TOR coordinates with transcriptional and chromatin machinery to regulate thermotolerance and thermomemory

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ABSTRACT

Global warming exhibits profound effects on plant fitness and productivity. To withstand stress, plants sacrifice their growth and activate protective stress responses for ensuring survival. However, the switch between growth and stress is largely elusive. In the past decade, emerging role of Target of Rapamycin (TOR) has been studied linking energy and stress signaling. Here, we have identified an important role of Glc-TOR signaling in plant adaptation to heat stress (HS). We show that Glc-TOR signaling regulates thermotolerance and thermomemory response through distinct mechanisms. Glc-TOR via E2Fa signaling module governs the transcriptome reprogramming of a myriad set of genes involved in HS protection and recovery. Glc also epigenetically governs the transcription of core HS signaling genes in a TOR-dependent manner. TOR acts in concert with p300/CREB HISTONE ACETYLTRANSFERASE1 (HAC1) and dictates the epigenetic landscape of HS loci to regulate thermotolerance. Arabidopsis plants defective in *TOR* and *HAC1* exhibited reduced thermotolerance with a decrease in expression of core HS signaling genes. TOR also promotes accumulation of histone H3K4me3 marks at the promoters of thermomemory-related genes and therefore, governs thermomemory. Further, genome-wide transcriptome of

Glc-TOR shows huge overlap with targets of chromatin remodeler BRAHMA, especially with genes enriched in stress, chromatin remodelling and histone/protein modifications. Collectively, our findings thus reveal a mechanistic framework in which Glc-TOR signalling through multiple modules determines the integration of stress and energy signaling to regulate thermotolerance and thermomemory.

INTRODUCTION

Plants are immobile in nature and are unable to escape the harmful conditions in the environment. Rather, over the course of evolution, they have evolved adaptive strategies to gauge and respond to these stressful conditions for survival and reproductive success. At the time of stress, plants sacrifice their growth to activate protective stress machinery and ensure survival. Once stress signal has vanished, plant quickly cease the stress machinery and activate plant growth and recovery. Sugars produced through photosynthesis are pivotal for plant growth and development. Global transcriptome profiling in response to sugars or mutants defective in sugar signaling showed alteration in stress induced gene transcripts such as heat shock proteins (Mishra et al., 2009; Xiong et al., 2013a; Gupta et al., 2015a). In the past two decades, researchers have established the crucial role of TOR Kinase in stress resilience and adaptation (Mahfouz et al., 2006; Deprost et al., 2007; Wang et al., 2016; Dong et al., 2018; Wang et al., 2018; Sharma et al., 2019). TOR is an evolutionary conserved ser/thr kinase, comprised of a multiprotein complex and is a central regulator of different environmental responses in eukaryotes. TOR kinase was first identified in mammals as a target of a molecule named rapamycin isolated from a bacteria *Streptomyces hygroscopicus* (Sehgal et al., 1975). In mammals and yeast, TOR kinase has been shown to play role in diverse biological processes and is composed of two large multiprotein complex named TORC1 and TORC2 in yeast and mTORC1 and mTORC2 in mammals (Laplante and Sabatini, 2012). Sequence and structure-based homology has identified several mTORC1 components in photosynthetic organisms that include Regulatory-Associated Protein of TOR (RAPTOR) and Lethal with Sec-13 protein 8 (LST8) (Deprost et al., 2005; Mahfouz et al., 2006; Xiong and Sheen, 2014). The complex domain architecture of Arabidopsis TOR is evolutionary conserved among eukaryotes including mammals and plants (Menand et al., 2002a; Zoncu et al., 2011). Mutants of *RAPTOR* and *LST8* exhibit defects in embryogenesis and post-embryonic development which resemble with *tor* mutant phenotype indicating the conserved role of these TOR targets in TOR-mediated growth and development (Anderson et al., 2005; Moreau et al., 2012; Shi et al., 2018). Previous reports have shown that TOR

transduces photosynthesis derived Glc/energy signals to activate progenitor stem cells in the root and shoot meristem. TOR through interaction and direct phosphorylation, activates E2Fa/b transcription factors in controlling root and shoot meristem (Xiong et al., 2013a; Li et al., 2017). The heterodimeric E2F-DP transcription factors are conserved regulator of cell cycle. E2Fa controls the transcriptional regulation of genes underlying DNA replication and cell cycle through recognition of conserved cis-acting TTTCCCGCC and other similar elements in their promoter region (Vandepoele et al., 2005; Xiong et al., 2013a).

In past years, TOR kinase has emerged as a central regulator of nutrient and stress responses. In Plants, roots absorb water, essential nutrients and minerals from the soil and slight changes in soil nutrient composition affect root system architecture. Soil nitrogen insufficiency leads to increased root growth whereas excess soil nitrate content causes strong root growth inhibition (Zhang et al., 1999; Deak and Malamy, 2005). TOR overexpressing (OE) plants showed longer primary roots under excessive nitrate and salinity, however, no detectable changes were observed under N-starvation. Moreover, plants with reduced TOR levels showed shorter primary roots with an increased sensitivity to osmotic stress (Deprost et al., 2007). In addition, TOR interacting protein RAPTOR1 controls the activity of S6K1 in response to osmotic stress (Mahfouz et al., 2006). Cold stress caused the transient inhibition of TOR kinase activity which was increased during long term cold exposure. Cold exposure converted the purple hypocotyl of *tor RNAi* lines into green which was supposed to be due to decreased translation in *tor RNAi* lines and their ability to survive under cold stress (Wang et al., 2016). TOR kinase regulates ABA signaling under non-stress condition, however, ABA controlled SnRK2 phosphorylates RAPTOR1 in response to stress. TOR kinase phosphorylates ABA receptor PYL at conserved Ser residue which dissociates the link between ABA and PP2C phosphatase, conferring inactivation of SnRK2. Onset of stress leads to phosphorylation of RAPTOR1 through ABA activated SnRK2 which disrupts TOR complex association and therefore, inhibition of TOR kinase activity. Plants employ this conserved phospho-regulatory loop to adjust the homeostasis of growth and stress response (Wang et al., 2018). Ectopic expression of Arabidopsis TOR in a cultivated Indica rice variety increased plant growth and water use efficiency under water-limiting conditions (Bakshi et al., 2017). Limitation of sulphur precursor is transduced by TOR through down-regulation of Glc metabolism, with a decrease in TOR activity leading to decreased translation, lowered meristematic activity and increased autophagy (Dong et al., 2017). In addition to abiotic stresses, TOR negatively regulates defence response to bacterial and

fungal pathogens through an antagonistic interaction with jasmonic and salicylic acid signaling (De Vleesschauwer et al., 2017).

Plants possess an inherent ability to withstand certain levels of stress, an ability called basal tolerance. In nature, plants are not only challenged by a single exposure of stress, rather they are exposed by multiple and chronic, sometimes, irregular stresses. Thus, plants have evolved strategies to cope up that enables them to withstand stress in a superior manner if prior confrontation with the same stress, called acquired tolerance. The preservation of acquired tolerance in the leisure of interfering stress is called stress memory (Balmer et al., 2015). Numerous reports have described priming or stress memory in response to salt, drought, heat and pathogen attack (Conrath, 2011; Ding et al., 2012a; Sani et al., 2013; Lämke et al., 2016; Sharma et al., 2019). The majority of stress responses revert back to pre-stress stage shortly after stress, which allows them to forget stressful period and allocate resources to support plant growth. In contrast, stress memory enables plants to respond more efficiently to future stress suggesting essential temporal dynamics but underexplored. Therefore, a delicate balance between stress forgetfulness and memory is required for optimum growth and survival under ever-changing environment (Crisp et al., 2016).

Chromatin structures lie at the core of stress responses and play crucial roles ranging from sensory to downstream gene activation (Kumar and Wigge, 2010; Lämke and Bäurle, 2017). Structure of chromatin is remodelled at various levels such as alternation in histone variants, nucleosome positioning, gene looping and post-transcriptional histone and protein modifications (Deal and Henikoff, 2010; Luo et al., 2012; Struhl and Segal, 2013). Histone modifications can activate or repress gene transcriptional response depending on the nature and position of residues which are modified. In most cases, modification of histone lysine through acetylation correlates with active gene transcription whereas lysine methylation at different positions serve as activating or repressing marks, for example, H3K4me2 and H3K4me3 promote active gene transcription whereas H3K27me3 serve as repressive histone mark. In addition, histone demethylation serves as important determinant of chromatin structures and performs critical roles in developmental and stress responses (Cui et al., 2016; Liu et al., 2019). The member of SWI/SNF complex BRAHMA (BRM) acts in concert with histone H3K27me3 demethylase REGULATOR OF EARLY FLOWERING 6 (REF6) and performs active roles in gene transcription at the chromatin level. REF6 facilitates the recruitment of BRM at specific genomic loci containing a CTCTGYTY motif (Li et al., 2016).

Lately, Sharma et al., 2019 identified the pivotal role of Glc and Glc-activated TOR signaling in plant response to HS. Arabidopsis TOR overexpressing plants showed increased short term acquired thermotolerance (SAT) whereas plants with subdued TOR displayed reduced SAT (Sharma et al., 2019). However, the underlying mechanism how TOR controls thermotolerance response is unknown. Here, we show important regulatory role played by Glc-TOR in mediating thermotolerance response. Whole genome transcriptome analysis of Arabidopsis seedlings in response to heat and Glc sufficiency exhibited large synergistic interaction with TOR transcriptomic data. TOR, in concert with p300 histone acetyltransferase 1 (HAC1) modulates the acetylation of HS loci in conferring SAT. In addition to SAT, Arabidopsis TOR remembers the past exposure of HS for several days through maintenance of expression and protein levels of thermomemory associated gene(s). TOR promotes the accumulation of H3K4me3 methylation at the promoters of thermomemory-related genes and thus causing thermomemory.

RESULTS

Exogenous glucose mimics the natural environmental conditions in governing thermotolerance

Glucose (Glc) is a vital source of energy for most of the organisms on earth ranging from bacteria, yeast, animals, plants and humans. It serves as a fundamental metabolic and signaling molecule in regulating multifaceted roles including transcriptional reprogramming, translation, organism growth and development, cell cycle control, disease and stress responses. Recently, Sharma et al., 2019 revealed the important regulatory role of Glc signaling in thermotolerance. In addition, Glc activated TOR-E2Fa signaling is required to cope deleterious effects of HS (Sharma et al., 2019). However, the molecular mechanism how Glc driven TOR signaling governs temperature mediated response is hitherto unknown. Under natural environmental conditions, glucose production depends on the availability of light. This photosynthetically generated high glucose levels could modulate root architecture (Gupta et al., 2015b). Endogenous sugar levels in plants could be altered by fluctuating light conditions (Kircher and Schopfer, 2012). To test whether high light generated glucose could provide thermotolerance to Arabidopsis plants, we grew plants under low (2000 lux) and high light (10000 lux) conditions in presence of low and moderate glucose and effect of HS was analysed. High light intensity (10000 lux) could increase the HS tolerance in Arabidopsis seedling as compared to low light (2000 lux) (Figure 1A-D). High light along with moderate glucose (56mM) displayed synergistic effect on HS tolerance, as shown by robust plant

growth (Figure 1A-D). We assessed the phenotype of Arabidopsis Col-0, TOR OE lines (G166 and G548) and *tor 35-7* RNAi lines under high and low light intensity in presence of low and moderate Glc and challenged them for HS. Arabidopsis plant overexpressing TOR survived better under high light intensity (10000LUX) than Col-0 plants. In contrast, *tor35-7RNAi* lines exhibited severe death phenotype under these high light intensities (Figure 1A-D). Arabidopsis G166 and G548 displayed higher growth recovery as revealed by higher fresh weight, chlorophyll content and increased number of lateral roots under high light intensities whereas plant under-expressing TOR showed reduced growth and recovery (Figure 1A-C). Since photosynthesis produces sucrose that is converted to glucose and fructose before entering metabolism, it was important to test whether high light-mediated thermo-protection is specific to Glc. To test this, we blocked Glc signaling/metabolism by using Glc analogue 2-deoxy-Glucose (2-DG) and checked thermotolerance response under high light condition. 2-DG is actively transported through hexose transporters and phosphorylated but cannot be metabolised. 2-DG-phoshate interferes with Glc metabolism through inhibition of glycolytic enzymes (Ralser et al., 2008). Five-day-old MS grown Arabidopsis Col-0 seedlings under standard light intensity were transferred to Glc (56mM) containing MS media without or with Glc analogue 2-DG (2.5mM). After transfer, seedlings were transferred to high light intensity (approximately 12000 LUX) for 24h and then subjected to heat stress at 1h_37ºC, 2h_22ºC, 2.5h_45ºC and 3-7d_22ºC. Interestingly, we found that plants treated with 2-DG could not provide tolerance under high light intensity further substantiating specific role of photosynthesis derived Glc signaling/metabolism in governing thermotolerance response (Supplemental Figure 1A-D).

