Supplementary Table 1. Current state of epigenetics in trees for development, abiotic stress or priming, biotic stress or priming, and markers, breeding and biotechnology topics. 2,4-D: 2,4-dichlorophenoxyacetic acid; 5-Azac: 5-Azacytidine; 5-mC: 5-methylcytosine; 5-mdC: 5-methyldeoxycytidine; ABA: Abscisic acid; AcH3: Acetylated histone H3; AFLP: Amplified Fragment Length Polymorphism; AnAc: Anacardic Acid; AREB1: Abscisic acid-Responsive Element 1; BA: Benzyladenine; BS-seq: bisulfite sequencing; ChIP: Chromatin ImmunoPrecipitation; ChIP-seq: Chromatin ImmunoPrecipitation sequencing; CsDML*: Castanea sativa* DEMETER-like; DCL3: DICER-like 3; DDM1: Decreased DNA Methylation 1; GA: Gibberellic Acid; HATs: Histone Acetyl Transferases; HDACs: Histone DeACetylases; DNase-seq: DNase sequencing; GDM: Global DNA Methylation; GeLC-Orbitrap/MS: Gel enhanced Liquid Chromatography - Orbitrap/Mass Spectrometry; IAA: Indole Acetic Acid; IBA: Indole-3-butyric acid; HPCE: High-Performance Capillary Electrophoresis; HPLC: High-Performance Liquid Chromatography; MACE-seq: Massive Analysis of cDNA Ends sequencing; MeDIP: Methylated DNA ImmunoPrecipitation; MeDIP-chip: Methylated DNA ImmunoPrecipitation chip; MeDIP-seq: Methylated DNA ImmunoPrecipitation sequencing; miRNA: microRNA; miRNA-array: microRNA array; MRE-seq: Methylation-sensitive Restriction Enzyme sequencing; MS: Mass Spectrometry; MS-PCR: Methylation Sensitive PCR; MS-RAPD: Methylation Sensitive - Random Amplified Polymorphic DNA; MSAP: Methylation-Sensitive Amplification Polymorphism; PARE-seq: Parallel Analyses of RNA Ends sequencing; rasiRNA: repeat associated small interfering RNAs; RNAi: RNA interference; RNA-seq: RNA sequencing; RRBS: Reduced Representation Bisulfite Sequencing; RT-qPCR: Reverse Transcription quantitative PCR; SAHA: Suberolylanilide Hydroxamic Acid; SAMs: Shoot Apical Meristems; SEs: Somatic Embryos; SMVs: Single-Methylation Variants; SNP: Single Nucleotide Polymorphism; sRNA: small RNAs; sRNA-seq: small RNA sequencing; TEs: Transposable Elements; TF: Transcription Factor; TLC: Thin Layer Chromatography; TSA: TrichoStatin A; WGBS: Whole Genome Bisulfite Sequencing.

Topics	Species	Plant Material	Experimental Conditions	Epigenetic Analysis	Main Conclusions	Reference
			DEVELOPMENT			
AGING	*Sequoiadendron giganteum*	Clonal lines from a 100-years-old giant tree, apical shoot	*In vitro* meristem-tissue with juvenile-like characteristics vs. Mature-type outdoor lines from grafts and rooted microcuttings.	HPLC	Shoots with a juvenile-like leaf morphology showed higher DNA methylation than outdoor-origin shoots from the same clone.	[1]

