarose gel electrophoresis.

The real-time RT-PCR amplifications were done according to Mai et al.[34] with the specific primers reported in **Table 1**. The oligonucleotides primers were constructed based on specific *Populus* EST database [http://genome.jgi-psf.org] the EST detected with the BLAST program. The reference genes 18S RNA and Ubiquitin transcripts (POPTR_0012s01250, Phytozome http://www.phytozome. net) were amplified using the primers 18SF 5'-CTT CGG GAT CGG AGT AAT GA-3', 18SR 5'-GCG GAG TCC TAG AAG CAA CA-3', and UbiF 5'-CCC GGC TCT AAC CAT ATC CA-3', UbiR 5'-GGG TCC AGC TTC TTG CAG TC-3', respectively. The housekeeping genes were combined into an index using the BestKeeper software tool (http://www.wzw.tum.de/gene-quantifaccation/bestkeeper.html).[35] Target gene abundance was conventionally normalized using this BestKeeper Index (I) using the delta-delta method mathematical model[36] and genes 18S rRNA and Ubiquitin transcripts were used at the reference. Target gene abundance was conventionally normalized using this BestKeeper Index (I) using the delta-delta method mathematical model.[36]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Nakamura T, Negishi Y, Funada R, Yamada M. Sedimenʃ amyloplasts in starch sheath cells of woody stems of *Japanese cherry.* Adv Space Res 2001; 27:957-60; PMID:11596639; http://dx.doi.org/10.1016/S0273-1177(01)00171-5.
2. Perera IY, Hung CY, Brady S, Muday GK, Boss WF. A universal role for inositol 1,4,5-trisphosphate-mediated signaling in plant gravitropism. Plant Physiol 2006; 140:746-60; PMID:16384898; http://dx.doi.org/10.1104/pp.105.075119.
3. Chang SC, Cho MH, Kim S-K, Lee JS, Kirakosyan A, Kaufman PB. Changes in phosphorylation of 50 and 53 kDa soluble proteins in graviresponding oat (*Avena sativa*) shoots. J Exp Bot 2003; 54:1013-22; PMID:12598571; http://dx.doi.org/10.1093/jxb/erg104.
4. Azri W, Chambon C, Herbette S, Brunel N, Coutand C, Leplé J-C, et al. Proteome analysis of apical and basal regions of poplar stems under gravitropic stimulation. Physiol Plant 2009; 136:193-208; PMID:19453506; http://dx.doi.org/10.1111/j.1399-3054.2009.01230.x.
5. Krieger-Brauer HI, Kather H. Human fat cells possess a plasma membrane-bound H2O2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. J Clin Invest 1992; 89:1006-13; PMID:1311714; http://dx.doi.org/10.1172/JCI115641.
6. Finkel T. Oxygen radicals and signaling. Curr Opin Cell Biol 1998; 10:248-53; PMID:9561849; http://dx.doi.org/10.1016/S0955-0674(98)80147-6.
7. Rhee SG, Bae YS, Lee S-R, Kwon J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cystein oxidation. Science'STKE. 2000; www.stke.org/cgi/content/full/OCsigtrans;2000/53/pe1.
8. Laloi C, Apel K, Danon A. Reactive oxygen signalling: the latest news. Curr Opin Plant Biol 2004; 7:323-8; PMID:15134754; http://dx.doi.org/10.1016/j.pbi.2004.03.005.
9. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004; 55:373-99; PMID:15377225; http://dx.doi.org/10.1146/annurev.arplant.55.031903.141701.
10. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science 1995; 270:296-9; PMID:7569979; http://dx.doi.org/10.1126/science.270.5234.296.
11. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, et al. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. J Biol Chem 1997; 272:217-21; PMID:8995250; http://dx.doi.org/10.1074/jbc.272.1.217.
12. Joo JH, Bae YS, Lee JS. Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol 2001; 126:1055-60; PMID:11457956; http://dx.doi.org/10.1104/pp.126.3.1055.
13. Kwak JM, Nguyen V, Schroeder JI. The role of reactive oxygen species in hormonal responses. Plant Physiol 2006; 141:323-9; PMID:16760482; http://dx.doi.org/10.1104/pp.106.079004.
14. Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 2005; 17:1866-75; PMID:15987996; http://dx.doi.org/10.1105/tpc.105.033589.
15. Agrawal GK, Rakwal R. Rice proteomics: a cornerstone for cereal food crop proteomes. Mass Spectrom Rev 2006; 25:1-53; PMID:15957154; http://dx.doi.org/10.1002/mas.20056.