Presence of Glc profoundly alters the expression profile of HS regulated genes

To dissect the contribution of Glc/energy in HS transcriptional response, whole genome microarray analysis of Arabidopsis Col-0 seedlings was performed. Five-day-old Arabidopsis seedlings were subjected to Glc free (-Glc) MS media for 24h followed by 24h Glc treatment (+Glc; 167mM). Following acclimation to Glc medium, seedlings were transferred to HS (37°C_3h) and HS Rec (37°C_3h followed by 22°C_3h). The cRNA was prepared from RNA isolated from Glc and HS treated samples and were hybridized onto Affymetrix Arabidopsis whole genome ATH1 arrays. The extent and overlap of Glc and HS regulated genes at the transcriptional level was analysed to determine the unique and overlapping set of genes, which are crucial targets of Glc-HS signaling crosstalk. We used six treatment conditions and designated following terms for Glc free (-Glc), Glc only (+Glc), HS only (-Glc/+HS), Glc

and HS (+Glc/+HS), HS followed by recovery (-Glc/+HS Rec) and Glc and HS followed by recovery (+Glc/+HS Rec).We considered the differential expression of all HS genes affected 1.5-fold or more in the presence of Glc. Out of 2311 differentially regulated HS (-Glc/+HS) genes, 1124 (49%) genes were also found to be up- and down-regulated by Glc and HS (+Glc/+HS; Figure 2A; Supplemental Table S1A). Out of these 1124 genes, 663 (59%) genes were synergistically (down-regulated by both -Glc/+HS and +Glc/+HS) downregulated (Figure 2B and 2C; Supplemental Table S1A). Moreover, out of 460 (41%) –Glc/+HS up-regulated genes, presence of Glc synergistically (up-regulated by both -Glc/+HS and +Glc/+HS) up-regulated 452 genes (Figure 2B and 2C; Supplemental Table S1A). Only 9 (0.8%) genes were antagonistically (-Glc/+HS up and +Glc/+HS down; -Glc/+HS down and +Glc/+HS up) regulated by both –Glc/+HS and +Glc/+HS. The other feature of interest was to analyse how the –Glc/+HS mediated induction or suppression of genes is affected in seedlings treated with +Glc/+HS. Among 1124 –Glc/+HS and +Glc/+HS co-regulated genes, the extent of HS induction/suppression was affected (FC ≥ +/- 1.5) in 259 (24%) genes in the presence of Glc (Figure 2D; Supplemental Table S1B). Out of these 259 genes, Glc was able to affect the extent of HS regulation antagonistically in 174 (115+59; 67%) genes and agonistically in 85 (65+20; 33%) genes. Out of 460 –Glc/+HS up-regulated genes, Glc could affect the HS induction in 180 (115+65; 39%) genes of which 115 (64%) genes were affected antagonistically and only 65 (36%) genes were affected agonistically. Similarly, out of 664 -Glc/+HS down-regulated genes, presence of Glc could affect the extent of HS mediated suppression in 79 (59+20; 19%) genes. Out of these 79 genes, presence of Glc could affect 59 (75%) genes antagonistically while only 20 (25%) genes were agonistically regulated by Glc (Figure 2D). Interestingly, an exclusive set of genes was also regulated by both –Glc/+HS (971 genes) and +Glc/+HS (1408 genes) category (Supplemental Table S1C and S1D). GO up-regulated categories enriched under –Glc/+HS conditions mostly belonged to stress responses whereas growth related processes such as root, embryo, seed and fruit development, fatty acid, lipids and other organic acids and amino acid biosynthetic processes required for organism growth were severely down-regulated (Figure 2E and Supplemental Figure S3A). In contrast, processes related to nucleotide biosynthesis, rRNA processing and ribonucleoprotein complex biogenesis required for ribosome synthesis were highly up-regulated only in +Glc/+HS (Figure 2E). TOR, as a master regulator, senses and transduces cellular energy signals and regulates thousands of genes involved in many biological and metabolic processes such as cell, cycle, translation, growth, signaling, and stress response. We, therefore, compared the transcriptome of TOR regulated genes from the public

microarray database with our own microarray data of Glc-HS. We found that Glc-TOR and +Glc/+HS regulate transcriptome reprogramming largely by synergistic interaction (total 560 genes; 310 genes agonistically down-regulated and 250 genes agonistically up-regulated by both Glc-TOR and +Glc/+HS) and only a small number were regulated antagonistically (total 89; 59 genes up-regulated by Glc-TOR and down-regulated by +Glc/+HS and 30 genes down-regulated by Glc-TOR and up-regulated by +Glc/+HS) (Figure 2F; Supplemental Table S2A). GO category enriched under synergistic down-regulation were involved in sucrose starvation and catabolic processes such as carbohydrate, amino acids, organic and carboxylic acid catabolism whereas processes related to protein folding, de novo posttranslational protein folding, peptidyl-prolyl isomerization and protein complex oligomerization were synergistically up-regulated by Glc-TOR and +Glc/+HS (Supplemental Figure S3B and S3C). In contrast, –Glc/+HS category showed major antagonism with Glc-TOR microarray (total 312 genes; 173 genes were up-regulated by Glc-TOR and down-regulated by –Glc/+HS and 139 genes were down-regulated by Glc-TOR and up-regulated by –Glc/+HS) (Figure 2G; Supplemental Table S2B). In contrast, 176 genes were agonistically regulated by both Glc-TOR and –Glc/+HS (97 genes synergistically down-regulated and 79 genes were synergistically up-regulated by both Glc-TOR and –Glc/+HS) (Figure 2G; Supplemental Table S2B). GO analysis of these genes showed that 139 genes (TOR down and –Glc/+HS up) mostly enriched in HS acclimation and stress response (Supplemental Figure S4A). Moreover, 173 genes (up by TOR and down by –Glc/+HS) enriched mostly belong to biosynthetic processes and DNA replication (Supplemental Figure S4B). In addition, 97 genes underlying starvation and catabolic processes were simultaneously down-regulated by both TOR and –Glc/+HS whereas 79 core genes involved in protein folding and HS acclimation were commonly up-regulated by TOR and –Glc/+HS (Supplemental Figure S4C and S4D).

Glc triggers HS transcriptome for better growth and recovery after harmful stress

To better understand how Glc regulates HS response under stress and post stress period, we analysed the overlap of HS transcriptome with post-HS (HS followed by recovery at 22°C) transcriptome data. We compared the genes regulated by +Glc/+HS with that of +Glc/+HS Rec. Intriguingly, we observed that Glc maintained the sustenance of expression in 1332 genes (Figure 3A; Supplemental Table S3A). In addition, out of 2913 +Glc/+HS regulated genes, 702 genes were found to be overlapping with +Glc/+HS Rec (Figure 3A; Supplemental Table S3B). Functional categorization of genes co-regulated by +Glc/+HS and

+Glc/+HS Rec enriched many important biological processes involved in plant tolerance to HS. Most genes belonging to de novo protein folding, protein refolding, chaperone-dependent protein folding, post-translational protein folding, rRNA metabolism and processing, rRNA transcription and ribosome biogenesis were up-regulated by both +Glc/+HS and +Glc/+HS Rec whereas processes related to sucrose starvation and catabolism were down-regulated (Figure 3C and 3E, Supplemental Figure S5A). Stress response leads to cessation of plant growth and once the stress has gone, plants regain their growth. Growth requires cell division and proliferation from meristematic organs. Remarkably, 630 genes were exclusively regulated by +Glc/+HS Rec with enrichment of processes related to cell division, cell wall modification, DNA unwinding, geometric changes and replication together with histone and protein methylation indicating major role of Glc/energy in post stress growth recovery process which was abrogated in heat stressed plants lacking Glc/energy (Figure 3A and 3B; Figure 3F; Supplemental Table S3C). Astonishingly, out of 2311 –Glc/+HS differentially regulated genes, only 19 (0.8%) genes showed their sustained expression when recovered at 22°C in absence of Glc. (Figure 3B). Out of these 19 genes, 12 genes exhibited a synergistic relation (10 genes up-regulated and 2 genes down-regulated by both –Glc/+HS and –Glc/+HS Rec) whereas, seven genes showed antagonistic interaction (5 genes up by –Glc/+HS and down by –Glc/+HS Rec; 2 genes down by –Glc/+HS and up by –Glc/+HS Rec) (Figure 3B and 3D; Supplemental Table S3D and S3E).

Next, we compared the transcriptome of -Glc/+HS Rec and +Glc/+HS Rec with Glc-TOR transcriptome data. We observed a large synergistic interaction between Glc-TOR and +Glc/+HS Rec (total 539 genes; 254 genes were synergistically down and 285 up-regulated by both Glc-TOR and +Glc/+HS Rec), however, very few genes (7 genes) were antagonistically regulated (Figure 3G; Supplemental Table S2C). Functional categorisation of 285 up-regulated genes showed emergence of peptide and histone arginine methylation together with processes related to DNA replication, RNA modification, rRNA processing and protein folding (Supplemental Figure S6A). Arginine methylation is a most common posttranslational modification functioning as an activator or a repressor of gene transcription and plays key roles in many biological processes including RNA processing, transcription, DNA damage & repair, ribosome biogenesis and translation (Liu et al., 2010; Fulton et al., 2018). In addition, processes such as sucrose starvation and catabolism were commonly down-regulated by +Glc/+HS and Glc-TOR (Supplemental Figure S6B). Moreover, we checked whether Glc-TOR transcriptome exhibits any overlap with –Glc/+HS Rec. Only very

few genes were found to be overlapping with –Glc/+HS Rec and Glc-TOR, further suggesting prerequisite requirement of Glc mediated signaling to cope with lethal HS (Figure 3H; Supplemental Table S2D). Taken together, these results suggest that Glc-TOR and +Glc/+HS Rec signaling governs synergistic regulation of HS transcriptome and therefore, Glc-TOR signaling pathway is obligatory for plants survival under extreme HS and post-stress period for improved growth and recovery.

TOR target E2Fa binds directly to HSFA1 gene promoters to regulate thermotolerance

In our previous report, TOR target E2Fa binds directly to *HLP1* promoter to activate HS gene expression (Sharma et al., 2019). Moreover, genes which are shown to play roles in stress and energy signaling demonstrated E2Fa binding sites in their promoters (Ramirez-Parra et al., 2003; Vandepoele et al., 2005; Xiong et al., 2013a). E2Fa is a transcription factor and is a direct downstream target of TOR which undergoes phosphorylation through TOR upon sugar/energy sufficiency (Xiong et al., 2013a). It has previously been shown that E2Fa motifs are present in gene promoters of HSFA1 class of HSFs (Ramirez-Parra et al., 2003; Naouar et al., 2009; Xiong et al., 2013a). To investigate whether E2Fa directly regulates heat shock gene transcription by binding to their promoters, we performed *in vivo* ChIP using protoplasts from four-week old Col-0 plants. Anti-E2Fa serum was used to immunoprecipitate E2Fa/promoter and approximately 200-250 bp *HSFA1s* promoter comprising E2Fa binding sites were analysed using qPCR. Two or more regions containing similar or identical E2Fa motifs were chosen. The Amount of chromatin enriched by anti-E2Fa was significantly higher in all four *HSFA1s* (*HFSA1a*, *HSFA1b*, *HSFA1d* and *HSFA1e*; Figure 4A-D). Interestingly, E2Fa also binds directly to *HSFA2* gene promoter (Figure 4E). Furthermore, transcript levels of all four *HSFA1 and HSFA2* genes were significantly reduced in *e2fa-1* [Figure 4F; (Sharma et al., 2019)]. These findings thus indicate that E2Fa transcriptionally activates *HSFA1s* and *HSFA2* genes by binding directly to their promoters, suggesting crosstalk between TOR-E2Fa and HSFA1/HSFA2 mediated HS signaling. Next important question was to answer whether Glc-TOR signalling regulates global HS transcriptome reprogramming through E2F transcription factors binding. To address this, we examined the overlap of genes regulated by Glc/HS from our microarray with publicly available genes containing cis-acting E2Fa motifs in their promoters. Interestingly, we observed that approximately 30% genes were found to contain E2F binding cis-acting elements in their promoter region (Figure 4G-I). Consistent with our microarray results, GO biological process of common genes up-regulated by -Glc/+HS and E2F motifs exhibited enrichment of mostly

stress-related pathways whereas +Glc/+HS and +Glc/+HS Rec up-regulated genes showed enrichment of processes required for growth and recovery after HS. These include protein folding and refolding, DNA replication, DNA unwinding and geometric changes, ribosome biogenesis and chromatin organization (Figure 2E, 3E and 3F; Supplemental Figure S7A-C). Collectively, TOR through E2Fs govern transcriptome reprogramming of HS-related genes as well as genes required for post HS growth and recovery.

Glucose epigenetically regulates the transcription of HS genes in Arabidopsis

According to previous microarray reports, glucose controls the transcription of a large number of heat shock proteins (HSP) genes (Mishra et al., 2009; Xiong et al., 2013a; Gupta et al., 2015a). To further confirm these microarray results, we performed quantitative real time PCR (RT-qPCR) under same experimental set up. Five-day-old MS grown Arabidopsis Col-0 seedlings were starved without glucose for 24h and then treated with glucose (167mM) for 3h under light grown conditions. We assessed the expression of five key HS genes (HSFA2, HSP70, HSP20-I, HSP20-II and HSP101). Glucose highly induced the expression of these HS genes than plants without glucose (Figure 5A). Next, we examined HSP expression in Arabidopsis TOR estradiol-inducible RNAi line (*tor es1*) under glucose sufficiency. Arabidopsis *tor es1* showed perturbation in these HS genes expression under glucose+estradiol treatment than mock (DMSO) treated seedlings (Figure 5A). These results suggest that glucose regulates HS gene transcription in a TOR-dependent manner. We hypothesize that glucose is a prerequisite signal and primes plants for better survival and stress mitigation under detrimental stress condition. As Glc regulates the large transcript of the HS genes through the TOR-E2Fa module, it was interesting to investigate whether Glc employs other factors that control this response or is the only mechanism governing this response. Therefore, to understand how glucose regulates HS gene transcription at the molecular level, we assessed the promoters of heat shock genes. Histone and chromatin modifications are important epigenetic mechanisms controlling gene expression underlying growth and stress responses (Chinnusamy and Zhu, 2009; Weng et al., 2014; Lee et al., 2017). There are numerous reports which suggested the role of histone acetylation and methylation (specifically H3K4 di- and tri-methylation) in gene transcriptional activation underlying stress responses (Saleh et al., 2008; Hu et al., 2015; Hu et al., 2019; Ueda and Seki, 2020). Recently, Chen et al., 2019 showed that p300/CREB HISTONE ACETYLTRANSFERASE 1 (HAC1) in association with WRKY18/53 transcription factors

regulate sugar responsive gene expression by modulation of histone acetylation (Chen et al., 2019). We, therefore, tested the histone acetylation and methylation at the promoters of HS genes in plants treated with and without glucose (+/- Glc). Nine-day-old Arabidopsis seedlings were starved in Glc free media for 24h followed by 3h glucose treatment. ChIP assay was performed using anti-histone 3 (K9+14+18+23+27) acetylation and anti-H3K4me2 antibodies and approximately 200 bp upsteram promoters of HS genes were analysed using ChIP-qPCR. Glucose alters the H3 acetylation (K9+14+18+23+27) and H3K4me2 at the promoters of HS genes (Figure 5B, Supplemental Figure S8). Arabidopsis plants treated with glucose showed more accumulation of these epigenetic marks at the promoters of HS genes encompassing HSEs whereas plants without glucose displayed less accumulation of these histone marks (Figure 5B, Supplemental Figure S8). We next asked whether TOR is required for glucose induced epigenetic changes at HS gene promoters. Five-day-old Arabidopsis *tor es-1* seedlings were transferred to estradiol (20µM) containing MS medium and kept for four days followed by starvation for 24h and 3h Glc treatment. Mock seedlings under Glc sufficiency showed increased accumulation of these histone acetylation and methylation marks at the HS gene promoters. On the contrary, glucose could not induce the accumulation of these epigenetic marks in seedlings treated with estradiol (Figure 5B, Supplemental Figure S8). Collectively, these results suggest the epigenetic mode of HS gene regulation by glucose-TOR signaling.

