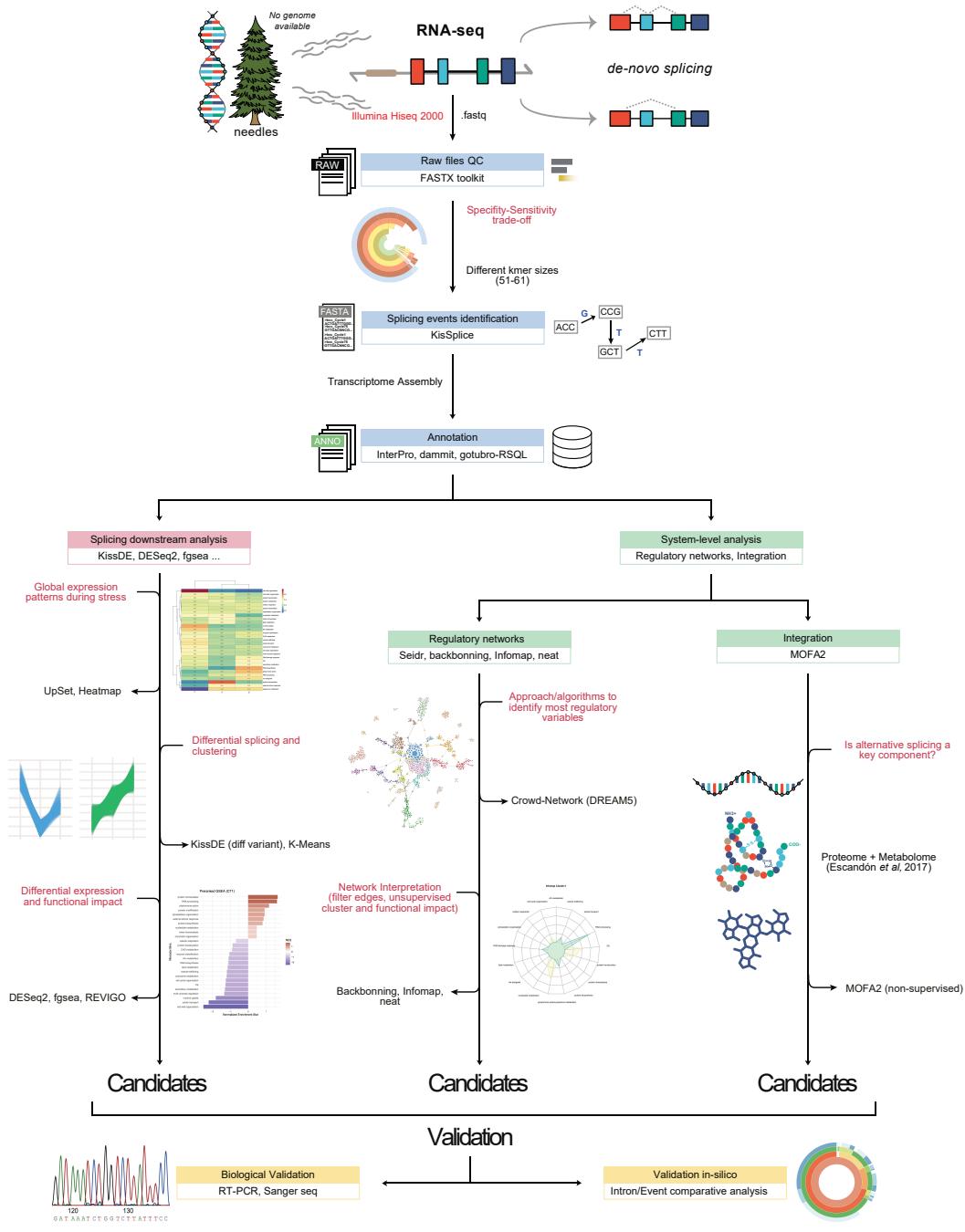


Supplementary Table 1. All genome data sources for the species used in intron comparative analysis.

