

Supplementary files

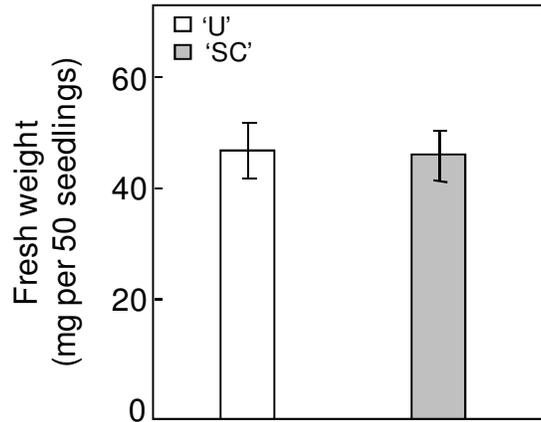


Figure S1. Effect of solvent-control (i.e. ethanol) on tobacco seedlings growth parameters. Fresh weight of 'U' and 'SC' plantlets at the end of test-period (28th day post-treatment). For seedlings weight measurement, 50 seedlings per biological replicate were used. Means \pm SDs, $n = 3$. 'U' - Untreated and 'SC' - GA₃ solvent-control (i.e. <0.1% *v/v* ethanol) treated plantlets.

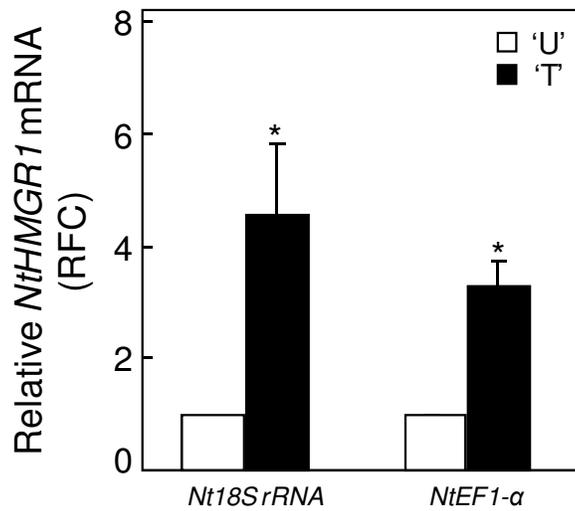


Figure S2. Effect of GA₃ application on *NtHMGR1* transcript levels. Differential *NtHMGR1* mRNA was measured by qRT-PCR using DNA-free RNA samples from 'U' and 'T'. Data analysed by 2^{-ΔΔCT} was depicted as RFC in *NtHMGR1* transcripts with respect to that of corresponding endogenous genes, 18S rRNA and *NtEF-1α*. The *y*-axis showed the *NtHMGR1* mRNA amount (in RFC) corresponding to 'U', which was set at 1. Means \pm SDs, $n = 3$, wherein * indicate the significant differences ($p \leq 0.05$). RFC - Relative fold changes, 'U' - Untreated and 'T' - GA₃ treated plantlets.