AGING	*Acacia mangium*	Clones, buds	**(1)** apical buds from juvenile- and mature-like microshoots of juvenile and mature sources. **(2)** mature source clones: apical and axillary buds collected from field elongating shoots vs. apical buds from *in vitro* microshoots exhibiting the same mature-like phyllode morphology.	HPLC	The higher rate of DNA methylation in juvenile buds and shoots vs. mature ones in *A. mangium* supports that DNA methylation in plants does not always increase with aging.	[2]
BUD	*Castanea sativa*	3-year-old trees, buds	Buds from different positions on trees from several provenances grown in the same location were collected at different stages of bud burst and set.	HPCE, Protein Gel Blot, Immunolocalization	The increased global DNA methylation and decreased H4 histone acetylation levels patterns during bud set were inverted during bud burst. These patterns differ in apical vs. axillary buds.	[3]
ORGANOGENESIS	*Pinus radiata*	Cultured and mature embryos	Collection of cones over 3 consecutive years. *In vitro* culture of embryos under growth regulator treatments (BA, ABA, IBA, 2,4-D).	Genomic DNA methylation, Immunofluorescence	Several growth regulators in apical meristems are epigenetically regulated during organogenic processes.	[4]
DAY/NIGHT CYCLE	*Populus nigra*	4-month-old clones, mature leaves	Greenhouse conditions. Sampling	MeDIP-seq	Methylated genes were prevalent in the poplar	[5]

			at 8:00 (day) and 24:00 (night).		genome but only a few of these participated in diurnal genes expression regulation.	
DEVELOPMENTAL REGULATION	*Quercus lobata*	5-month-old seedlings, leaves	Collection of acorns from 8 localities (California, USA). Greenhouse: foliar application of 5-Azac (a methylation inhibitor) for 27 days.	WGBS	The reduction in genome-wide methylation resulted in differential gene expression and substantial reduction in new growth. DNA methylation is involved in seedlings' gene expression and phenotypic variations and the removal of DNA methylation affects plant development.	[6]
DEVELOPMENTAL REGULATION	*Populus trichocarpa* and *Populus deltoides x Populus nigra*	Shoot apical meristematic cells	Rooted stem cuttings from 10-year-old mother plants were grown in a glasshouse (15-27°C).	MeDIP, BS-seq	DNA methylation is widespread and variable among genes in open chromatin of meristematic cells.	[7]
EVOLUTION	*Populus balsamifera*	Mature trees, leaves and vegetative buds	Trees grown under natural conditions. Weekly sampling during June and July.	sRNA-seq	A large fraction of miRNAs varies in comparison with *Populus trichocarpa*. Non-conserved miRNAs may regulate cellular, physiological or developmental taxa-specific processes. All miRNAs seem to target genes with similar biological functions indicating similar selection pressures on both miRNA types.	[8]

EVOLUTION	*Populus trichocarpa*	Several genotypes, roots and leaves	Sampling of clonally replicated genotypes (across USA). Plants grown in hydroponic systems.	MeDIP-seq	Geographic differentiation at multiple scale of structure populations (i.e. allele frequency,) effective population size) reveal that genetic drift has played a significant role in the recent evolutionary history of *P. trichocarpa*.	[9]
LEAF	*Eucalyptus globulus*	Ramets of a selected clone produced by cuttings, leaves	Field genetic trial (Chile): juvenile leaves (after 6 months) vs. adult leaves (more than 2 years).	MeDIP-seq, MRE-seq	Locus-specific methylation could be major regulators of vegetative phase change, which may be useful in conservation programs, e.g. selecting the best methylomes for a particular environment in a restoration project. Further investigations are required to define the loci associated with heterochrony/heteroblasty (the change in the timing or rate of developmental events during ontogeny) regulated by DNA methylation.	[10]
NEEDLE MATURATION	*Pinus radiata*	App. 15-year-old adult trees, needles	Field plantation: mature (12-month-old) vs. immature (3–5-week-old, active growth) needles.	HPCE, MSAP, Protein Blot, 5mC-Immunolocalization	Needle maturation (associated with decreased organogenic capability) is related to an increase in heterochromatin-related epigenetic markers (high DNA methylation and	[11]