TOR coordinates with histone acetylation machinery to regulate sugar-mediated acetylation

Since Glc and TOR regulate HS genes expression by modulating epigenetic status, it was important to assess how Glc/TOR controls these responses. In mammals, TOR through phosphorylation activates a CREB-binding protein/p300-type histone acetyltransferase (Wan et al., 2017). In Arabidopsis, ortholog of p300 histone acetyltransferase HAC1 undergoes phosphorylation in response to sucrose (Van Leene et al., 2019a). Moreover, HAC1 in combination with WRKY18/53 is recruited at the promoters of sugar responsive genes and regulate their acetylation under sugar sufficiency (Chen et al., 2019). To examine whether TOR mediated epigenetic changes at HS gene promoters are governed through HAC1, we first assessed the expression and co-expression of Arabidopsis HAC1 with TOR and RAPTOR1. Plants gauge and adapt to diurnal light-dark cycle through transcriptional networks which facilitates plant's response to external environment (Ferrari et al., 2019). We, therefore, assessed the comparative expression of *TOR* and *HAC1* in diurnal light-dark cycle

transcriptome data obtained from public resources and observed that *TOR* concurs in expression with *HAC1* throughout diurnal cycle (Ferrari et al., 2019) (Supplemental Figure 9A). In addition, data obtained from Genevestigator (https://genevestigator.com/) and string (http://string-db.org/), we observed that HAC1 shares huge similarity with TOR and RAPTOR1 expression in anatomy, development and perturbations (Supplemental Figure 9B-D, Supplemental Figure 12A). Next, to assess the HS hypersensitivity of *hac1* mutants, we examined the phenotype of Arabidopsis plants defective in *HAC1* expression in response to HS. Seven-day-old Arabidopsis Col-0 and *hac1* mutants (*hac1-2* and *hac1-6*) were subjected to Glc free and Glc containing MS media for 24h followed by short term acquired thermotolerance (SAT) HS treatment. As expected, Col-0 and *hac1* mutants treated without Glc could not survive the lethal HS. However, Col-0 plants supplemented with Glc showed improved thermotolerance (showed higher fresh weight, increased chlorophyll [Chl] and higher lateral root numbers) (Figure 6A and 6B). Interestingly, *hac1* mutants showed reduced fresh weight and Chl content and fewer lateral roots after HS (Figure 6A and 6B). Next, we assessed the expression levels of HS genes underlying thermotolerance response in Col-0 and *hac1* mutants using RT-qPCR. Col-0 plants subjected to Glc and HS showed increased expression of these HS genes. In contrast, alleles of *hac1* (*hac1-2*, *hac1-3* and *hac1-6*) revealed greater perturbation in HS gene expression following sugar and HS treatment (Figure 6C; Supplemental Figure S10A and S10B). The next important question was to assess the epigenetic status of HS gene promoters upon sugar and HS treatment in Col-0 and *hac1* mutants. We, therefore, performed ChIP assay to examine the acetylation levels of HS gene promoters using anti-H3K (9+14+18+23+27) acetylation antibody coupled with qPCR. Intriguingly, Arabidopsis Col-0 plants showed increased accumulation of H3K acetylation at HS gene promoters upon sugar and HS treatment, whereas plants having mutation in *HAC1* showed reduced acetylation at these gene promoters (Figure 6D, Supplemental Figure S10C). In addition, global transcriptome comparison of *tor* differentially expressed genes (DEGs) with *hac1/5* obtained from public resources (Jin et al., 2018) identified 269 commonly down-regulated genes, with high enrichment of processes related to protein folding or heat shock responses (Supplemental Figure S11A-C). Furthermore, TOR and RAPTOR1 exhibits huge overlap of co-expression network genes with HAC1 with high enrichment of processes involved in chromatin remodelling, histone modification and gene transcriptional/posttranscriptional regulation (Supplemental Figure S11D-F). Since Glc through TOR-E2Fa regulates transcriptome reprogramming of large HS genes and Glc using acetylation machinery also regulates HS gene transcription, it was necessary to investigate

whether these two mechanisms target similar or different genes. To address this, we compared the genes regulated by TOR and HAC1/5 with E2F motif containing genes (Xiong et al., 2013a; Jin et al., 2018). Interestingly, we observed that out of 269 genes commonly down-regulated between TOR and HAC1, 190 genes were specific to TOR and HAC1 whereas 79 genes were found to be overlapping between TOR, HAC1 and E2F motifs with high enrichment of GO processes related to protein folding and HS response (Supplemental Figure S12). Collectively, these findings suggest that Glc-TOR signaling via E2Fa and the acetylation machinery employs common as well as different targets to regulate the thermotolerance response.

Glucose activated TOR is also required for maintaining short and long term thermomemory

Under natural environment, plants are constantly challenged by recurrent often irregular exposure of heat waves. Therefore, it is important to remember the environmental history as primed plants are more resistant to future stresses than naïve plants. It has already been proposed that Glc establishes and maintains the sustained expression of thermomemory-associated genes, and thereby causing thermomemory (Sharma et al., 2019). It is also necessary to test whether Arabidopsis TOR regulates acquired thermotolerance or to recall previous exposure to heat stress. To check this, we analysed the transcript levels of thermomemory associated genes in *tor35-7 RNAi* line. Seven-day-old Arabidopsis plants grown under moderate Glc (56mM) were treated with HS and recovered for various time points (3h, 24h and 48h). HS was applied as follows: 1h_37ºC, 90min_22ºC, 45min_45ºC and then recovered at 22ºC for 3h, 24h and 48h. Intriguingly, we observed that Arabidopsis plants under-expressing TOR showed reduced expression of thermomemory genes after extended recovery (24h and 48h) as compared to Col-0 (Figure 7A).

Chromatin structures are crucial factors which remember past experience of stress and show quick and heightened response to future stresses. These structures can be altered by nucleosome positioning, incorporation of histone variants and post-translational modifications (Struhl and Segal, 2013; Zentner and Henikoff, 2013; Tessarz and Kouzarides, 2014; Venkatesh and Workman, 2015). Enthralling evidences revealed the critical roles of histone methylation specifically H3K4me3 in chromatin based transcriptional memory (Ding et al., 2012a; Ding et al., 2012b; Lämke et al., 2016; Sharma et al., 2019). We, therefore, analysed the H3K4me3 epigenetic marks at the promoters of thermomemory associated loci

after 24h of HS recovery in Col-0 and *tor35-7* plants. Arabidopsis Col-0 plants showed higher accumulation of H3K4me3 memory-associated marks at the promoters of thermomemory loci whereas *tor35-7 RNAi* plants failed to accumulate these methylation marks (Figure 7B). Next, we asked whether TOR is only able to remember short duration of HS (SAT) or is involved in long term acquired thermotolerance (LAT). To address this, we examined the phenotype of Arabidopsis Col-0 and *tor35-7RNAi* plants after LAT HS. Seven-day-old Arabidopsis plants grown under moderate Glc (56mM) conditions were subjected to HS. HS was applied as follows: 1h_37ºC, 2d_22ºC, 2.5h_45ºC, 3-4d_22ºC. Arabidopsis Col-0 plants showed higher seedling survival (had higher fresh weight, increased Chl and higher number of lateral roots) whereas plants under-expressing TOR (*tor35-7*) failed to recover after LAT (Figure 7C-F). In addition, we checked the phenotype of TOR OE lines following LAT. Remarkably, plants overexpressing TOR (G166 and G548) showed higher seedling survival in comparison with Col-0 and *tor35-7* after LAT HS (Supplemental Figure S13A-C). Moreover, we analysed the phenotype of Col-0 and TOR overexpression (G166 and G548) seedlings after priming and un-priming HS. Arabidopsis G166 and G548 survived better (as shown by robust phenotype) than Col-0 under priming HS, whereas no major changes were observed in Arabidopsis Col-0 and G166 seedlings without priming (Supplemental Figure S14A-D). However, G548 showed better growth recovery even after unpriming HS than Col-0 plants (Supplemental Figure S14A-D). Next, to assess whether high temperature affects TOR promoter activity, we examined transgenic line carrying TOR promoter-reporter fusion construct. In line with the previous reports (Menand et al., 2002b), *pTOR::GUS* activity was largely restricted to shoot apical meristem (SAM). Testing *pTOR::GUS* without and with Glc alone could not further enhance reporter activity. Interestingly, *pTOR::GUS* activity was unchanged immediately after HS in the absence as well as in presence of Glc. Unexpectedly, *pTOR::GUS* plants treated with Glc and recovered for 24h after HS showed strong reporter activity in the zones of primary meristem and at the leaf base (Supplemental Figure S15).

Furthermore, we also checked expression of thermomemory associated genes in *e2fa-1* mutants after extended recovery following HS. Similar to *tor* mutants, *e2fa-1* plants did not show sustained expression of memory genes when recovered than WT plants (Supplemental Figure S16). We next checked the long-term acquired thermotolerance (LAT) in Arabidopsis Col-0 and *e2fa-1* plants. Arabidopsis *e2fa-1* plants showed reduced leaf greening and chlorophyll retention than Col-0 plants further supporting the role of TOR/E2Fa signaling in providing memory response (Supplemental Figure S17A and S17B). Since plants defective in

e2fa-1 showed reduced memory gene expression, we were interested to know whether E2Fa binds directly to thermomemory gene promoters in order to activate their expression. To examine this, we performed ChIP assay in Arabidopsis Col-0 plants using anti-E2Fa antibody. Interestingly, E2Fa did not bind directly to these gene promoters (Supplemental Figure S17C). We also checked binding of E2Fa to its known targets such as MCM5 and ATXR6 as positive control (Raynaud et al., 2006; Xiong et al., 2013a). E2Fa showed binding to MCM5 and ATXR6 promoters than memory associated gene promoters (Supplemental Figure S17C). Taken together, these results suggest that glucose involves TOR-E2Fa signaling in sustaining thermomemory associated gene expression to provide thermotolerance.

Arabidopsis TOR controls the length of the transcriptional memory persistence

Induction of key thermomemory-related genes and their sustained expression is crucial and considered as potential factor in establishing thermomemory (Lämke et al., 2016; Sharma et al., 2019). We, therefore, looked at the expression of thermomemory-associated genes in Col-0 and *tor35-7* after extending intervening recovery time from two hours (SAT) to two days (LAT). HS was applied as follows: 1h_37ºC, 2d_22ºC, 45min_45ºC and then recovered at 22ºC for 3h, 24h and 48h. Plants with subdued TOR showed greater reduction in sustenance of thermomemory-related gene expression following 24h and 48h of recovery than Col-0 plants (Figure 8A and 8B). Furthermore, we examined the persistence of thermomemory-associated HSP21 protein in Col-0 and *tor35-7* plants after extending memory phase from two days to seven days. Unexpectedly, *tor35-7* plants showed significantly higher levels of HSP21 protein after two days of HS recovery than Col-0 plants (Figure 8C). However, degradation of HSP21 was significantly faster after five and seven days of HS recovery in *tor35-7* plants and we could not observe any detectable level of HSP21 after seven days, which correlated with HS hypersensitivity of *tor35-7*, leading to higher death than Col-0 (Figure 8C). Next, to test how long TOR controls the length of transcriptional memory, we primed Col-0, G166 and *tor35-7* plants with mild non-lethal stress (37°C_3h) and extended the duration of intervening non-stress phase (22°C) up to seven days and then subjected to lethal HS (45°C_2.5h). Fascinatingly, plants overexpressing TOR (G166) survived and recovered well from the deleterious HS. In contrast, plants with subdued TOR forgot their HS memory and showed greater sensitivity to HS than Col-0, which caused death of the *tor35-7* plants (Figure 8D). Collectively, these several lines of evidence suggest the pivotal role of Arabidopsis TOR in establishment and maintenance of thermomemory response.

TOR co-expression and transcriptome share huge overlap with transcriptome of chromatin remodeler BRAHMA

Since TOR governs the epigenetics of non-memory and thermomemory-associated gene loci and +Glc/+HS and +Glc/+HS Rec transcriptome data revealed higher enrichment of chromatin machinery/posttranslational histone modification, it was apparent to assess the relationship between TOR and chromatin machinery. We analysed the co-expression data of TOR using *ATTED-II* database (http://atted.jp). Unexpectedly, we observed that TOR co-expresses with members of SWI/SNF family transcriptional regulators CHR2/BRAHMA (BRM) and SYD (CHR3; Figure 9A). Moreover, in a high throughput interactome data extracted from BIOGRID (https://thebiogrid.org/), TOR target E2Fa was found to be an interactor of BRM, SYD and SWI3C chromatin remodelers (Efroni et al., 2013) (Supplemental Figure 18E). In past few years, Brzezinka et al., (2016) showed that mutants of BRM display reduced sustenance of thermomemory gene expression and therefore, reduced thermomemory. To examine whether TOR triggered thermomemory is mediated through BRM, we analysed the co-expression network of BRM with that of TOR. Amazingly, out of 2000 TOR co-expressed genes, 1474 (74%) genes were found to be co-expressed with BRM and 1236 (62%) genes were co-expressed between TOR, BRM and CHR3 (Figure 9B; Supplemental Table S4A). Similarly, 966 genes were commonly co-expressed between TOR, BRM and RAPTOR1B (Supplemental Figure 19A-C; Supplemental Table S4B). Gene ontology (GO) enrichment of these commonly co-expressed genes between TOR, BRM, SYD and RAPTOR1B exhibited up-regulation of processes involved in chromatin remodelling/organization and histone posttranslational modifications (Figure 9B; Supplemental Figure S19A and S20A). BRM regulates target gene expression through concerted actions of REGULATOR OF EARLY FLOWERING 6 (REF6). REF6 is a H3K27 demethylase which facilitates the recruitment of BRM to the specific genomic sequence CTCTGYTY (Lu et al., 2011; Li et al., 2016). Moreover, GO molecular function of these genes enriched categories such as histone demethylase activity, chromatin binding and transcription coregulator activity (Figure 9B; Supplemental Figure S19B and S20B). Next, to test whether BRM and TOR regulates similar targets, we tested the DNA motifs recognised by BRM and REF6 in the genes co-expressed with TOR, *tor* differentially expressed genes *(tor_DEG)* and genes having E2F motifs in their promoter region. Interestingly, in all three categories, we found that approximately one third genes contain BRM-REF6 binding CTCTGYTY motifs in their 1000 bp upstream and 200 bp downstream promoters (Figure

9C; Supplemental Table S5). Next, to check whether BRM remembers the previous exposure of heat for longer duration of time, we treated Col-0 and *brm-5* plants for seven-day-LAT treatment and checked the phenotype. Intriguingly, plants defective in BRM and REF6 could not remember long periods of HS memory and died compared to Col-0 plants (Figure 9D and 9E). Further, we overlap *tor_DEG* with BRM and REF6 targets. Similar to co-expression data, *tor_DEG* shares huge overlap with BRM and REF6 targets. Out of 4979 *tor_DEG*, 1392 and 611 genes were found to be overlapping with BRM and REF6 targets, respectively (Figure 9F; Supplemental Table S4C). GO enrichment of these common genes showed categories with phosphorelay signal transduction, abiotic stress response and response to temperature, osmotic, salt and oxidative stress (Figure 9F-H). Further, GO molecular functions exhibited classes such as DNA binding, transcriptional regulatory DNA binding, transcription factor and transcriptional regulator activity (Figure 9F-H). In addition, we also compared the genes containing E2F motifs with BRM and REF6 targets and found GO enrichment exactly similar to what we observed in *tor_DEG* comparison with BRM and REF6 (Supplemental Figure S20C-E; Supplemental Table S4D). In addition, only 173 (8.65%) genes were found to be overlapping between TOR, BRM and E2Fa co-expression (Supplemental Figure S19D-F; Supplemental Table S4E). Among these 2000 co-expressed genes of TOR, RAPTOR, E2Fa and BRM, we fetched the genes involved in chromatin remodelling/organization, posttranslational modification (acetylation, deacetylation, histone and arginine methylation), transcriptional regulator of HS response and histone coding genes. Interestingly, we observed that TOR and RAPTOR exhibited co-expression pattern similar to BRM for most of these genes in contrast to E2Fa (Supplemental Table S6). Moreover, phospho-proteome data of sucrose sufficiency/AZD-8055 treatment identified 4,988 sugar induced phospho-peptides in 2,119 proteins (Van Leene et al., 2019b). Among these phospho-peptides, sucrose treatment induced phosphorylation in 26 proteins (containing 83 phospho-peptides) involved in chromatin machinery including large number of phospho-peptides in BRM (15 peptides), SYD (8 peptides) and REF6 (7 peptides; Supplemental Figure S18F). Collectively, these comprehensive analyses of overlap using TOR co-expression, *tor_DEG* and E2F motifs containing genes with BRM indicate that TOR might function in combination with BRM to control downstream processes, including thermotolerance/thermomemory.