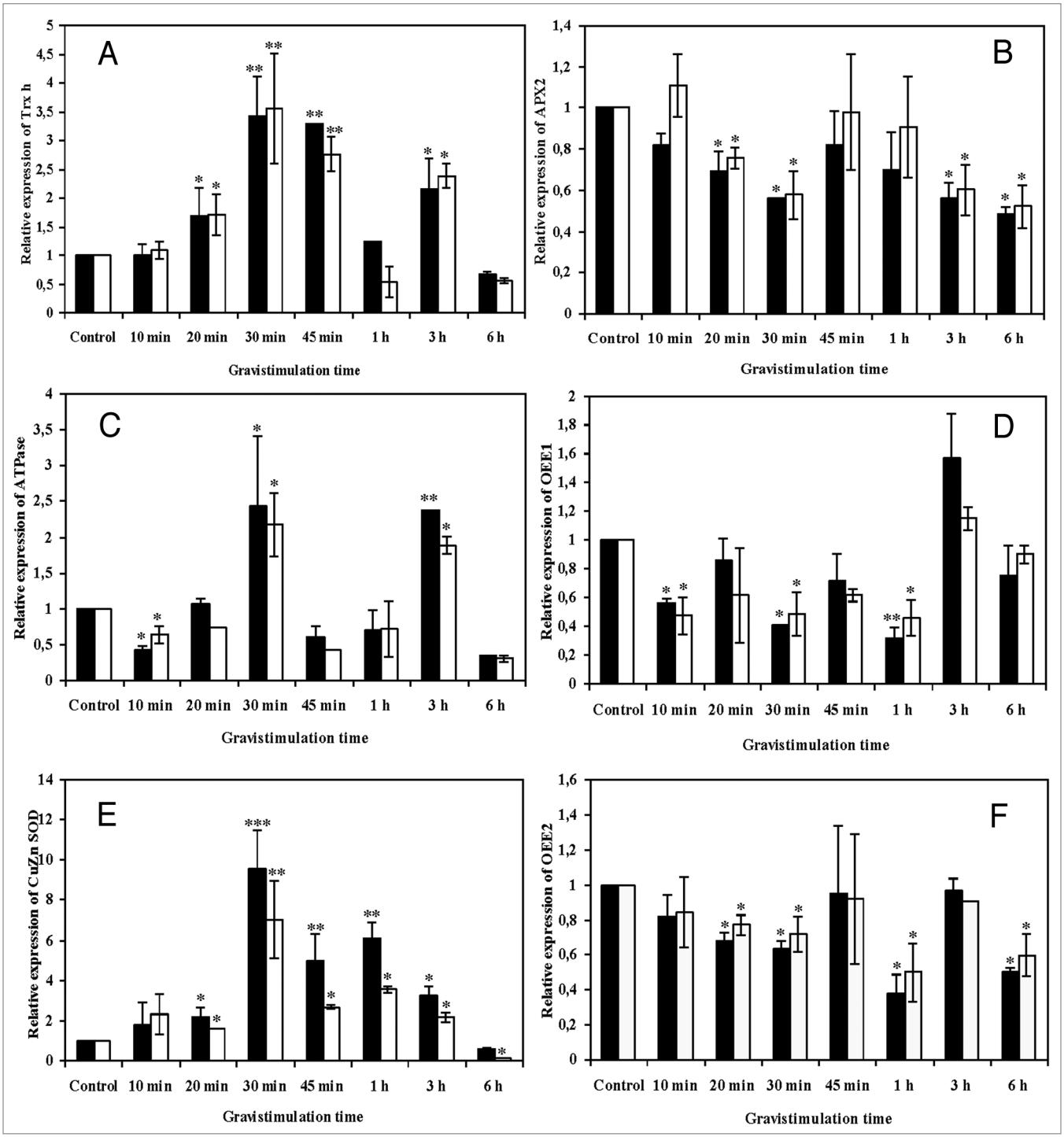

Figure 2. Time course accumulation of transcripts *Trx h* (A), *APX2* (B), *ATPase* (C), *OEE1* (D), *CuZn SOD* (E) and *OEE2* (F) after gravitational stimulus. Total RNAs were extracted from basal internodes of inclined plants for 0 (control), and 10, 20, 30, 45 min, 1 h, 3 h and 6h. The accumulation of relative transcripts was determined by RT-qPCR. Mean values (+SE) of three technical replicates are shown. For each time, one plant is analyzed. Similar results were obtained on a second series of plants (white histograms). Asterisks (*) represent Student's t-test significant at $p \leq 0.05$. Double asterisks (**) represent Student's t-test significant at $p \leq 0.01$. Asterisks (***) represent Student's t-test significant at $p \leq 0.001$.

16. Zhang CJ, Zhao BC, Ge WN, Zhang YF, Song Y, Sun DY, et al. An apoplastic h-type thioredoxin is involved in the stress response through regulation of the apoplastic reactive oxygen species in rice. Plant Physiol 2011; 157:1884-99; PMID:22010108; http://dx.doi.org/10.1104/pp.111.182808.

17. Joo JH, Yoo HJ, Hwang I, Lee JS, Nam KH, Bae YS. Auxin-induced reactive oxygen species production requires the activation of phosphatidylinositol 3-kinase. FEBS Lett 2005; 579:1243-8; PMID:15710420; http://dx.doi.org/10.1016/j.febslet.2005.01.018.

18. Kimbrough JM, Salinas-Mondragon R, Boss WF, Brown CS, Sederoff HW. The fast and transient transcriptional network of gravity and mechanical stimulation in the *Arabidopsis* root apex. Plant Physiol 2004; 136:2790-805; PMID:15347791; http://dx.doi.org/10.1104/pp.104.044594.