NEEDLE MATURATION	*Pinus radiata*	Mature, developed and immature needles, calli	Calli induced from needles collected in test-garden. 1-year-old seedlings grown in greenhouse were submitted twice to SAHA (inhibitor of HDACs) or to AnAc (inhibitor of HATs).	RT-qPCR, BS-seq, ChIP	low acetylated histone H4 levels, and the presence of histone H3 methylated at lys 9). DNA methylation of palisade parenchyma cell layers during the transition from immature to mature scions is associated with the loss of the capacity to induce adventitious organs. Needle maturation correlates with changes in global DNA methylation and histones levels. Photosynthetic carbon fixation regulation is associated to a crosstalk between histone H4 acetylation and H3K9me3 at the promoter level.	[12]
PHOTOSYNTHESIS	*Populus simonii x Populus nigra*	5-month-old plants - leaves, stem chlorenchyma and vascular tissues, roots	Seedlings grown in greenhouse conditions. Spraying with 2.5 and 5 μM of the HDAC specific inhibitor TSA for 2 days.	ChIP	Histone acetylation positively regulates the tissue-dependent expression pattern of the poplar homologs of C4 photosynthetic enzymes genes. This regulatory mechanism seems to be conserved among the C3 and C4 species.	[13]
HORMONE REGULATION	*Populus tomentosa*	1-year-old clones, mature leaves	Greenhouse conditions. 100μM IAA treatment daily	WGBS, HPLC, MSAP, RNA-seq	IAA treatment induces a change in DNA methylation pattern and is manifested by a	[14]

			for 1 week.		long-term growth inhibition.	
POLLEN	*Pinus taeda*	Pollen grains	Pollen cones (in dehydration for dormancy and dispersal) collected from field grown pines. Mature (ungerminated) vs. germinated pollen.	miRNA-array	Many conserved miRNAs showed stage-dependent expression in mature and germinated loblolly pine pollen, indicating that the two stages of the male gametophyte examined are regulated at the miRNA level.	[15]
POLLEN	*Cryptomeria japonica*	Second-generation offspring male trees, strobili	Strobili from fertile vs. sterile male trees from the field (Japan). Sampling at early stages of pollen development.	sRNA-seq	Both conserved and species-specific sRNAs contribute to the development of male strobili.	[16]
REPRODUCTIVE DEVELOPMENT	*Pinus tabuliformis*	Genetically distinct individuals, immature cones	Collection of immature cones in botanic gardens (China): male vs. female.	sRNA-seq, PARE-seq	The sRNA pathways have higher activity in female than in male cones, and the miRNA pathways are the main sRNA pathways.	[17]
REPRODUCTIVE DEVELOPMENT	*Ginkgo biloba*	30-year-old trees, mature leaves	Trees grown under natural conditions: male vs. female.	sRNA-seq	Identification of a large number of miRNAs in mature female and male leaves, which are likely involved in the regulation of primary biological processes such as plant-pathogen interactions, plant hormone signal transduction, and flavonoid biosynthesis.	[18]
REPRODUCTIVE	*Populus*	Several 29-years-old	Trees from natural	BS-seq	miRNA 172b might play an	[19]

DEVELOPMENT	*tomentosa*	clone trees, flower (last phase of development, before pollination)	populations representative of the geographic distribution (China): male vs. female flowers from andromonoecious trees. Validation with flowers from gynomonoecious clones, male poplar flowers, and female poplar flowers.		important role in the regulation of bisexual flower development-related gene expression in andromonoecious poplar, via modification of methylation. Hyper-methylation in andromonoecious and gynomonoecious poplar might function as an important regulator in bisexual flower development.	
ROOT	*Populus trichocarpa*	Seedlings, roots	*In vitro* stem segments. Shoots developed from the axillary buds were treated with 0, 1, and 2.5 μM of the HDAC specific inhibitor TSA.	HDACs Colorimetric Assay, RNA-seq	HDACs were required for *de novo* organogenesis and normal growth of poplar roots. Several genes differentially expressed depending on TSA concentration are probably regulated by HDACs during root development.	[20]
SEED	*Picea glauca*	Three populations (from the same orchard), seeds	Populations with different fertilization timing and seed set duration. Collection and dissection of seeds at early, middle and late seed set.	sRNA-seq	Lacking of 24-nt sRNAs at the late conifer seed developmental phase may result in less constraints in TE activities, thus contributing to the massive expansion of genome size.	[21]
SOMATIC	*Quercus suber*	SEs	Developmental	RT-qPCR, DNA	Change in the expression of	[22]