DISCUSSION

During their life cycle, plants are faced with adverse growth conditions. An environmental factor that significantly affects plant growth and reproduction is an increase in temperature above optimum. Due to their stable nature, plants cannot move to more favourable locations and therefore must adapt to these stressful temperatures through an inherent potential called basal or acquired thermotolerance. A well-studied mechanism is the induction of HSPs that protect the cellular proteome against heat-induced damage. Furthermore, unlike animals, plants do not have the brain or antibodies to recall previous exposure to adverse conditions, for example illness or stress. As they are constantly threatened by recurrent and irregular stresses, importance of stress priming and memory response is being recognized. However, the underlying mechanisms how plants remember the past experience and convey this information to ensure survival under stressful condition remain largely underexplored. Recent reports provide enthralling evidences of "epigenetic memory" which requires changes through alteration in nucleosome positioning, chromatin modification and posttranslational histone modifications (Zentner and Henikoff, 2013; Tessarz and Kouzarides, 2014; Venkatesh and Workman, 2015; Brzezinka et al., 2016). Chromatin and histone modifications specifically (H3K9/14Ac, H3K56Ac, H3K4me3 and H3K27me3) are most plausible regulatory mechanisms which facilitates global changes in gene expression underlying stress tolerance and memory (Ding et al., 2012a; Sani et al., 2013; Lämke et al., 2016; Liu et al., 2019; Sharma et al., 2019). Another form of stress memory could be RNA polymerase II stalling at gene promoters underlying drought response (Ding et al., 2012a; Kwak and Lis, 2013). Mechanistic understanding of transcriptional memory of recurrent stresses is most advanced in yeast which involves lasting alteration in chromatin structures either through histone variant incorporation, histone modifications, nucleosome positioning or gene looping at memory associated genes (Brickner et al., 2007; Tan-Wong et al., 2009; Light et al., 2010). In plants, transcriptional memory is underexplored and mostly described for plant defence responses (Bruce et al., 2007; Conrath, 2011; Jaskiewicz et al., 2011; Avramova, 2015). A number of reports exhibited involvement of epigenetic regulators in plant stress responses (Hu et al., 2015; Liu et al., 2015), however, few examples of transcriptional memory have been known in case of plant memory to abiotic stresses (Ding et al., 2012a; Lämke et al., 2016; Lämke and Bäurle, 2017; Liu et al., 2019; Sharma et al., 2019). Through a comprehensive chemical, genetic, transcriptome and epigenomic analysis, our results presented here provided the pivotal role of Arabidopsis Glc-TOR signaling in heat stress adaptation and maintaining thermomemory. Previously, numerous researchers have identified the involvement of TOR in stress resilience and adaptation (Mahfouz et al., 2006;

Deprost et al., 2007; Wang et al., 2016; Bakshi et al., 2017; Dong et al., 2017; Dong et al., 2018; Wang et al., 2018; Fu et al., 2020). Recently, Sharma et al., (2019) revealed that Arabidopsis TOR controls transcription of many heat shock protein (HSP) genes including HSFA2 and HSP70 (Sharma et al., 2019). In addition, global transcriptome study of Arabidopsis plants under Glc sufficiency or plants underexpressing TOR (*tor35-7 RNAi*) showed that Glc-TOR signaling governs transcriptome reprogramming of HS genes (Mishra et al., 2009; Xiong et al., 2013b; Gupta et al., 2015a). However, the underlying mechanism how TOR controls thermotolerance response is not well understood. In line with the previous observation, whole genome transcriptome study of Arabidopsis seedlings under Glc lacking/sufficiency demonstrated that Glc can largely affect the expression of HS-related genes. At the whole genome level, 1124 genes were co-regulated by -Glc/+HS and +Glc/+HS. As expected, several metabolic processes related to primary and secondary metabolism, microtubules disassembly, sulphur compound biosynthetic processes and biotic stimulus were severely down-regulated whereas genes involved in HS protection such as HS responsive molecular chaperones, de novo protein folding, HS acclimation and de novo post-translational protein folding were up-regulated (Supplemental Figure S2A and S2B). Interestingly, apart from common genes regulated by -Glc/+HS and +Glc/+HS, presence of Glc exclusively regulates 1408 genes. GO enrichment of these genes showed high enrichment of protein deneddylation process (Figure 2E). Protein neddylation and deneddylation upon stress stimuli and stress recovery is required for the assembly and disassembly of stress granules (SG), respectively (Jayabalan et al., 2016). Like ubiquitylation, neddylation is a post-translational modification which requires a small ubiquitin-like protein NEDD8. Under stress, organisms show inhibition in protein synthesis, blocks translation initiation and triggers polysome disassembly and sequestration of translation stalled mRNA into cytoplasmic non-membranous aggregates called SG for storage or in processing bodies (PBs) for decay (Chantarachot and Bailey-Serres, 2018). SGs prevent the degradation of mRNA and store them which can reversibly be engaged into the translation once stress has removed. Cell employ these mechanisms to repress the translation of housekeeping genes and preserve stress protective genes mRNA to ensure cell survival. Recently, Merret et al., (2017) showed that mutants of *HSP101* showed perturbation in translational recovery and SG disassembly following HS (Merret et al., 2017). The mRNA encoding ribosomal proteins (RPs) were stored into SGs and subsequent release of these mRNA in re-engaging translation process was dependent on HSP101 (Merret et al., 2017). In agreement with the previous reports, our data strongly suggest that Glc signaling drives regulation of SG assembly and disassembly

through expression of genes underlying neddylation and deneddylation which are crucial for stress recovery. In addition, Glc through TOR promotes the transcriptional regulation of *HSP101* which is crucial for SGs disassembly and hence provide an adaptive strategy of plant survival under deleterious HS. Further, we observed major antagonistic interaction between TOR and HS transcriptome under energy deficient conditions (-Glc/+HS). Unlike, transcriptome reprogramming of HS genes under Glc sufficiency exhibited major synergistic interaction (310 down- and 250 simultaneously up-regulated) with TOR regulated genes suggesting that Glc governs transcriptome reprogramming of HS genes mainly through TOR pathway.

Another important feature of HS response was to study the transcriptome of genes induced/suppressed when the HS signal was absent, i.e. HS recovery. A recent report by our group has shown that Glc maintains the sustained expression of HS genes, thereby regulates thermomemory (Sharma et al., 2019). At the whole genome level, Glc controls a large number of genes implicated in growth and stress recovery. We found that a huge number of genes (1332; 46%) demonstrated their sustained expression when HS stressed plants were recovered under Glc sufficiency. These include GO processes related to DNA unwinding, geometrical and conformational changes, initiation of DNA replication, ribosome biogenesis and cell wall modifications suggesting accelerated cell division and proliferation leading to better growth and recovery (Supplemental Figure S5B and S5C). Chromatin and histone modifications are central regulatory mechanism implicated in transcriptome reprogramming of stress memory (Ding et al., 2012a; Singh et al., 2014; Brzezinka et al., 2016; Lämke et al., 2016). HS recovery under Glc sufficiency specifically induced GO processes related to histone and protein methylation and DNA confirmation and geometric changes which perhaps required to facilitate stress memory response (Figure 3F; Supplemental Figure S5B). In contrast, expression profile of genes induced by –Glc/+HS was completely vanished (only 28 genes; 1.2%) when plants were recovered in absence of Glc. Interestingly, comparison of transcriptome underlying HS recovery under Glc lacking/sufficiency with TOR revealed that Glc induction and sustenance of genes primarily governs through TOR. We observed a large synergistic interaction between +Glc/+HS Rec with TOR (285 genes up- and 254 genes simultaneously down-regulated). Moreover, whole genome transcriptome data of *tor_DEG* exhibits 48 genes encode for chromatin maintenance/remodelling (Xiong et al., 2013b). Interestingly, we found that genes exclusively induced by -Glc/+HS, +Glc/+HS and genes commonly induced by -Glc/+HS and +Glc/+HS did not exhibit any overlap with these genes

(Supplemental Figure S18A-C). In contrast, genes exclusively up-regulated under +Glc/+HS Rec showed significant overlap (20 out of 48 genes) with these genes suggesting that Glc-TOR signaling governs transcriptional control of chromatin machinery during stress recovery (Supplemental Figure S18D).

Earlier reports have shown that Glc regulates transcriptome reprogramming of myriad set of genes via E2Fa transcription factor (Xiong et al., 2013a; Li et al., 2017). E2Fa regulates target genes via conserved cis-acting TTTCCCGCC or similar motifs through direct binding at their genomic sites (Vandepoele et al., 2005; Naouar et al., 2009). We observed that E2Fa recruited at HSFA1 and HSFA2 upstream genomic regions and positively regulates their expression. In addition, we noticed high enrichment of cis-acting E2Fa motifs in genes regulated by Glc and HS. About one third of the genes regulated by Glc and HS display E2Fa binding motifs at their upstream promoters, promoting processes related to protein folding and refolding, DNA geometric and conformational changes, chromatin organisation and ribosome biogenesis which are thought to necessary for cell growth after stress.

Numerous reports revealed that dynamic nature of chromatin structure is a crucial determinant of gene expression and can be modulated by nucleosome rearrangement, histone variant and posttranslational modification of histone tails (Berr et al., 2009; Tamada et al., 2009; Kumar and Wigge, 2010; Brzezinka et al., 2016). Depending on the position of histone residues, which are acetylated or methylated, these modifications can promote or repress transcription. A recent study showed that WRKY18/53 control sugar induced accumulation of histone acetylation at the promoters of sugar responsive genes (Chen et al., 2019). Our study provides strong evidence supporting that Glc signaling regulates HS transcriptome reprogramming by accumulation of histone acetylation marks at the promoters of HS loci. We observed a strong defect of *tor* on Glc promoted HS expression, demonstrating that Glc induction of HS transcriptome is majorly regulated through TOR pathway. Lately, an interactome and phosphoproteome study of sugar and TOR identified CBP/p300 protein HAC1 and GCN5 as plausible TOR targets (Van Leene et al., 2019a). In another report, HAC1 acts together with WRKY18/53 and recruits H3K acetylation at sugar responsive genes under sugar sufficiency (Chen et al., 2019). We observed that mutants of *HAC1* showed higher sensitivity to heat stress with a strong defect in Glc promoted accumulation of H3K acetylation which ultimately resulted into less expression of HS genes. Moreover, transcriptome comparison of genes regulated by TOR, HAC1 and E2F showed that TOR employs similar or different sets of HS targets via HAC1 and E2Fa, which are implicated in

the temperature stress response. Collectively, these results demonstrate that Glc-TOR acts in concert with histone acetylation machinery to regulate genes underlying thermotolerance response.

Plant stress research has largely been focussed on basal or acquired tolerance to certain levels of stresses. However, the impact of environmental history on the plant performance to recurrent and irregular stresses has been under-researched. The importance of transcriptional memory was described for yeast, mammals and plants where gene transcription was highly induced upon repeated exposure of stress signal and prepares them for future stresses in a fast or superior manner (D'Urso and Brickner, 2014; Iwasaki and Paszkowski, 2014; D'Urso and Brickner, 2017; Kim and Costello, 2017). Plant memory to recurrent stresses can be described as non-epigenetic such as RNA metabolism (e.g., decay or stabilization), protein turnover and epigenetic mechanisms which involve lasting changes in chromatin either through posttranslational histone modification or nucleosome occupancy (Charng, 2006; Ding et al., 2012a; Brzezinka et al., 2016; Crisp et al., 2016; Sedaghatmehr et al., 2016). TOR, as being an important regulatory gene governs numerous biological processes ranging from embryo development to senescence (Sheen, 2014). The involvement of plant TOR kinase has already been proposed in several biotic and abiotic stress responses (Deprost et al., 2007; Bakshi et al., 2017; De Vleesschauwer et al., 2018; Wang et al., 2018; Fu et al., 2020). We previously reported that Arabidopsis TOR overexpressing plant showed increased acquired thermotolerance whereas *tor 35-7 RNAi* lines showed hypersensitivity to HS (Sharma et al., 2019). However, the involvement of Arabidopsis TOR in plant response to recurrent or repeated HS is hitherto unexplored. Here, we demonstrated that TOR modulates the dynamics of chromatin status in repetitively facing HS. Numerous reports revealed preservation of chromatin modification specifically H3K4me3 at trained genes in response to variety of environmental stresses such as drought, salinity, hypoxia, heat and defence (Tsuji et al., 2006; Sokol et al., 2007; Ding et al., 2012a; Singh et al., 2014; Lämke et al., 2016). Correlation between chromatin modification and priming has been observed during osmotic response, drought and pathogen response (Jaskiewicz et al., 2011; Ding et al., 2012a; Sani et al., 2013). Interestingly, Arabidopsis plants exposed to HS demonstrated increased accumulation of histone H3 tri-methylation marks, leading to the open chromatin configuration in the promoters of thermomemory associated genes which was greatly suppressed in plants defective in TOR. This increased enrichment of H3K4me3 at thermomemory associated gene promoters correlated with the sustained expression over a

period of days, leading to increased thermomemory. We have also shown that Arabidopsis TOR not only demonstrated thermomemory response to SAT, LAT responses were also governed by TOR. Arabidopsis WT plants exposed to LAT HS showed increased sustenance of thermomemory associated *HSP21* and *HSP22* gene transcripts which was compromised in *tor35-7* plants. Moreover, we observed that WT plants showed increased abundance of HSP21 protein even after seven-day of HS, while *tor35-7* displayed higher degradation of HSP21, thereby causing greater sensitivity to repeated HS. Given the importance of chromatin remodelling in stress and memory response, we observed significant overlap of TOR co-expression and differentially expressed genes with BRM. In addition, we found the significant presence of BRM cis-acting motifs in the genomic loci of TOR co-expression, *tor_DEG* and E2F motifs containing genes. Collectively, these results suggest a central role of Glc-TOR signalling along with several mechanisms which are thought to act in concert to control thermotolerance/thermomemory (Figure 10). This work identified the crucial function of TOR in shaping the environmental history of plants and provides a mechanistic framework to confront ever challenging and fluctuating environment. This approach would be exploited to improve temperature tolerant crop varieties in order to feed ever growing human population.