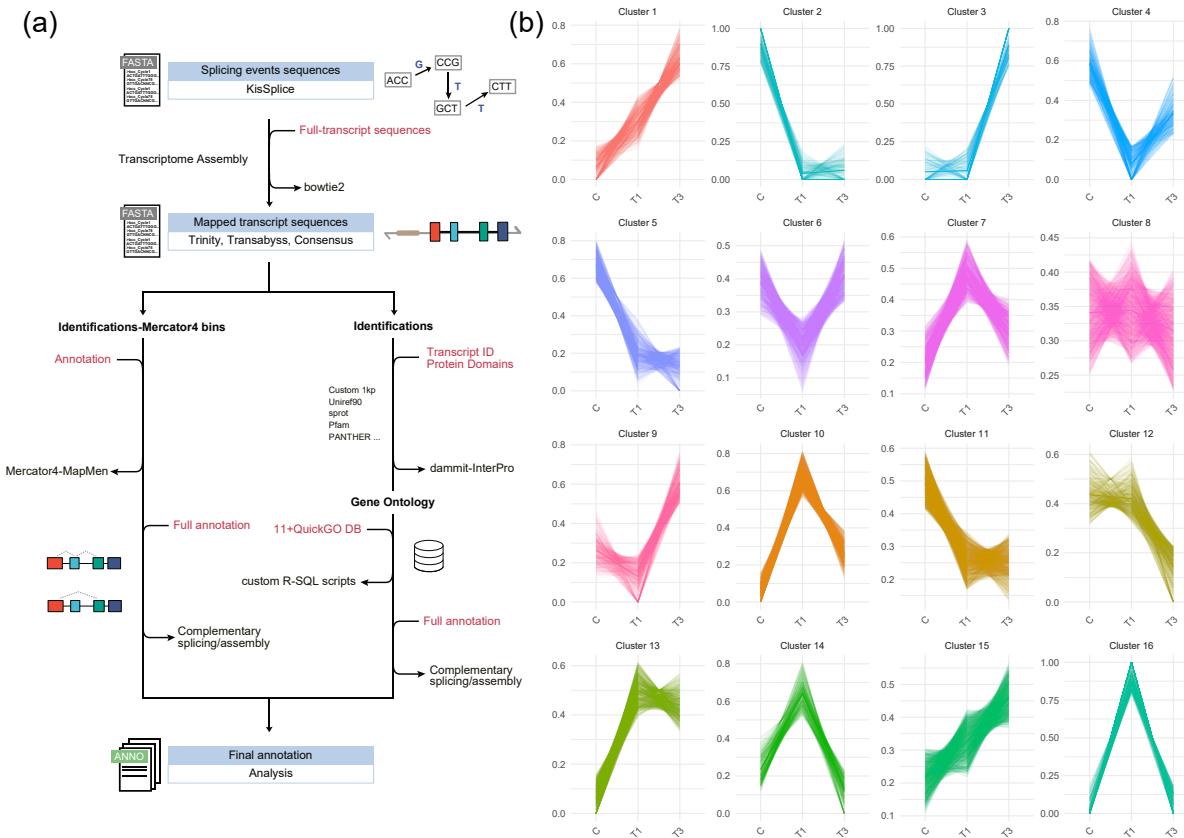
Species	Source
<i>Arabidopsis thaliana</i>	Ensembl Plants 49 (https://plants.ensembl.org/index.html)
<i>Oryza sativa</i>	Ensembl Plants 49 (https://plants.ensembl.org/index.html)
<i>Zea mays</i>	Ensembl Plants 49 (https://plants.ensembl.org/index.html)
<i>Amborella trichopoda</i>	Ensembl Plants 49 (https://plants.ensembl.org/index.html)
<i>Ginkgo biloba</i>	TreeGenesDb (https://treegenesdb.org)
<i>Gnetum montanum</i>	TreeGenesDb (https://treegenesdb.org)
<i>Pseudotsuga menziesii</i>	TreeGenesDb (https://treegenesdb.org)
<i>Pinus lambertiana</i>	TreeGenesDb (https://treegenesdb.org)
<i>Pinus taeda</i>	TreeGenesDb (https://treegenesdb.org)

Supplementary Table 2. List of primers used for RT-PCR.