EMBRYOGENESIS			stages from immature to fully developed embryos were studied.	sequencing	genes associated with epigenetic regulation is needed for the correct development of *Q. suber* SEs.	
SOMATIC EMBRYOGENESIS	*Quercus suber*	SEs	Immature acorns were collected during fruit development, isolated and cultured under sterile conditions.	Genomic DNA methylation, Immunofluorescence	There is a specific epigenetic-related spatial-temporal regulation during embryogenesis, which play an important role in correct maturation and germination of SEs.	[23]
TISSUE	*Cunninghamia lanceolata*	Seedlings, adult leaves, stems, calli	Plants grown under greenhouse conditions: seedlings, adult leaves, and stems; dry seeds; and calli derived from immature seeds (*in vitro*).	sRNA-seq	Four conserved and one novel miRNAs displayed developmental stage-specific expression patterns. The DCL3-dependent rasiRNA generation pathway, which had been considered absent in conifers, was found in Chinese fir.	[24]
TISSUE	*Populus trichocarpa*	Mature leaves, vegetative buds, fine roots, xylem, phloem, male and female catkins (inflorescence)	Tissues obtained from 2-years-old clones or mature trees.	MeDIP-seq	DNA methylation is tissue-specific and gene-body DNA methylation (i.e. in transcribed regions) has a more repressive effect on transcription than promoter methylation.	[25]
XYLEM	*Eucalyptus grandis*	7-years-old ramets, developing secondary xylem	Field-grown clonal trees. Sampling in early Spring.	ChIP-seq	The enrichment of the activating histone modification H3K4me3 is an indicator of active	[26]

					transcription in developing xylem.	
ABIOTIC STRESS OR PRIMING						
WINTER DORMANCY	*Populus tremula x Populus alba*	4-year-old hybrids, 2-year-old branches	Hybrids grown in common garden (Spain). Temperature during harvesting were 3.6°C in Winter and 22.5°C in Summer.	Immunofluorescence	Higher 5-mC signal and lower H4 signal in winter may reveal an epigenetic control of winter dormancy in poplar stems.	[27]
WINTER DORMANCY	*Populus tremula* × *Populus alba*	Plantlets, shoot apices	24 short-day induction of wild-type and CsDML-overexpressing lines vs. Non-induction.	5-mC Immunodetection	DEMETER-like CsDML gene induces bud formation needed for the survival of the apical meristem under winter conditions.	[28]
WINTER DORMANCY	*Populus tremula* × *Populus alba*	Clonal trees, apical meristems	Wild-type and knockout lines shoot apical meristems collected close to bud burst (from January to April, Spain).	HPLC, WGBS	A chilling-dependent DEMETER-like DNA demethylase is a component of the mechanism underlying the shift from winter dormancy to a condition that precedes shoot apical vegetative growth.	[29]
WINTER DORMANCY	*Pinus sylvestris*	Mature seeds, megagametophytes and embryos	Three populations located in northern and southern Finland.	GDM	Differential DNA methylation and expression of adaptation-related genes contribute to local adaptation in Scots pine populations under climate change conditions.	[30]
CLIMATE/GEOGRAPHIC DISTRIBUTION	*Populus simonii*	Natural populations	Clonal arboretum from root segments	MSAP, MS-PCR, HPLC	Population epigenetic distance and geographic	[31]

			from natural populations (representative of the geographic distribution in China, including several provenances).		distance showed a significant correlation, suggesting that environmental factors affect epigenetics. DNA methylation markers associated with phenotypic traits, explaining part of the phenotypic variance. The differentially methylated genes found may play important roles in leaf development and regulation of photosynthesis.	
CLIMATE/GEOGRAPHIC DISTRIBUTION	*Quercus lobata*	Natural populations, mature leaves	Sampling in 58 localities throughout its entire distribution (California, USA), covering the entire climate gradient.	RRBS	Climate and spatial variables explain more overall variance in CG-SMVs among individuals than in SNPs, CHG-SMVs or CHH-SMVs. This suggests a role of CG methylation in locally adaptive evolution or plasticity in plant response.	[32]
CLIMATE/GEOGRAPHIC DISTRIBUTION	*Quercus lobata*	Expanding leaf/flower bud tissue or mature leaves	Three climatologically distinct populations.	RRBS	Patterns of (epi)genetic differentiation indicate that local adaptation is operating on large portions of the oak genome: while CHG methyl polymorphisms do not play a significant role and would make poor targets for natural selection, CpG methyl polymorphisms are involved	[33]