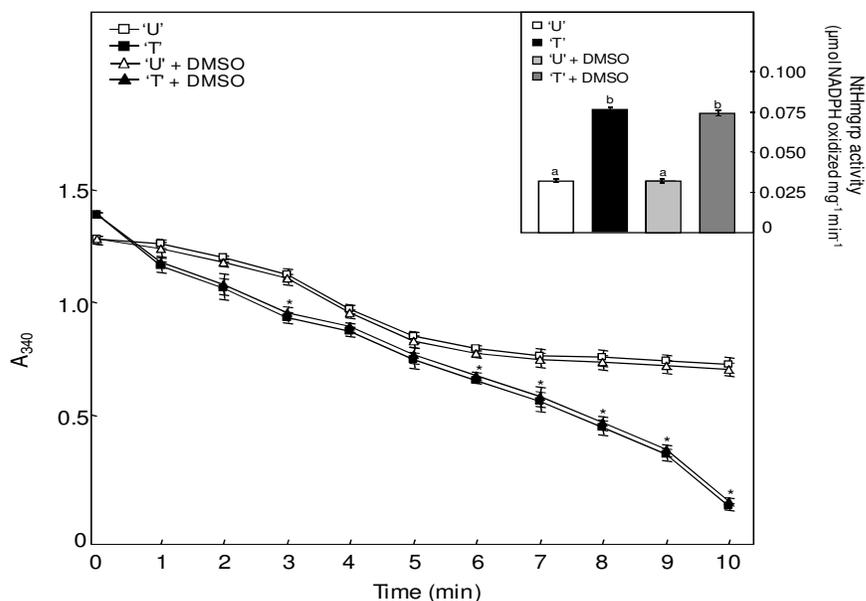


Figure S3. Effect of solvent-control (i.e. DMSO) on NtHMGR activity

Differential NtHMGR activity from 'U' and 'T' cellular-extract (~50 µg) in the absence or presence of solvent (i.e. <math><0.1\% v/v</math> DMSO) of reference statin drug, PRV was measured. NADPH consumption rate was monitored at 37 °C at 340 nm by spectrophotometric-scanning for 10 min. The specific activity of 'U' and 'T' calculated between 60-600 s was observed to be 0.03 and 0.08 µmol NADPH oxidized mg⁻¹ (protein) min⁻¹, respectively. The inset depicts HMGR specific activity of each sample at the end of the test period (10 min). Means ± SDs, $n = 3$, wherein * indicate the significant differences ($p \leq 0.05$). DMSO - Dimethyl sulfoxide, PRV - Pravastatin, 'U' - Untreated and 'T' - GA₃ treated plantlets.

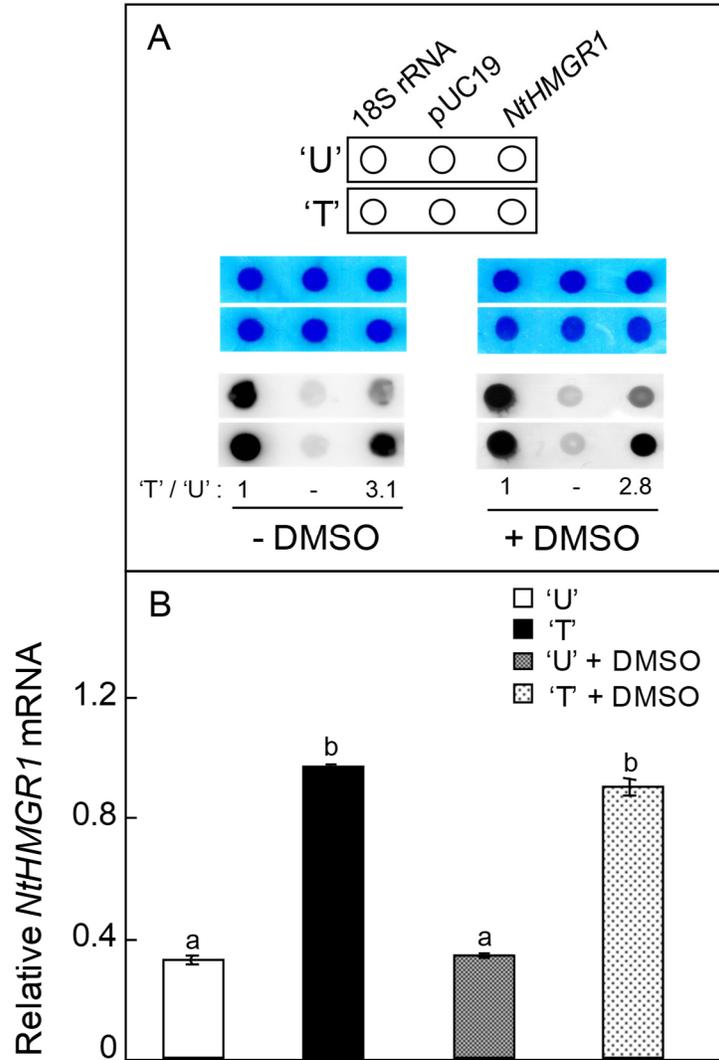


Figure S4. Effect of solvent-control (i.e. DMSO) on transcriptional rate of *NtHMGR1*