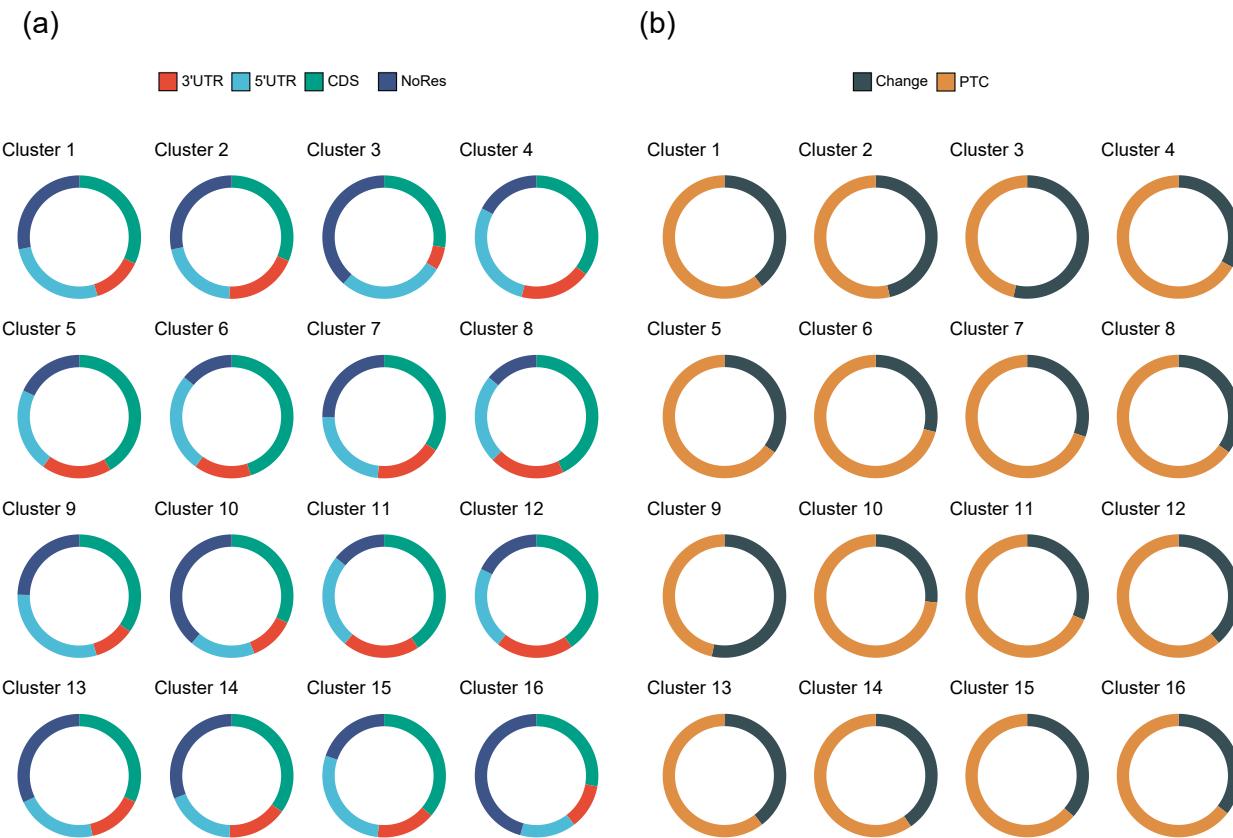
ID	Name	Forward	Reverse	Tm (°C)
bcc_3998 Cycle_0	Histone deacetylase HDT2 (HDT2)	AGGAAGCGTCTTCATTGG	CGATCATCATCAGAACAGACTG	61
bcc_17045 Cycle_0	Magnesium, protoporphyrin IX methyltransferase, chloroplastic (CHLM)	CCTCCAGAGGCCATCATT	GTTCGCAGACCCTGAGACTAC	61
bcc_29432 Cycle_2	Uncharacterized protein, DUF4050 (DUF4050)	CACAGTTGACAAATGACAGTAC	GTGGAAAAGGCTGGTGGTT	61
bcc_9458 Cycle_1	ESCRT-related protein CHMP1B (CHMP1B)	ATTCAGCTCAGGGTTCCACG	GAAAGGCTGAGGAAGATAACC	61