HEAVY METALS	*Populus alba*	Cuttings of a selected clone, leaves	Greenhouse conditions: plants (1) inoculated or not with arbuscular mycorrhizal fungi (AMF) and (2) grown on heavymetal polluted (HM) or unpolluted soil. Sampling 4 and 6 months after the start of the experiment.	MSAP	in local adaptation, either directly or through linkage to regions under selection. Modest cytosine methylation changes at the first sampling, followed by extensive hypomethylation after 6 months in mycorrhizal plants grown in HM soils. The expression of genes selected based on DNA methylation status varied in response to HMs and/or AMF inoculation, with the upregulation of genes involved in RNA processing, cell wall and amino acid metabolism in the presence of AMF.	[34]
NUTRIENT-EPIGENETIC MEMORY	*Populus trichocarpa*	Clonal cuttings from different sites	Cuttings transferred into a fully nutrient supplied environment - phosphorus nutrition.	WGBS	Differential site-dependent growth was associated with DNA methylation, and few differentially methylated miRNA and its target genes were dependent on phosphorous nutrition. This may explain habitat or seasonal memory and site-dependent growth.	[35]
RADIATION	*Pinus sylvestris*	20-25-year-old trees, needles	Populations growing in the Chernobyl-affected zone	RNA-seq, SNP	Adaptive responses of Scots pine under chronic exposure of radiation involve	[36]

			(contaminated with radionuclides): Reference plot vs. Low contaminated vs. Highly contaminated.		modulation of redox process, enhanced expression of chaperones and histones and control of ion balance.	
RADIATION	*Acer palmatum*	Yellow-leaves mutant	Leaves grown in different light conditions: half of the plant in full sunlight condition vs. 30% of full sunlight.	RNA-seq, sRNA-seq	Gene differentiation associates with A. palmatum leaf coloration in different light conditions.	[37]
RADIATION	*Pinus radiata*	5-6-month-old selected seedlings, needles	Grown in controlled environment greenhouse and submitted to UV-B stress. Samplings at 24, 48, 72 or 96 h and after 1 month recovery.	RT-qPCR	The expression levels of stress-related genes were upregulated, while genes involved in photosynthesis and epigenetic regulation were downregulated.	[38]
SALINITY	*Laguncularia racemosa*	Adult trees, young and undamaged leaves	Two nearby habitats: riverside (RS) vs. near a salt marsh (SM).	MSAP	In spite of SM plants being smaller, little genetic but abundant DNA methylation differentiation was found between RS and SM plants, suggesting that epigenetic variation in natural populations is crucial for plants to cope with different environments.	[39]
SALINITY	*Phoenix*	5-week-old	Growth chamber:	sRNA-seq	Date palm contains a large	[40]

	dactylifera	seedlings, leaves and roots	watered regularly or treated with a 300 mM NaCl solution at 72h intervals. Sampling 1 week after treatment.		population of conserved and non-conserved miRNAs that function at the post-transcriptional level and are important for adaptation to salinity.	
SALINITY	*Populus alba x Populus glandulosa*	Hybrid clone, defoliated stems	Liquid culture system: 24h 100 mM NaCl treatment, followed by 3-days recover and another 24h stress cycle. Sampling at 0, 1, 3, 6, and 12h for each stress cycle.	RNA-seq	Important transcriptional reprogramming and finding of new genes involved in salt stress response and adaptation in Populus after repeated stress cycles, mainly including genes involved in hormone signaling, cell wall biosynthesis and modification, negative regulation of growth, and epigenetic regulation.	[41]
TEMPERATURE	*Hevea brasiliensis*	Three high-yield clones with different sensitivities to cold conditions, tender sprouts	Plants from each clone selected from two different climatic regions: control vs. cold stress.	BS-seq, RAPD	Highly divergent phenotypic characters and epigenetic variations in responses to environmental variations among Hevea clones.	[42]
TEMPERATURE	*Populus simonii*	50 cm tall plants of one clone, leaves	Greenhouse: control vs. heat (42°C) or cold (4°C) stress. Sampling after 3, 6, 12, and 24 h for each stress.	MSAP, PARE-seq, BS-seq	DNA methylation probably regulates the expression of miRNA genes, thus affecting expression of their target genes, likely through the gene-silencing function of miRNAs, to maintain cell survival under abiotic stress	[43]