MATERIAL AND METHODS

Plant Materials and Growth Condition

The Arabidopsis (*Arabidopsis thaliana*) mutant lines estradiol-inducible TOR RNAi (CS69829; *tor es-1*; Col-0 background), TOR overexpression lines (AT1G50030, GK-548G07- 020632; Col-0 background) and *e2fa* (AT2G36010, WiscDsLox434F1; Col-0 background) were obtained from The Arabidopsis Biological Resource Centre at Ohio State University. The *TOR* (AT1G50030, G166; Col-0 background) line was obtained from the Nottingham Arabidopsis Stock Centre. The following lines were obtained from the published sources: *tor 35-7* RNAi (Col-0 background (Deprost et al., 2007) and *HAC1* mutants (*hac1-2*, *hac1-3* and *hac1-6*; Col-0 background (Heisel et al., 2013), *proTOR::GUS* (Col-0 background (Menand et al., 2002a). Seeds of wild type and mutants were surface-sterilized and imbibed at 4°C for 2 days. Seeds were sown on square petri plates (120*120mm) containing 0.5% MS medium with 1% Suc (29mM) and 0.8% agar (24mM), under sterile conditions. Seed germination was carried out in a culture room maintained under long-day

photoperiod (16h light and 8h dark, 60mM m^{-2} s^{-1} light intensity otherwise specifically mentioned) at 22°C±2°C temperature.

GUS Assay

GUS assay was performed using seven-day-old *proTOR::GUS* reporter transgenic line treated without or with Glc (-/+ Glc) followed by HS. HS was applied as 1 h at 37°C, 2 h at 22°C, and 2.5 h at 45°C. After HS, *proTOR::GUS* seedlings were transferred immediately (0h) after 24h of HS recovery at 22°C to GUS buffer. Images were taken using stereo zoom microscope (GUS treatment was described by Mishra et al., 2009).

ChIP Assays

ChIP assays were performed using protocol described by Saleh et al. (2008) with minor modifications. Protoplasts were harvested from Col-0 plants and treated with Glc (5mM) for 3 h followed by chromatin isolation. For ChIP assay in seedlings, seven-day-old Arabidopsis seedlings were starved with Glc free medium for 24 h followed by 3h of Glc treatment. For ChIP assay used in thermomemory, seven-day-old Arabidopsis seedlings were transferred directly to Glc-containing media followed by HS treatment. HS was applied as 1 h at 37°C, 1.5 h at 22°C, and 45 min at 45°C. Following HS, seedlings were recovered for 24h at 22°C. Seedlings were cross-linked with 1% formaldehyde for 10 min followed by quenching with 0.1M glycine. The resuspended chromatin was sonicated in a 4°C water sonicator (Diagenode Bioruptor Plus). Serum containing anti-E2Fa antibodies was obtained from Prof. Lieven De Veylder, VIB Department of Plant Systems Biology, Ghent University (Takahashi et al., 2008). Antibodies against H3K (9+14+18+23+27) acetylation were purchased from Abcam (Cat. no. ab47915). Antibodies against H3K4me2 (Cat. no. 07-030) and H3K4me3 (Cat. no. 07-473) were purchased from Millipore. All primers used are mentioned in the Supplemental Table S7.

Western blot Assay

The total plant protein from seven-day-old Arabidopsis seedlings was extracted in cold extraction buffer (137mM of NaCl, 2.7mM of KCl, 4.3mM of Na2HPO4, 1.47mM of KH2PO4, 10% glycerol [1.1 M], and 1mM phenyl methyl sulfonyl fluoride [PMSF]) together with plant protease inhibitor cocktail (Sigma-Aldrich, http://www.sigmaald rich.com/). Protein concentration was measured using nanodrop. 50 µg of protein samples were loaded in

each well. The antibody for HSP21 (Anti- HSP21; chloroplastic heat shock protein Cat. no. AS08 285) and (HSP90-2; Cat. no. AS11 1629) were purchased from Agrisera.

Thermotolerance/thermomemory assays

Arabidopsis thaliana seedlings grown in standard culture room and maintained under long day photoperiod conditions were used for thermotolerance assays. Seven-day-old seedlings were grown initially in MS media using 1% sucrose (29mM) and 0.8% Agar and then accimatized to Glc free and Glc (167mM) containing solid MS media for 24 h followed by SAT and LAT HS treatments. For SAT treatment, seedlings were transferred to the heat chamber which was pre-set at 37°C. Seedlings were kept in HS as 37°C for 1h, allowed to recover for 2h at 22°C and then transferred to 45°C for 2.5h, followed by recovery in the culture room for 3–4d. For phenotyping under LAT HS treatment, seven-day-old seedlings were transferred to the heat chamber at 37°C for 1h, allowed to recover for 2d at 22°C and then transferred to 45°C for 2.5h, followed by recovery in the culture room for 3–4d. To estimate the length of thermomemory, seven-day-old seedlings were transferred to the heat chamber at 37°C for 3h, allowed to recover for 7d at 22°C and then transferred to 45°C for 3h, followed by recovery in the culture room for 3–4d. For RT-qPCR under SAT HS, seedlings were treated at 37°C for 1h, 22°C for 90min, 45°C for 45min followed by recovery 22°C for 3h, 24h and 48h. For RT-qPCR under LAT HS, seedlings were treated at 37°C for 1h, 22°C for 2d, 45°C for 45min followed by recovery 22°C for 3h, 24h and 48h. For immunoblotting assay, seven-day-old Arabidopsis seedlings were transferred to HS at 37°C for 1h, 22°C for 90min, 45°C for 45min followed by recovery 22°C for 2d, 5d and 7d. The thermotolerance/thermomemory experiments were accomplished in a pre-set heat chamber maintained at the required temperature and recovery of the plants in between the acclimation and severe HS treatments was performed in the standard growth room condition (22°C) in the Arabidopsis culture room.

Gene expression analysis

Gene expression analysis was performed using RT-qPCR. Arabidopsis seedlings were initially germinated and grown for 5 days in ½ MS medium containing 1% (29mM) sucrose under long day photoperiod (16-h day/8-h night conditions). For RT-qPCR study in response to Glc, 5-d-old Arabidopsis seedlings were starved without Glc containing MS medium for 24h in dark followed by without or with Glc containing MS medium for 3h in light. For RT-qPCR study under thermomemory HS, Arabidopsis seedlings were germinated and grown

directly in medium containing 1% Glc (56mM) for seven days under standard 16-h day/8-h day night conditions. For SAT, seedlings were transferred to HS at 37°C for 1h, 22°C for 2h, 45°C for 45 min and 22°C for 3h, 24h and 48h. For LAT, seedlings were transferred to HS at 37°C for 1h, 22°C for 2d, 45°C for 45 min and 22°C for 3h, 24h and 48h. Following stress, whole seedlings were harvested in liquid nitrogen. Total plant RNA was isolated using RNAeasy Plant Mini Kit (Qiagen). Quantification of RNA was done using nanodrop and tested for quality before subsequent use. First-strand cDNA was synthesized by reverse transcription using 2 µg of total RNA with a high-capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers were designed by identifying a unique sequence within the genes preferably 3'UTR. All candidate gene primers were designed using the Primer Express (version 3.0; Applied Biosystems). RT-qPCR for 96 well plate, ABI Prism 7900 HP Fast Real-Time PCR system (Applied Biosystems) and for 384 well plate, Quant Studio version 6 Flex Real-Time PCR system (Applied Biosystems) was used. Normalization of genes was performed using 18S as a reference control. The fold-change for each candidate gene in different experimental conditions was determined using the quantitative DDCT method. All primers used are listed in the Supplemental Table S7.

Chl Estimation

Total Chl (Chl a and Chl b) estimation was performed with seedlings treated with HS. Seedlings were immersed in 2mL 80% (v/v) acetone and kept them in dark at 4°C overnight. Cell debris was pelleted by centrifugation at 8,000 rpm for 10min and the amount of Chl was estimated at 663 and 646 nm using a Smart Spec Plus spectrophotometer (Bio-Rad). The amount of Chl a (12.21*l,663-2.81*l,646) and Chl b (20.13*l,646-5.03*l,663) was quantified and the total amount of Chl (Chl a + Chl b) was normalized per seedling. Percentage Chl was estimated by normalizing HS treated seedling with Ctrl (no heat stress) or with respective WT seedlings.

Microarray Analysis

Whole genome microarray analysis of Arabidopsis Col-0 seedlings treated without or with Glc (-/+ Glc) and HS was performed. To minimize the endogenous sugar background, five-day-old Col-0 seedlings were first starved with ½ MS medium containing no sugar for 24h in the dark. Following starvation, seedlings were subjected to without or with Glc treatment for 24h followed by HS. HS treatment was applied at 37°C for 3h. Whole seedling tissue was

collected immediately as well as at 3h recovery after HS. Harvested samples were outsourced for transcriptome profiling. The data was analysed using the Transcriptome Analysis Console (v3.0; Affymetrix) using default parameters. Differentially expressed genes in all other conditions were compared to -Glc with fold change (FC) value greater than or equal to 1.5. One-Way Between-Subject ANOVA (unpaired) statistical test with p-value less than 0.05 and false discovery rate (FDR) p-value 0.1 were used to analyse the DEGs. Microarray data has been submitted to Array Express with accession no. E-MTAB-6980. Three biological replicates were used in this microarray study.

Bioinformatics analysis

BRM/REF6 promoter motifs in TOR co-expression genes, *tor_DEG* and E2Fa motif containing genes were extracted for a length of 1200 nucleotides (including an upstream region of 1000 nts and downstream region of 200 nts, from the TSS sites). These regions were then scanned for the presence of eight nucleotide length motif “CTCTGYTY" (where Y is any one of A/T/G/C) and this was done using in-house python scripts. Protein-Protein interaction (PPI) between HAC1 and TOR complexes was explored in String v.11 (Szklarczyk et al., 2019) which uses evidence from homologs in other species. Gene expression data for TOR, RAPTOR1, HAC1 and BRM in *A. thaliana* across various anatomical parts, developmental stages and stress related experiments was extracted using the mRNA-Seq dataset ‘AT_mRNASeq_ARABI_GL’ from Genevestigator platform (Hruz et al., 2008). The available time series expression data (in Transcripts per million TPM counts) for Arabidopsis diurnal range across the day-night cycle was downloaded from the Supplementary dataset in Ferrari et al., 2019 (Ferrari et al., 2019). Temporal expression patterns for all four genes were extracted from this dataset and compared using Normalized TPM values (rescaled between 0 and 1 via Min-Max scaling/normalization). Expression Heat maps were generated using MeV_4_9_0.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers: AT1G50030 (TOR), AT1G79000 (HAC1), AT2G36010 (E2FA), AT4G17750 (HSFA1A), AT2G26150 (HSFA2), AT3G12580 (HSP70), AT1G54050 (HSP20-LIKE), AT1G52560 (HSP20-LIKE), AT4G27670 (HSP21), AT5G59720 (HSP18.2), AT5G12020 (HSP17.6II), AT4G10250 (HSP22), AT1G74310 (HSP101) and AT3G18780 (ACT2).

Supplemental data

The following supplementary data are available:

Supplemental Figure S1. Photosynthesis generated Glc could not provide thermotolerance under high light intensity when blocked with Glc analogue 2-deoxy-glucose.

Supplemental Figure S2. Gene ontology of common genes between –Glc/+HS and +Glc/+HS exhibits up-regulation of genes involved in protein folding.

Supplemental Figure S3. Glc regulates HS transcriptome largely through TOR pathway.

Supplemental Figure S4. HS transcriptome induced in absence of Glc exhibits large antagonistic interaction with Glc-TOR target genes.

Supplemental Figure S5. Presence of Glc regulates sustenance of HS transcriptome for HS recovery.

Supplemental Figure S6. HS recovery under Glc induces processes required for growth/HS recovery in a TOR dependent manner.

Supplemental Figure S7. TOR through E2F regulates global expression of stress related genes as well as genes required for growth and recovery post-stress period.

Supplemental Figure S8. Glc through TOR induces enrichment of histone H3K4me2 marks at the promoters of HS genes.

Supplemental Figure S9. Diurnal light-dark cycle and Genevestigator transcriptome comparison shows similarity in expression between TOR, RAPTOR1, HAC1 and BRM.

Supplemental Figure S10. *hac1* mutant demonstrates less accumulation of H3K acetylation, leading to perturbation in HS induced gene expression.

Supplemental Figure S11. Transcriptome comparison of TOR microarray with differentially expressed genes in *hac1* and *gcn5* mutants.

Supplemental Figure S12. TOR regulates different set of heat stress genes via E2Fa and HAC1.

Supplemental Figure S13. TOR remembers past exposure of heat stress.

Supplemental Figure S14. TOR overexpression lines primed with HS display enhanced thermotolerance.

Supplemental Figure S15. HS recovery under Glc induces *pTOR::GUS* activity in the zones of primary meristems.

Supplemental Figure S16. Arabidopsis *e2fa-1* plants could not sustain thermomemory gene expression.

Supplemental Figure S17. *e2fa-1* mutants cause weaker LAT.

Supplemental Figure S18. TOR controls the transcription and protein phosphorylation of several chromatin remodelers.

Supplemental Figure S19. Similar to TOR, RAPTOR1B shares huge overlap with BRM.

Supplemental Figure S20. Arabidopsis TOR co-expression shares huge overlap with chromatin remodeler BRAHMA and SYD (CHR3).

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AUTHOR CONTRIBUTIONS

M.S. and A.L. conceived and designed the experiments. M.S. performed most of the experiments. M.S. and B.N.S. performed immuno-blotting experiment. M.S. and M.J.K. performed Microarray experiment. M.S., B.N.S., M.S. and P.A. performed physiology and RT-qPCR experiments. M.S., S.K.M. and G.Y. performed promoter motif analysis, Diurnal and Genevestigator analysis. M.S. and A.L. analysed the data. M.S wrote the manuscript. A.L. supervised and complemented the writing.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Figure legends

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Figure 7. Glc regulated TOR is required in maintaining thermomemory. A, RT-qPCR expression of thermomemory related genes in Arabidopsis Col-0 and *tor 35-7 RNAi* seedlings. Arabidopsis Col-0 and *tor 35-7* plants were grown for seven days in presence of 1% Glc (56mM) under long-day conditions (16h light and 8h dark, 100 µM m-2 s-1 light intensity) treated without or with HS. HS was applied as 1h_37ºC, 90min_22ºC, 45min_45ºC. After HS, seedlings were recovered for various time points (3h, 24h and 48h) at 22ºC. Data shown are representative of four technical replicates among two biological replicates. Experiments were repeated twice times with similar results. **B,** ChIP-qPCR enrichment of thermomemory related genes in Col-0 and *tor 35-7* seedlings treated without or with HS. Following HS, seedlings were recovered for 24h at 22°C. Amount of immunoprecipitated promoter DNA was calculated by comparing samples treated without or with anti-Histone H3K4me3 antibody. C_T Values without and with anti-Histone H3K4me3 antibody were normalized by input control. Data shown are representative of one biological replicate. Experiments were repeated four times with similar results. **C,** Picture showing seedling survival of Arabidopsis Col-0 and *tor 35-7* RNAi after long term acquired thermotolerance. **D-F,** Percentage Chl, fresh weight and LR count of Arabidopsis Col-0 and *tor 35-7* RNAi after LAT HS. Arabidopsis Col-0 and *tor 35-7* plants were grown for seven days in presence of 1% Glc (56mM) under long-day conditions (16h light and 8h dark, 100 µM m-2 s-1 light intensity) and subjected to HS. HS was applied as 1h_37ºC, 2d_22ºC, 2.5h_45ºC and 3-4d_22ºC. Data shown are representative of four biological replicates. Experiments were repeated four times with similar results.