bcc_9268 Cycle_9	Serine/arginine-rich splicing factor (RSZ22)	ACGTTAGGTGTAGCTCTTC	GGAGTCCCAGTCCTCGATAC	60
bcc_12324 Cycle_0	General transcription and DNA repair factor IIH subunit TFB5 (TFB5)	GCCTAGCAGCGTTCAAATG	TGCGCTACTTCTTCTCCAG	60
bcc_40022 Cycle_1	Glycine-rich RNA-binding protein RZ1A (RZ1A)	TGGCGGGCGTTCTATGATG	AATTCTTACTGAGGCATCACAGC	60
bcc_15225 Cycle_0	Ubiquitin-conjugating enzyme E2 36 (UBC36)	ACATTGCAAAACACTGGAAGAC	TTGAGACGGCGTCATTCAG	60



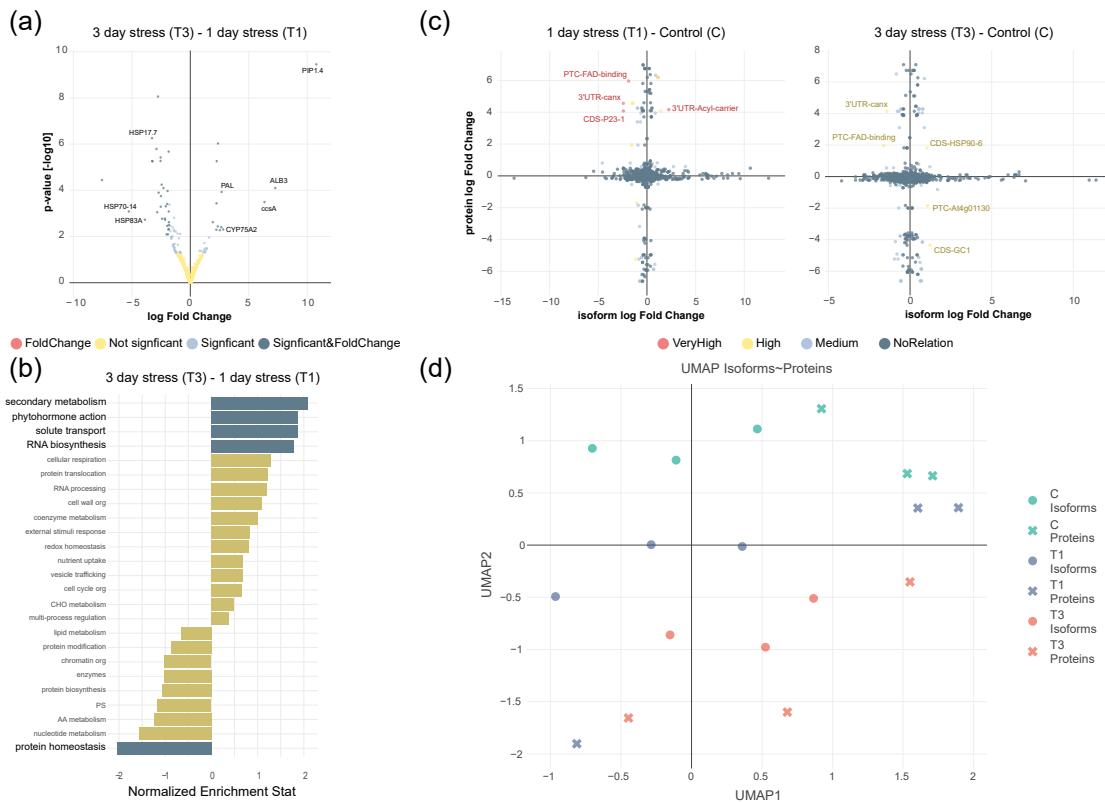
Supplementary Figure 1. Overview of the bioinformatics workflow.