					conditions.	
TEMPERATURE	*Quercus suber*	8-month-old plants, fully expanded leaves	Controlled climate chamber: gradual increase by 10 °C every 3 days from 25°C to 55°C, maintaining peak temperature for 3h. Sampling at 3rd day during peak heat hours at 25°C, 35°C, 45°C and 55°C.	HPCE, MS-RAPD, Protein Gel Blot, 5-mC and AcH3 Immunolocalization	Epigenetic mechanisms such as DNA methylation and histone H3 acetylation have opposite and particular dynamics that can be crucial for the stepwise establishment of cork oak into high heat stress, allowing its acclimation and survival.	[44]
TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	Single genotype, terminal buds and needles from the previous year	Field trial using two epitypes of the same genotype originated from cold (18°C) and warm (28°C) somatic embryogenesis environments.	RT-qPCR	Epigenetic memory affects the timing of bud burst phenology and the expression of bud burst related genes in genetically identical Norway spruce epitypes in a manner usually associated with ecotypes.	[45]
TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	Seedlings of two full-sib families	Seeds developed in a cold vs. warm environment.	sRNA-seq	Norway spruce contains a set of conserved miRNAs and a large proportion of novel non-conserved miRNAs. Only one family showed distinct epigenetic differences in bud set together with differential expression of specific miRNAs indicating its putative participation in epigenetic regulation.	[46]

TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	SEs (morphogenesis)	Epitype-inducing temperatures: 18°C vs. 30°C.	MACE-Seq	Temperature-dependent gene expression changes are putatively based on chromatin modifications.	[47]
TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	SEs	Epitype-inducing temperatures: 18, 23 and 28°C.	RNA-seq	The differential expression of epigenetic regulators during embryogenesis at different epitype-inducing conditions, mainly involved in DNA and histone methylation, and sRNA pathways supports that these mechanisms are crucial for the establishment of an epigenetic memory.	[48]
TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	SEs	Epitype-inducing temperatures: 18, 23 and 28°C.	sRNA-seq	miRNAs differentially expressed at different epitype-inducing temperatures putatively target transcripts of proteins involved in the signal- transduction of environmental stimuli into molecular responses. Fine-tuning of the miRNA production likely participates in both developmental regulation and epigenetic memory formation in Norway spruce.	[49]
TEMPERATURE - EPIGENETIC MEMORY	*Picea abies*	Terminal and lateral buds	2 epitypes originated from in vitro SEs cultured in cold	HPLC, Immunofluorescence	Presence of oxidized forms of 5-mC (5-hydroxymethylcytosine and	[50]