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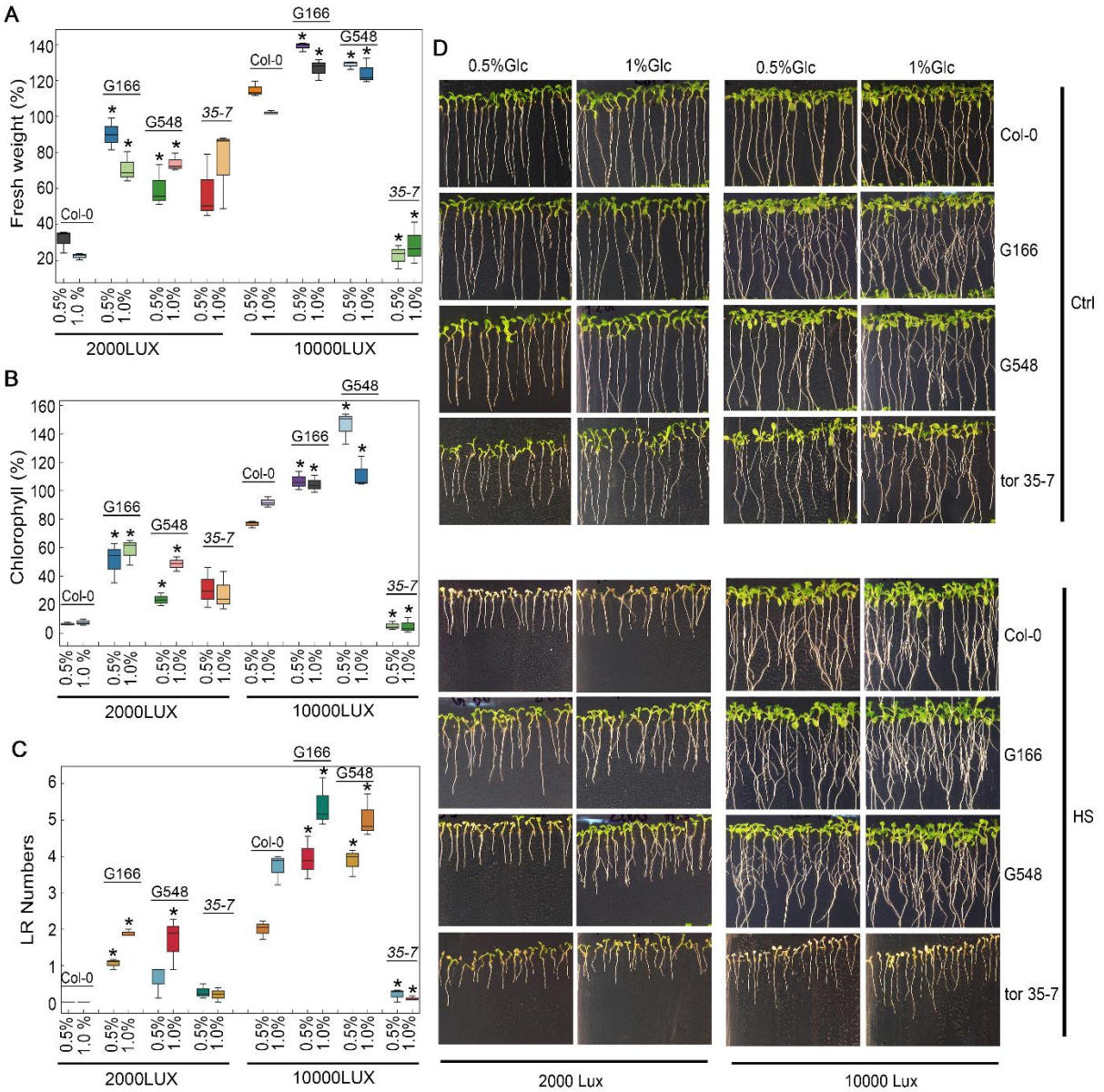
Figure 9. TOR co-expresses with chromatin remodeler BRM and overlaps common downstream targets. A, Network showing co-expression data of TOR with BRM and SYD (CHR3). Co-expression network was extracted from ATTED-II. **B,** Venn diagram showing overlap of TOR co-expressing genes with BRM and SYD. **C,** Pictures showing enrichment of BRM-REF6 recognising genomic motif CTCTGYTY in TOR co-expressing genes, tor_DEG and in genes containing E2Fa motifs in their promoters. 1000 bp upstream and 200 bp downstream Promoters relative to TSS was considered for promoter analysis. **D, E** Chlorophyll measurement and phenotype of Arabidopsis Col-0, *tor35-7*, *brm-5* and *ref6-2* plants after seven-day of LAT HS. Arabidopsis Col-0, *tor35-7*, *brm-5* and *ref6-2* plants were grown for five days in MS medium and subjected to HS. HS was applied as 3h_37ºC, 7d_22ºC, 2.5h_45ºC and 3-5d_22ºC. Picture shown are representative of one biological replicate. Experiment was repeated thrice with similar results. **F,** Venn diagram showing overlap of genes

differentially regulated by TOR with BRM and REF6 targets. **G, H,** GO fold enrichment of biological process and molecular function of 1392 genes commonly regulated by *tor_DEG* and BRM targets. Panther 15.0 tool was used to analyse GO fold enrichment using Bonferroni correction and Fisher's exact test type.

Figure 10. Proposed model shows how Glc-TOR signalling through distinct mechanisms regulates thermotolerance/thermomemory. A, Arabidopsis TOR regulated E2Fa transcription factor recruited at the promoters of HSFA1 and HSFA2 genes to control their expression which in turn regulate HS gene expression. **B,** Arabidopsis TOR-E2Fa module also regulates transcriptome reprogramming of genes involved in chromatin remodelling/epigenetic modifications which are crucial to regulate thermotolerance response. **C,** Glc through TOR promotes recruitment of histone H3 acetylation marks at the promoters of HS genes to induce their transcription, leading to thermotolerance. This Glc-TOR mediated histone acetylation is facilitated through HAC1 and GCN5. **D,** Sugar also controls interaction and phosphorylation of several chromatin proteins including BRM, SYD and REF6. TOR co-expresses with BRM & SYD and exhibits huge overlap with BRM target genes. TOR promotes the accumulation of histone methylation marks at the promoters of thermomemory associated gene promoters, and therefore regulates thermomemory.



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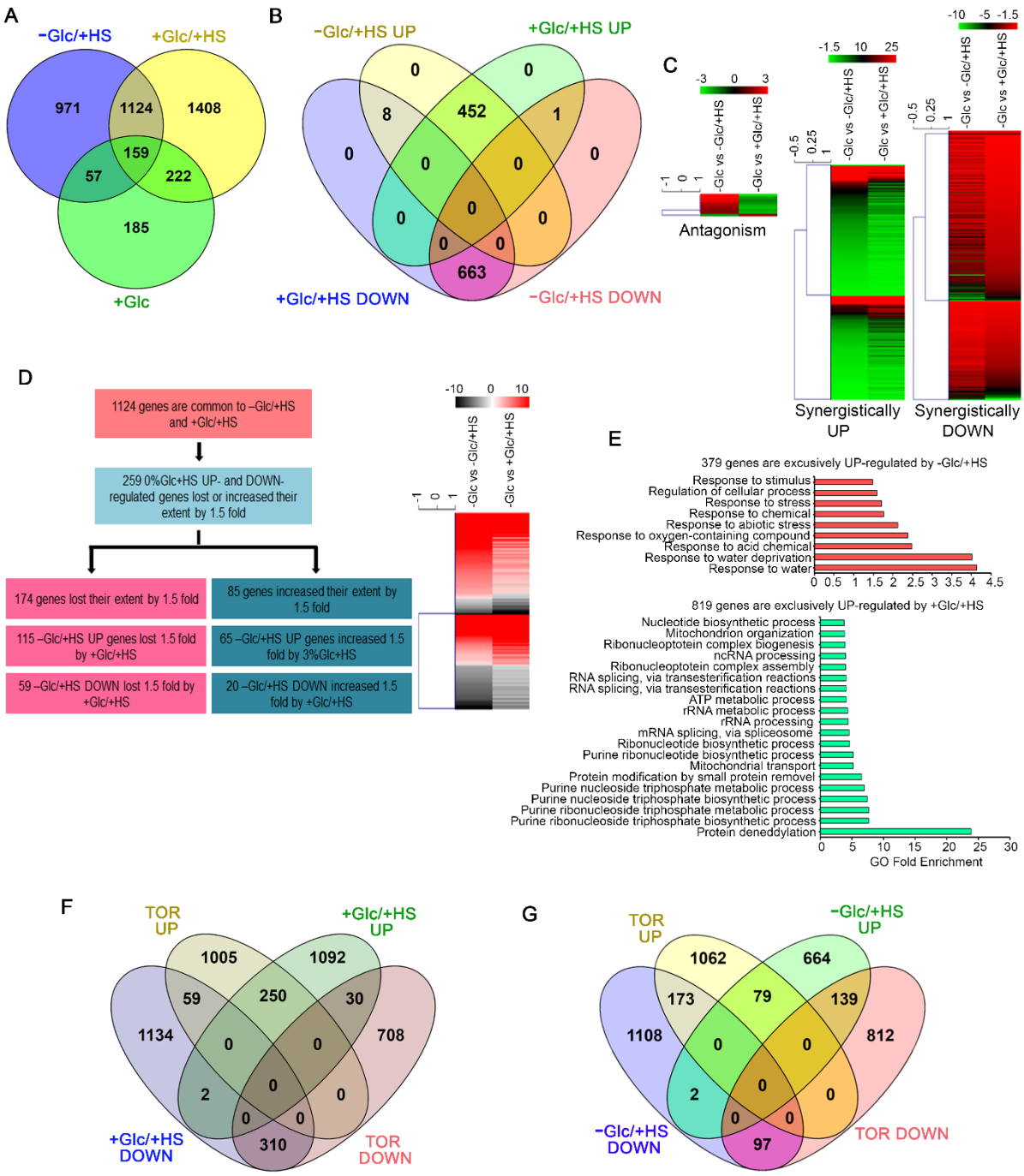

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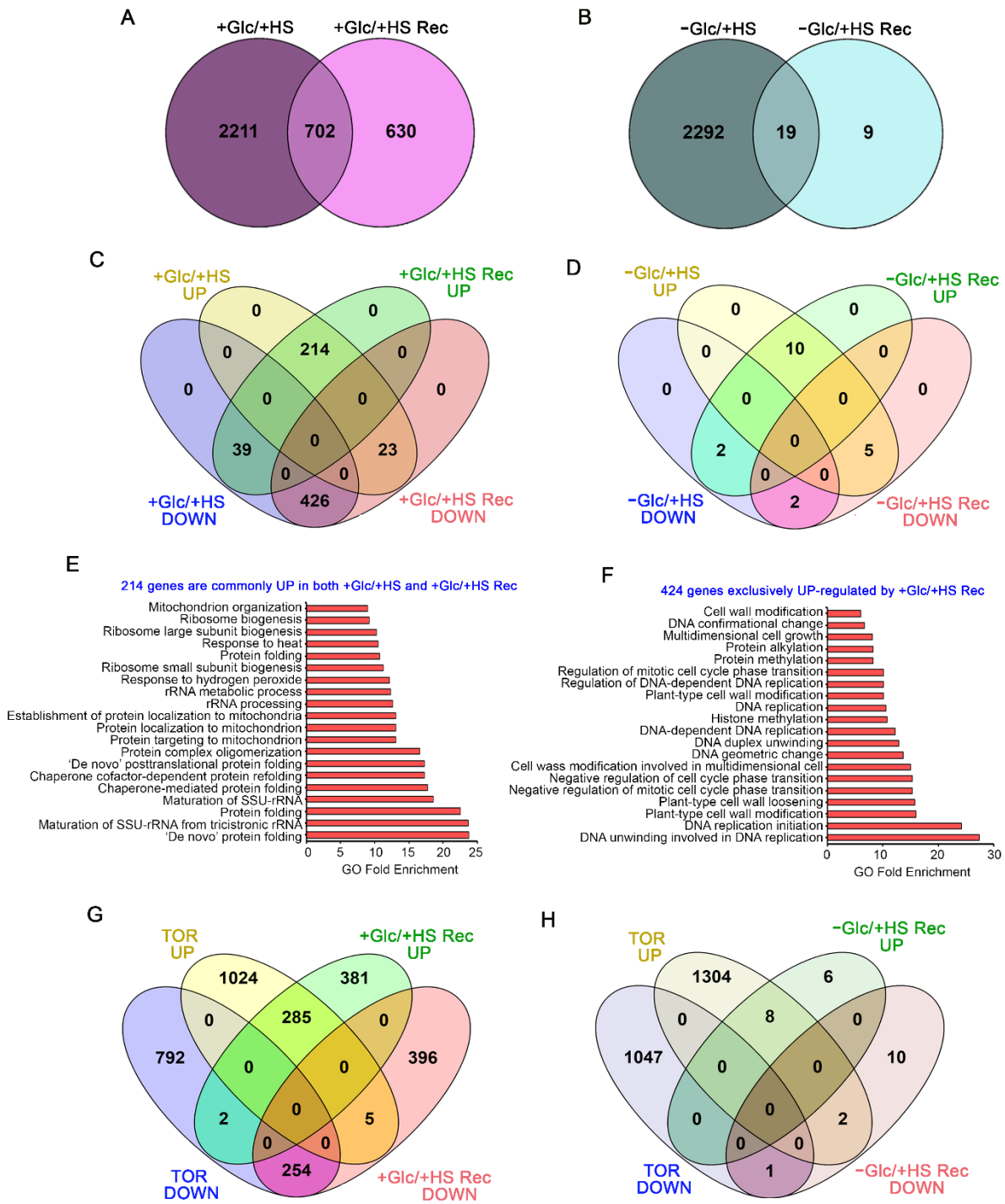