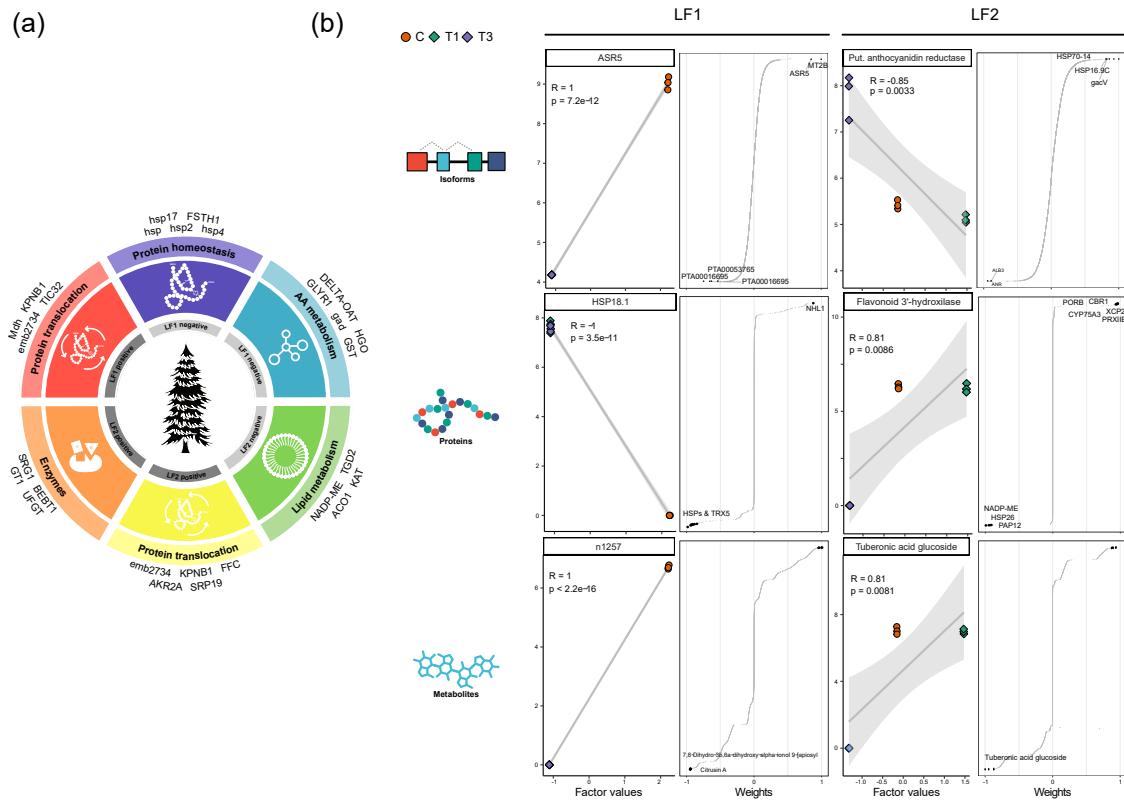
Supplementary Figure 2. Annotation pipeline and non-supervised behavior clusters. (a) KisSplice isoform sequences were mapped to different transcriptome assemblies using bowtie2. Then, full-transcript sequences were identified and annotated to achieve functional information using Mercator4-Mapmen and custom R-SQL script integrated with 12 gene ontology databases. Finally, all the annotations were completed with complementary assembly/splicing. (b) K-means clustering of all isoforms reported. The solid line shows the mean for each cluster. The sampling times correspond to the 40 °C assay shown in **Figure 1**.



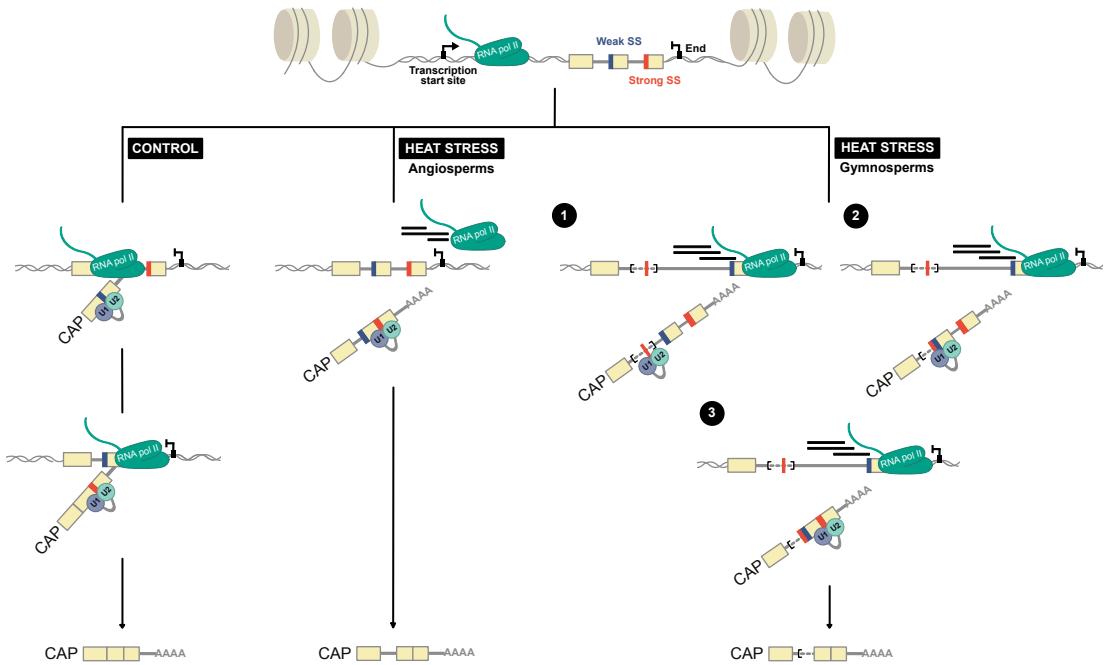
Supplementary Figure 3. Splicing variation effect proportions across non-supervised behaviour clusters. (a) Pie-charts showing proportions corresponding to regions where the splicing variation was produced for all the isoforms of each cluster. NoRes = neither full model nor full-transcript sequences were predicted/mapped so events could not be classified. Different colors reflect transcript regions where the splicing variation was produced. (b) Pie-charts illustrating proportions corresponding to splicing variation effect for all the CDS isoforms of each cluster. Change = lead to protein sequence changes (gray). PTC = introduce premature termination codons (orange).