19. Requejo R, Tena M. Proteome analysis of maize roots reveals that oxidative stress is a main contributing factor to plant arsenic toxicity. Phytochemistry 2005; 66:1519-28; PMID:15964037; http://dx.doi.org/10.1016/j.phytochem.2005.05.003.
20. Moseyko N, Zhu T, Chang HS, Wang X, Feldman LJ. Transcription profiling of the early gravitropic response in Arabidopsis using high-density oligonucleotide probe microarrays. Plant Physiol 2002; 130:720-8; PMID:12376639; http://dx.doi.org/10.1104/pp.009688.
21. Horling F, Lamkemeyer P, König J, Finkemeier I, Kandlbinder A, Baier M, et al. Divergent light-, ascorbate-, and oxidative stress-dependent regulation of expression of the peroxiredoxin gene family in *Arabidopsis*. Plant Physiol 2003; 131:317-25; PMID:12529539; http://dx.doi.org/10.1104/pp.010017.
22. Sweetlove LJ, Heazlewood JL, Herald V, Holtzapffel R, Day DA, Leaver CJ, et al. The impact of oxidative stress on *Arabidopsis* mitochondria. Plant J 2002; 32:891-904; PMID:12492832; http://dx.doi.org/10.1046/j.1365-313X.2002.01474.x.
23. Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa KI, Yokota A, et al. Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts. FEBS Lett 1998; 428:47-51; PMID:9645472; http://dx.doi.org/10.1016/S0014-5793(98)00483-9.
24. Pnueli L, Liang H, Rozenberg M, Mittler R. Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)-deficient *Arabidopsis* plants. Plant J 2003; 34:187-203; PMID:12694594; http://dx.doi.org/10.1046/j.1365-313X.2003.01715.x.
25. Baier M, Dietz K-J. Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. J Exp Bot 2005; 56:1449-62; PMID:15863449; http://dx.doi.org/10.1093/jxb/eri161.
26. Sugihara K, Hanagata N, Dubinsky Z, Baba S, Karube I. Molecular characterization of cDNA encoding oxygen evolving enhancer protein 1 increased by salt treatment in the mangrove Bruguiera gymnorrhiza. Plant Cell Physiol 2000; 41:1279-85; PMID:11092914; http://dx.doi.org/10.1093/pcp/pcd061.
27. Yang EJ, Oh YA, Lee ES, Park AR, Cho SK, Yoo YJ, et al. Oxygen-evolving enhancer protein 2 is phosphorylated by glycine-rich protein 3/wall-associated kinase 1 in Arabidopsis. Biochem Biophys Res Commun 2003; 305:862-8; PMID:12767910; http://dx.doi.org/10.1016/S0006-291X(03)00851-9.
28. Bradley DJ, Kjellbom P, Lamb CJ. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell 1992; 70:21-30; PMID:1623521; http://dx.doi.org/10.1016/0092-8674(92)90530-P.
29. Depège N, Varenne M, Boyer N. Induction of oxidative stress and GPX-like protein activation in tomato plants after mechanical stimulation. Physiol Plant 2000; 110:209-14; http://dx.doi.org/10.1034/j.1399-3054.2000.110210.x.
30. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 1962; 15:473-92; http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x.
31. Herbette S, Brunel N, Prensier G, Julien JL, Drevet JR, Roeckel-Drevet P. Immunolocalization of a plant glutathione peroxidase-like protein. Planta 2004; 219:784-9; PMID:15164287; http://dx.doi.org/10.1007/s00425-004-1284-8.
32. Marengo E, Robotti E, Antonucci F, Cecconi D, Campostrini N, Righetti PG. Numerical approaches for quantitative analysis of two-dimensional maps: a review of commercial software and home-made systems. Proteomics 2005; 5:654-66; PMID:15669000; http://dx.doi.org/10.1002/pmic.200401015.
33. Chang S, Puryear J, Cairney J. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 1993; 11:113-6; http://dx.doi.org/10.1007/BF02670468.
34. Mai J, Herbette S, Vandame M, Kositsup BA, Kasemsap P, Cavaloc E, et al. Effect of chilling on photosynthesis and antioxidant enzymes in Hevea brasiliensis Muell. *Arg.* Trees – Struct Funct 2009; 23: 863-874.
35. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper--Excel-based tool using pair-wise correlations. Biotechnol Lett 2004; 26:509-15; PMID:15127793; http://dx.doi.org/10.1023/B:BILE.0000019559.84305.47.
36. McMaugh SJ, Lyon BR. Real-time quantitative RT-PCR assay of gene expression in plant roots during fungal pathogenesis. Biotechniques 2003; 34:982-6; PMID:12765025.
37. Yahraus T, Chandra S, Legendre L, Low PS. Evidence for mechanically induced oxidative burst. Plant Physiol 1995; 109:1259-66; PMID:12228667.
38. Foyer CH, Lopez-Delgado H, Dat JF, Scott IM. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant 1997; 100:241-54; http://dx.doi.org/10.1111/j.1399-3054.1997.tb04780.x.
39. Cazalé A-C, Rouet-Mayer M-A, Barbier-Brygoo H, Mathieu Y, Laurière C. Oxidative burst and hypoosmotic stress in tobacco cell suspensions. Plant Physiol 1998; 116:659-69; PMID:9490766; http://dx.doi.org/10.1104/pp.116.2.659.