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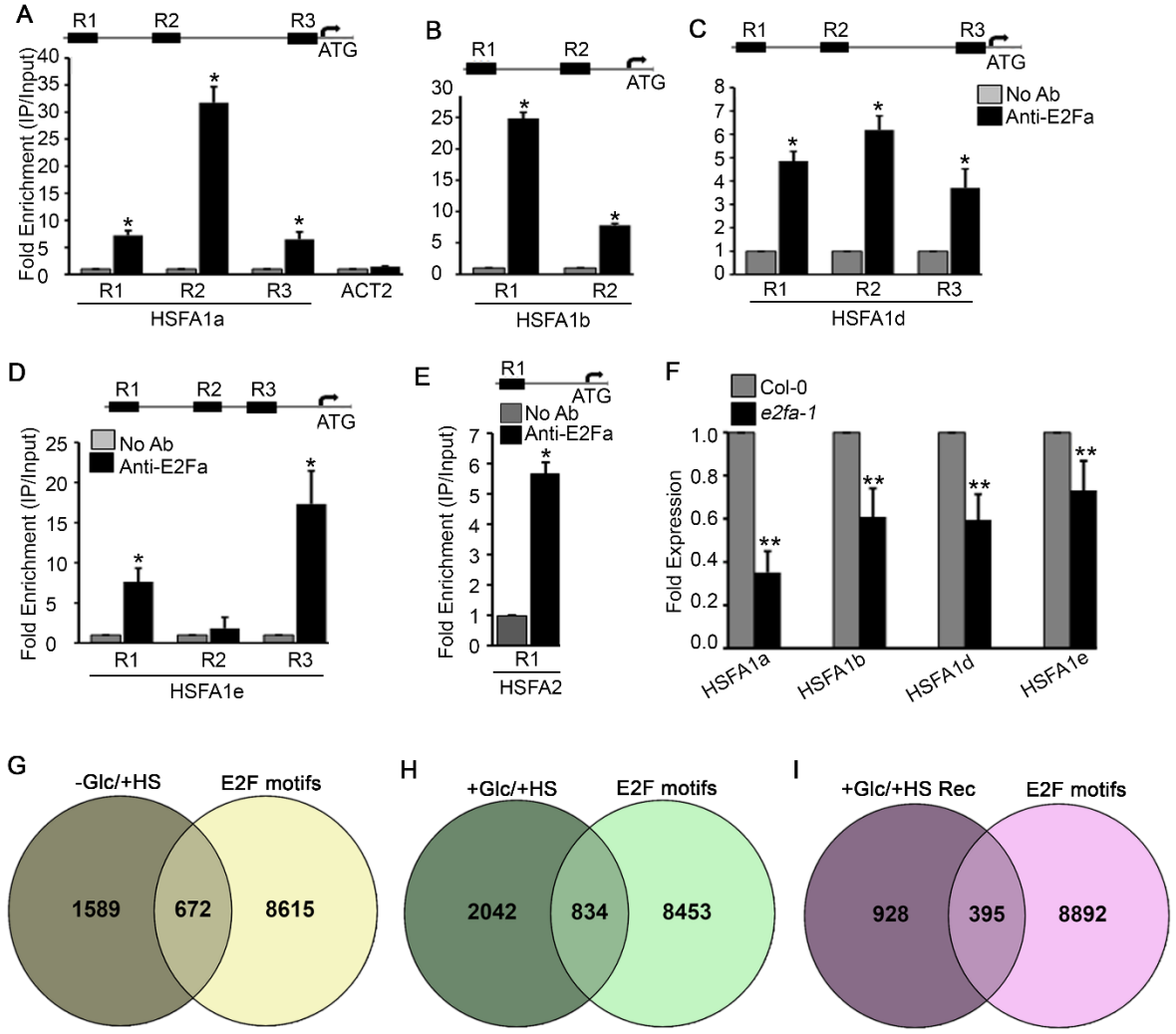

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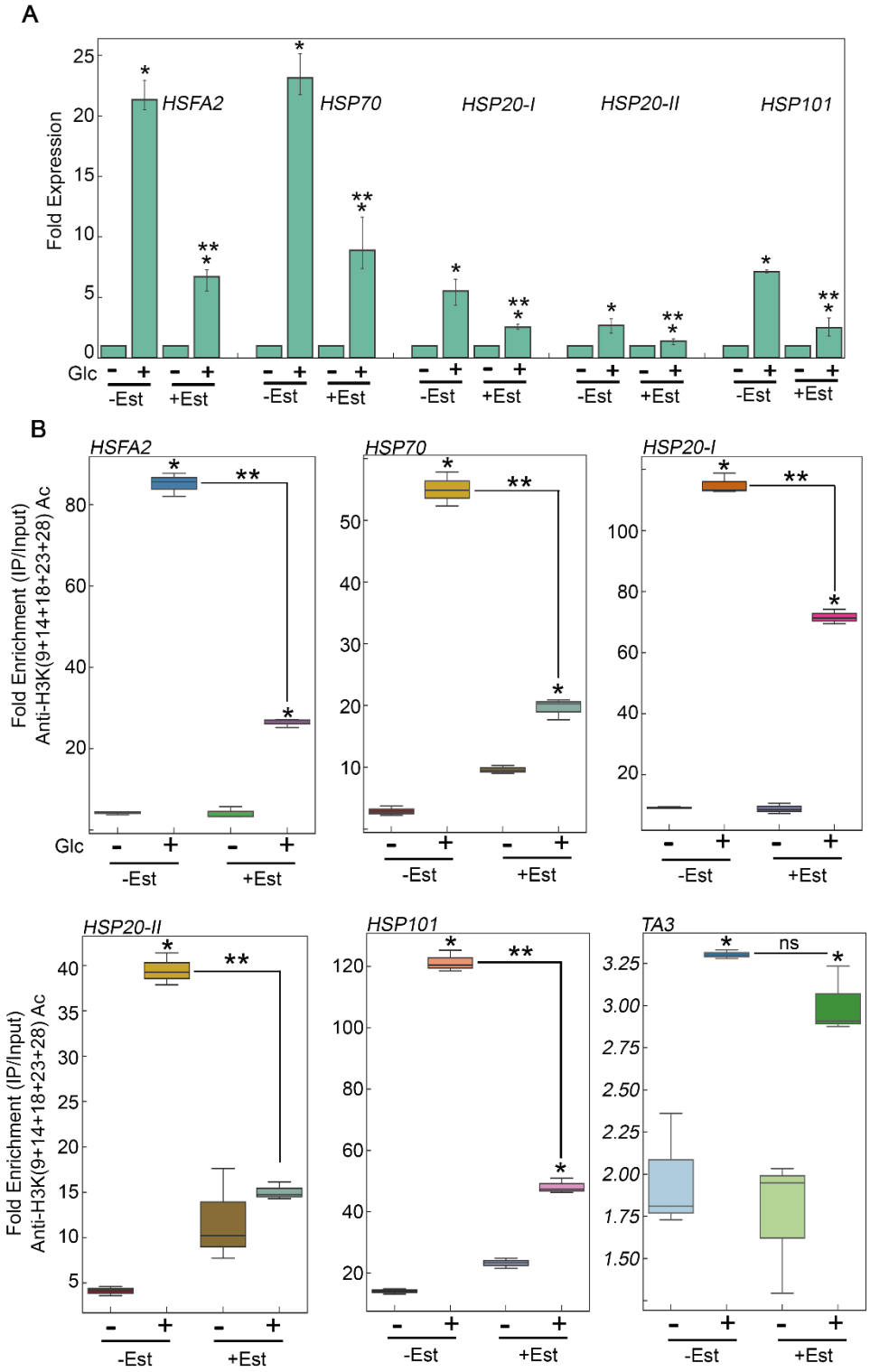

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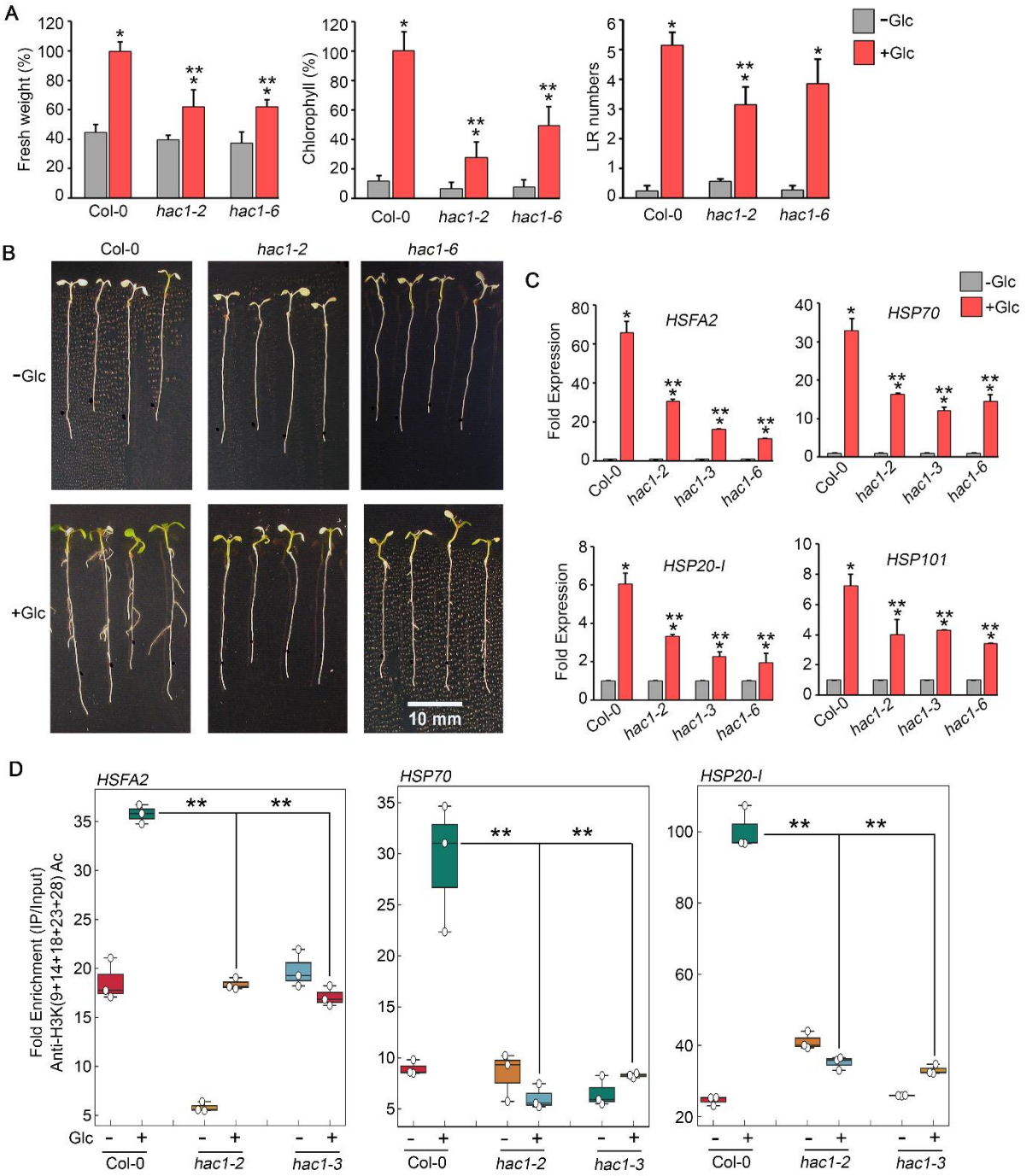

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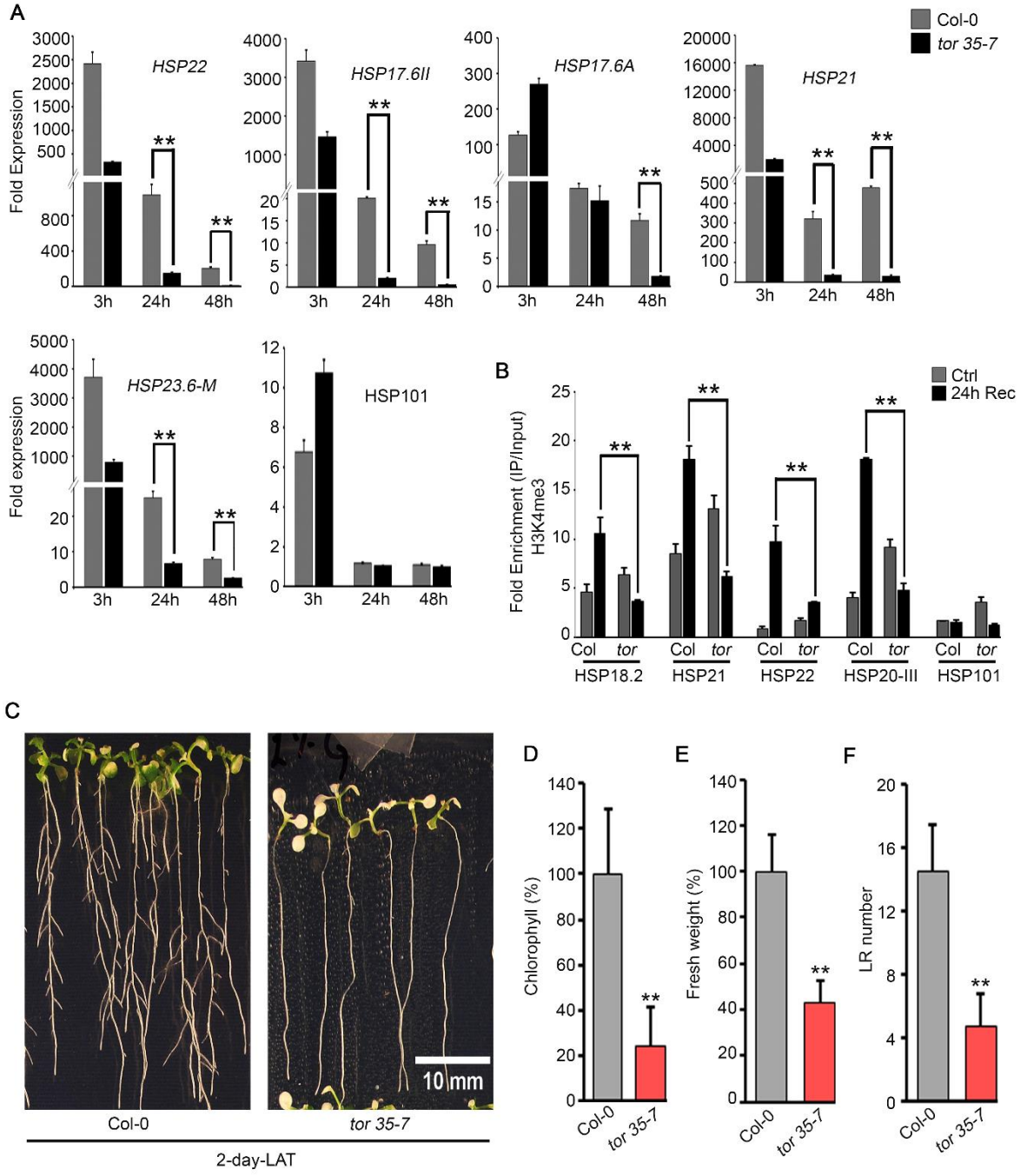

Figure 7. Glc regulated TOR is required in maintaining thermomemory. A, RT-qPCR expression of thermomemory related genes in Arabidopsis Col-0 and *tor 35-7 RNAi* seedlings. Arabidopsis Col-0 and *tor 35-7* plants were grown for seven days in presence of 1% Glc (56mM) under long-day conditions (16h light and 8h dark, 100 µM m-2 s-1 light intensity) treated without or with HS. HS was applied as 1h_37ºC, 90min_22ºC, 45min_45ºC. After HS, seedlings were recovered for various time points (3h, 24h and 48h) at 22ºC. Data shown are representative of four technical replicates among two biological replicates. Experiments were repeated twice times with similar results. **B,** ChIP-qPCR enrichment of thermomemory related genes in Col-0 and *tor 35-7* seedlings treated without or with HS. Following HS, seedlings were recovered for 24h at 22°C. Amount of immunoprecipitated promoter DNA was calculated by comparing samples treated without or with anti-Histone H3K4me3 antibody. C_T Values without and with anti-Histone H3K4me3 antibody were normalized by input control. Data shown are representative of one biological replicate. Experiments were repeated four times with

similar results. **C,** Picture showing seedling survival of Arabidopsis Col-0 and *tor 35-7* RNAi after long term acquired thermotolerance. **D-F,** Percentage Chl, fresh weight and LR count of Arabidopsis Col-0 and *tor 35-7* RNAi after LAT HS. Arabidopsis Col-0 and *tor 35-7* plants were grown for seven days in presence of 1% Glc (56mM) under long-day conditions (16h light and 8h dark, 100 µM m-2 s-1 light intensity) and subjected to HS. HS was applied as 1h_37ºC, 2d_22ºC, 2.5h_45ºC and 3-4d_22ºC. Data shown are representative of four biological replicates. Experiments were repeated four times with similar results.