Supplementary Figure 4. Stress-specific contrast functional description and isoform-protein relationships. (a) Volcano analysis of DD event isoforms. Not significant = FDR-adjusted $P > 0.05$ and absolute log fold change < 1.8 ; Significant = FDR-adjusted $P < 0.05$ and absolute log fold change < 1.8 ; Fold Change = FDR-adjusted $P > 0.05$ and absolute log fold change > 1.8 ; Significant & Fold Change = FDR-adjusted $P < 0.05$ and absolute log fold change > 1.8 . (b) Gene set enrichment analysis of DD event isoforms using Mercator4 functional categories. Blue and gold indicate significative and non-significative enriched terms, respectively. (c) Volcano analysis of isoform-protein relationships. NoRelation = absolute isoform and protein log fold change < 0.5 ; Medium = absolute isoform and protein log fold change > 0.5 ; High = absolute isoform and protein log fold change > 1 ; VeryHigh = absolute isoform and protein log fold change > 1.8 . (d) Scatter-plot of UMAP1 (x axis) and UMAP2 (y axis) illustrating the proteins projection over isoforms. Samples are coloured/shaped according to treatment (C, T1, T3) and molecular level (isoforms, proteins), respectively. The sampling times correspond to the 40 °C assay shown in **Figure 1**.



Supplementary Figure 5. Functional characterization of proteins and all regulatory layers biomarkers for each MOFA2 latent factor. (a) Enriched Mercator4 functional categories and top weight proteins associated to latent factor 1 (top) and latent factor 2 (bottom). Positive = functional terms linked to samples with > 0 scores; Negative = functional terms linked to samples with < 0 scores. (b) For each regulatory layer (rows) and each biologically relevant latent factor (columns), weights distribution (right panel) and scatterplot of factor values (x-axis) versus expression/abundance values (y-axis) for the top features with largest weight (left panel) are displayed. Samples are coloured according to treatment (C, T1, T3). The sampling times correspond to the 40 °C assay shown in **Figure 1**.



Supplementary Figure 6. Heat stress-responsive intron retention hypothesis in gymnosperms. Under control conditions, the spliceosome (represented only by U1 and U2 pre-spliceosome complex for a easier interpretation) binds co-transcriptionally to the unique splicing sites available, leading to constitutive splicing (Control). During heat stress, several factors, such as nucleus hypomethylation and faster kinetics, result in transcription loss of control which leads to simultaneous availability of multiple splice sites, thus, splice sites compete for spliceosome/splicing factors recruitment depending on their strength (Heat stress). As a product of this, a general increase of retained introns, especially those ones flanked by weak splice sites, would be induced (angiosperms). However, intron length divergence between seed plants (angiosperms and gymnosperms) may have crucial implications in this type of RNA processing. Despite being flanked by weak splice sites, gymnosperms long introns (indicated by a dashed line) might hinder intron recognition as a single unit to be retained because of their length or the potential appearance of strong cryptic splice sites (gymnosperms). All of this could ultimately cause the retention of fragmented instead of complete introns and/or a lower prevalence of IR in gymnosperms.