A, approximately 100 ng of empty-vector plasmid DNA, pUC19 (negative-control), 18S rDNA (positive-control) and *NtHMGR1* PCR amplicons were immobilized on positive-charged nylon membranes. The blots were probed with α -[³²P] UTP-labelled nascent NRO transcripts derived from permeabilized nuclear-fractions of 'U' and 'T'. As a solvent control for nascent transcript synthesis inhibitor, COR, the pre-treatment of isolated nuclear-fraction of 'U' and 'T' with DMSO (<0.1% *v/v*) was performed. The *upper*-, *middle*- and *bottom*-panel showed the relative position of individual dot-blotted DNA, loading-control (methylene-blue stain) and recognition of dotted DNA by hybridized labelled nuclear RNA, respectively. The signal-intensity of hybridized nuclear RNA of *NtHMGR1* from 'U' and 'T' was quantified using densitometry-scanning of phosphoimages. 'T' / 'U' ratio under the blot indicates the normalised nuclear RNA intensity between the indicated samples following background-correction. *B*, Relative signal-intensity of *NtHMGR1* transcript with respect to corresponding 18S rRNA was plotted as bar-graph. Means \pm SDs, *n* = 2, wherein * indicate the significant differences (*p* \leq 0.05). DMSO - Dimethyl sulfoxide, TRO - Transcription Run-On, '- DMSO' - without DMSO, '+ DMSO' - with DMSO addition, 'U'- Untreated and 'T' - GA₃ treated plantlets.

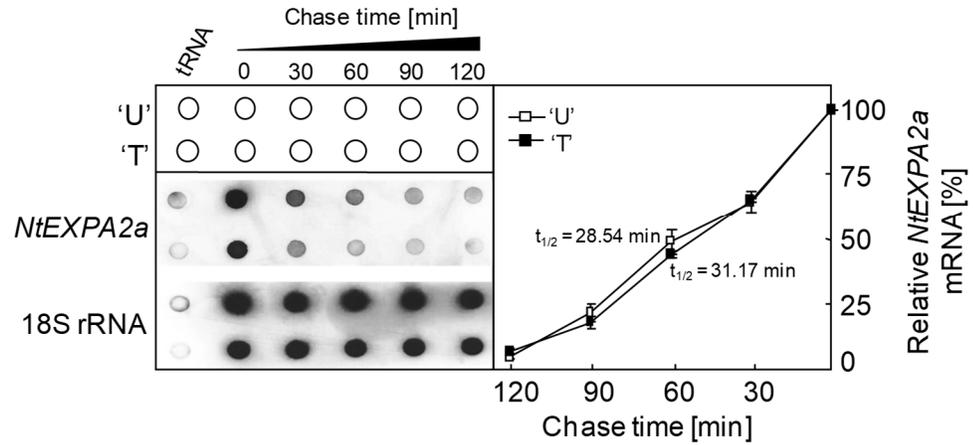


Figure S5. Measurement of *NtEXPA2a* mRNA decay

Post-addition of an optimized concentration ($200 \mu\text{g ml}^{-1}$) of COR, an *in vivo* transcriptional-inhibitor, total RNAs was isolated from 'U' and 'T' at the indicated time of chase (0, 30, 60, 90 and 120 min) and thereafter subjected to dot-blot hybridization ($\sim 2 \mu\text{g dot}^{-1}$). The upper-, middle- and bottom-panel showed the relative position of individual dot-blotted RNA and recognition of dotted RNA by hybridized α - ^{32}P -labelled DNA probes of *NtEXPA2a* (nucleotides: 276-444 from the start codon) and 18S rDNA, respectively (*left-panel*). The signal-intensity of hybridized *NtEXPA2a* transcripts from 'U' and 'T' at each time-point was quantified by densitometry-scanning and base-corrected to corresponding constitutively expressed 18S rRNA. Normalized signal-intensity of *NtEXPA2a* transcripts with respect to corresponding time T_0 was plotted as line-graph for both 'U' and 'T' and expressed in % (*right-panel*). *NtEXPA2a* mRNA $t_{1/2}$ was calculated by fitting non-linear regression. Means \pm SDs, $n = 2$. COR - Cordycepin, $t_{1/2}$ - half-life, 'U' - Untreated and 'T' - GA_3 treated plantlets.