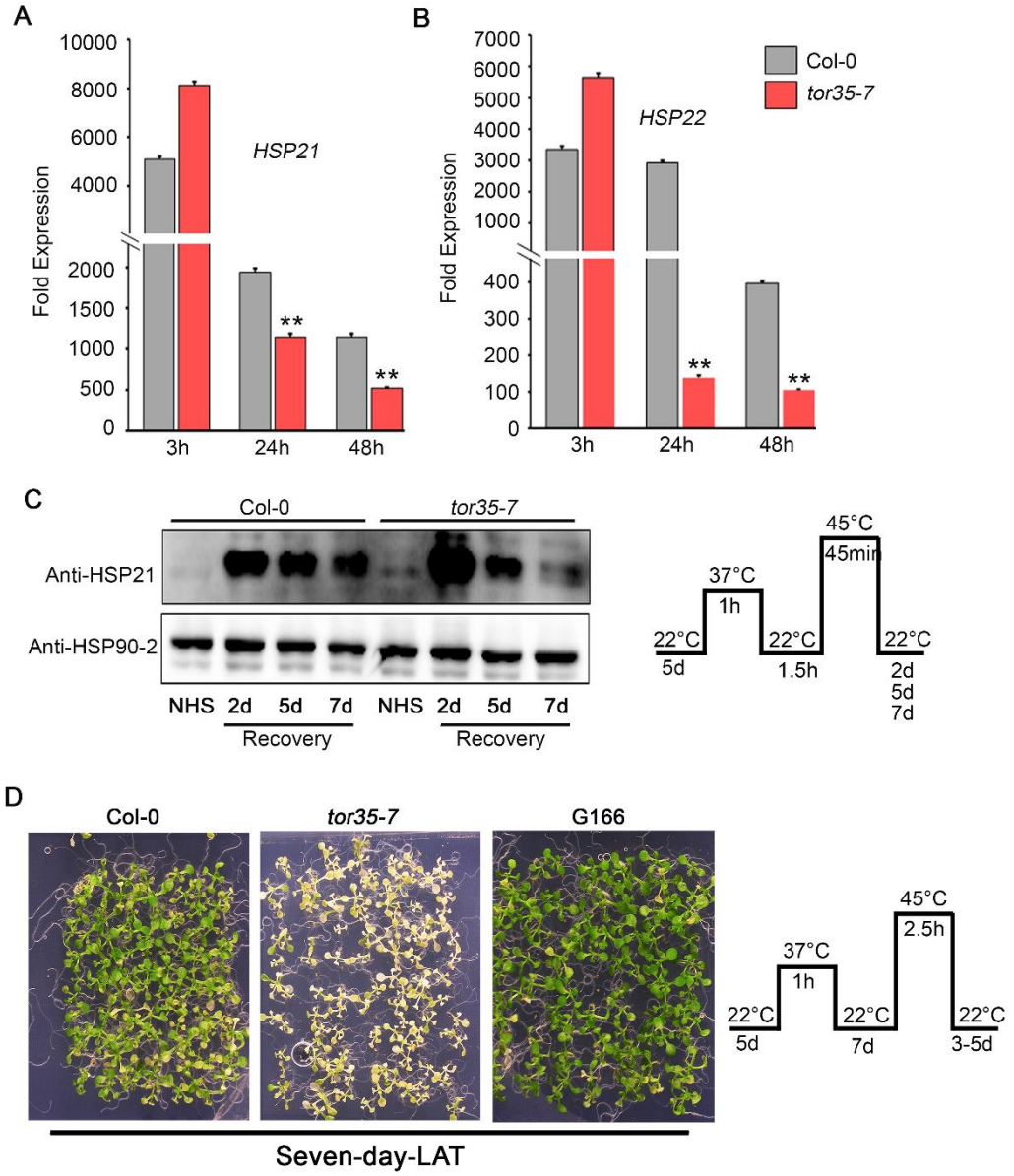

Figure 8. TOR controls the duration of thermomemory. A, B, RT-qPCR expression of thermomemory related genes in Arabidopsis Col-0 and *tor 35-7 RNAi* seedlings after LAT HS. Arabidopsis Col-0 and *tor 35-7* plants were grown for seven days in presence of 1% Glc (56mM) under long-day conditions (16h light and 8h dark, 100 µM m-2 s-1 light intensity) treated without or with HS. HS was applied as 1h_37ºC, 2d_22ºC, 45min_45ºC. After HS, seedlings were recovered for various time points (3h, 24h and 48h) at 22ºC. Data shown are representative of one biological replicate. Experiments were repeated twice with similar results. **C,** Immunoblot detection of thermomemory-associated HSP21 protein in Arabidopsis Col-0 and tor 35-7 seedlings after 2-7d of HS recovery. Arabidopsis plants were grown for five days in MS medium containing 1%Glc (56mM) and subjected to HS as 1h_37ºC, 1.5h_22ºC, 45min_45ºC. Following HS, seedlings were recovered for 2d, 5d and 7d at 22ºC. HSP21 protein was detected using anti-HSP21 specific antibody and HSP90 was detected using Anti-HSP90-2 specific antibody and used as a loading control. Data shown are representative of one biological replicate. Experiment was repeated thrice with similar result. **D,** Phenotype of

Arabidopsis Col-0 and tor 35-7 plants after seven-day of LAT HS. Arabidopsis Col-0 and *tor 35-7* plants were grown for five days in MS medium containing 1% Glc (56mM) and subjected to HS. HS was applied as 1h_37ºC, 7d_22ºC, 2.5h_45ºC and 3-5d_22ºC. Picture shown are representative of one biological replicate. Experiment was repeated twice with similar results.

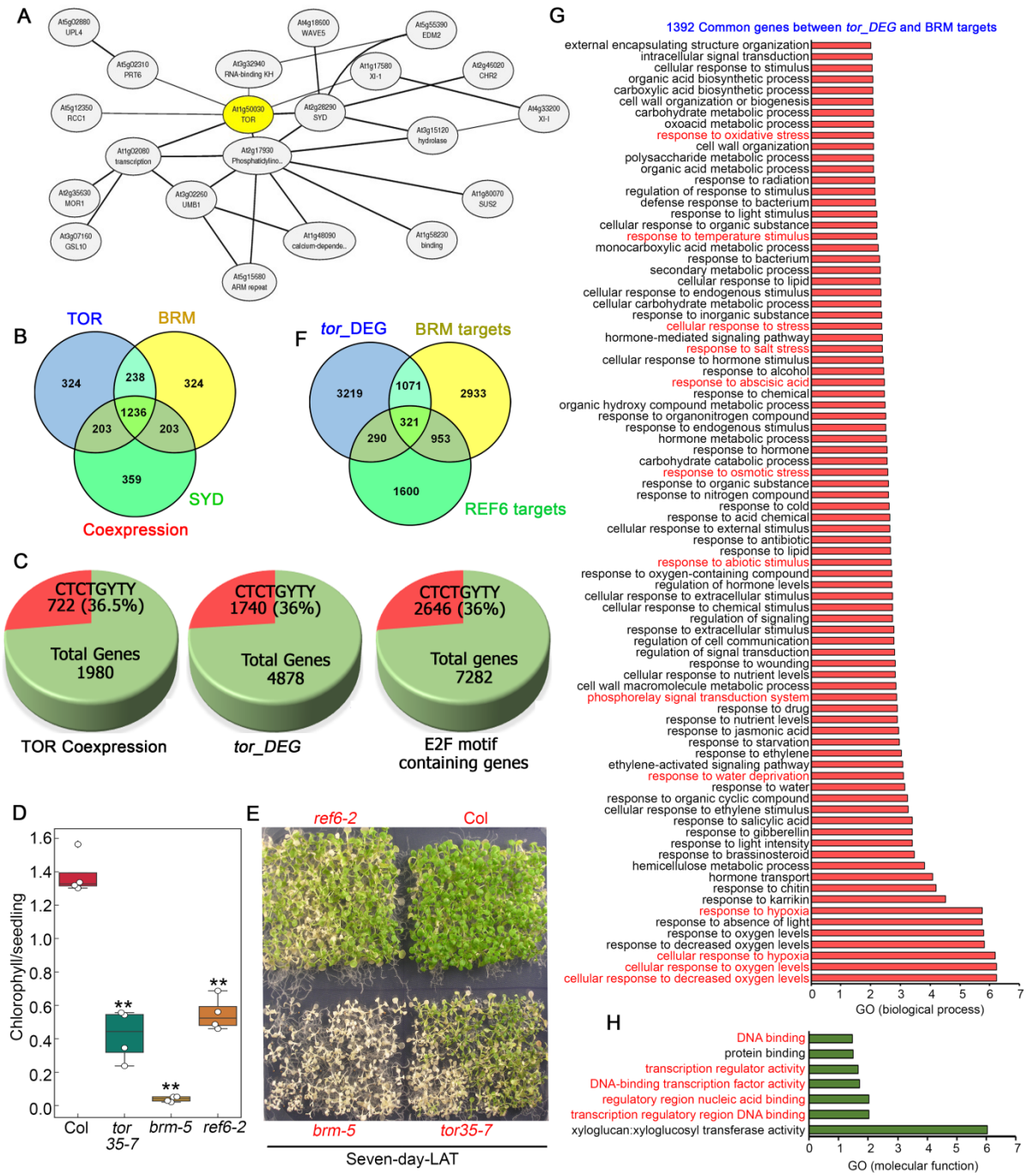

Figure 9. TOR co-expresses with chromatin remodeler BRM and overlaps common downstream targets. A, Network showing co-expression data of TOR with BRM and SYD (CHR3). Co-expression network was extracted from ATTED-II. **B,** Venn diagram showing overlap of TOR co-expressing genes with BRM and SYD. **C,** Pictures showing enrichment of BRM-REF6 recognising genomic motif CTCTGYTY in TOR co-expressing genes, tor_DEG and in genes containing E2Fa motifs in their promoters. 1000 bp upstream and 200 bp downstream Promoters relative to TSS was considered for promoter analysis. **D, E** Chlorophyll measurement and phenotype of Arabidopsis Col-0, *tor35-7*, *brm-5* and *ref6-2* plants after seven-day of LAT HS. Arabidopsis

Col-0, *tor35-7*, *brm-5* and *ref6-2* plants were grown for five days in MS medium and subjected to HS. HS was applied as 3h_37ºC, 7d_22ºC, 2.5h_45ºC and 3-5d_22ºC. Picture shown are representative of one biological replicate. Experiment was repeated thrice with similar results. **F,** Venn diagram showing overlap of genes differentially regulated by TOR with BRM and REF6 targets. **G, H,** GO fold enrichment of biological process and molecular function of 1392 genes commonly regulated by *tor_DEG* and BRM targets. Panther 15.0 tool was used to analyse GO fold enrichment using Bonferroni correction and Fisher's exact test type.

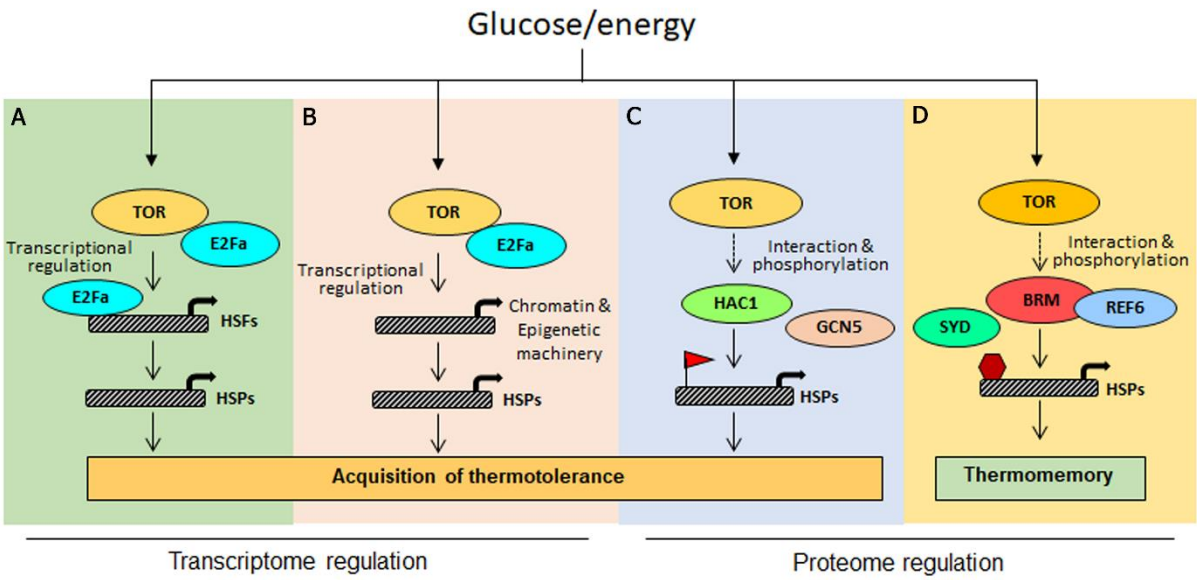

Figure 10. Proposed model shows how Glc-TOR signalling through distinct mechanisms regulates thermotolerance/thermomemory. A, Arabidopsis TOR regulated E2Fa transcription factor recruited at the promoters of HSFA1 and HSFA2 genes to control their expression which in turn regulate HS gene expression. **B,** Arabidopsis TOR-E2Fa module also regulates transcriptome reprogramming of genes involved in chromatin remodelling/epigenetic modifications which are crucial to regulate thermotolerance response. **C,** Glc through TOR promotes recruitment of histone H3 acetylation marks at the promoters of HS genes to induce their transcription, leading to thermotolerance. This Glc-TOR mediated histone acetylation is facilitated through HAC1 and GCN5. **D,** Sugar also controls interaction and phosphorylation of several chromatin proteins including BRM, SYD and REF6. TOR co-expresses with BRM & SYD and exhibits huge overlap with BRM target genes. TOR promotes the accumulation of histone methylation marks at the promoters of thermomemory associated gene promoters, and therefore regulates thermomemory.