			(18°C) and warm (28°C) conditions. Buds were collected from 13-year-old trees.		5-formylcytosine) in the P. abies genome implying their probable non-spontaneous generation, which may play a role to sense environmental changes and cope with harsh conditions.	
TEMPERATURE - PRIMING	*Pinus radiata*	6-month-old seedlings, leaves nuclei	Climate chamber: seedlings exposed to 45°C for 10 days followed by recovery.	GeLC-Orbitrap/MS, Immunolocalization of 5-mdC	Nuclei proteome profiles revealed an accumulation of H2A histone and methyl cycle enzymes after recovery, indicating that thermopriming may be linked to H2A histone abundance and overaccumulation of spliceosome elements. Epigenetic mechanisms seem to play a key role in heat stress tolerance and priming mechanisms.	[51]
SEVERAL STRESSES	*Populus simonii*	Stress-tolerant genotype, leaves	Greenhouse: control vs. 150mM NaCl, 30% PEG 6000, 42 °C, or 4 °C. Sampling after 3, 6, 12, and 24h. Long-term changes evaluated after 1 and 2 (treated leaves), and 6 months (newly emerged leaves after dormancy) of stress	HPLC, MSAP	Different patterns of cytosine methylation in response to cold, osmotic, heat and salt stresses. Methylation levels decreased progressively after stress relief, with the exception of the methylation-regulated gene MIRNA6445a which showed long-term expression stability.	[52]

			relief.			
SEVERAL STRESSES	*Jatropha curcas*	Young leaves	56 samples from Thailand and other countries, including samples with high-yield production, γ-irradiation and non-toxic varieties.	MSAP	Differences in DNA methylation levels were observed among samples, with samples from saline areas and some hybrids showed specific patterns. Some DNA methylation polymorphisms differed between toxic and non-toxic samples. MSAP is a powerful technique to study the genetic diversity of organisms with a narrow genetic base.	[53]
SEVERAL STRESSES	*Populus alba x Populus tremula and Populus trichocarpa*	Xylem, phloem, bark vascular tissues	(1) gravitropism experiment on xylem tissues from hybrid, wild-type, and mutants with GA treatment; (2) xylem and bark vascular tissues from well-watered, drought stressed and drought recovered trees; (3) collection of xylem from 20 common gardens; (4) collection of xylem and phloem from a riparian site.	ChIP-seq, DNase-seq, RNA-seq	Conserved gene coexpression modules associated with biological processes in wood formation were identified as highly preserved across diverse environmental conditions and genetic origin.	[54]

WATER AVAILABILITY	*Acer platanoides and Acer pseudoplatanus*	Embryonic axes, cotyledons, and 3-month-old seedlings	Gradual dissecation of orthodox (desiccation-tolerant) vs. recalcitrant (desiccation-sensitive) seeds.	TLC	Variations of DNA methylation level during water stress are both tissue and seed specific and highly correlated with recalcitrant seed viability. Global 5-mC changes in response to desiccation were only retained in the DNA isolated from seedlings derived from strongly desiccated orthodox seeds.	[55]
WATER AVAILABILITY	*Eucalyptus globulus*	5-month-old rooted cuttings, leaves	Climate chamber: acute drought stress (7 and 11 days after water withholding) and relief (2h and 3 days after rewatering).	RAPD, 5-mC Immunolocalization	A parallel induction of redox (i.e. shift in the major antioxidant pools, increase of lipid peroxidation) and complex DNA methylation (i.e. increase of 5-mC, specific demethylation events) changes occurred during drought stress and recovery.	[56]
WATER AVAILABILITY	*Pinus halepensis*	Cuttings of a mature tree from a semi-arid area with suboptimal growth conditions, needles	Greenhouse with semi-controlled conditions: Well-irrigated plants vs. Water withholding for 34 days vs. Recovery.	RNA-seq	Drought adaptative responses and recovery involve several transcripts related with redox activity, photosynthesis and phytohormones and a differential expression of methylation-related transcripts.	[57]
WATER AVAILABILITY	*Populus tomentosa*	57-day-old plantlets	Untreated vs. dehydration) vs.	sRNA-seq, RT-qPCR	Significant changes in the expression of both conserved	[58]

			flooding.		miRNA families and novel miRNAs were observed in response to drought and flooding. These were involved in plant regulation targeting genes encoding TFs, enzymes, and signal transduction components implicated in the abiotic stress response.	
WATER AVAILABILITY	*Populus trichocarpa*	3-month-old clonally propagated plants, debarked stem	Controlled growth chamber: control vs. 5- and 7-days drought treatment.	RNA-seq, ChIP-seq, RT-qPCR	AREB1 protein establishes a coordinated histone acetylation and TF-mediated gene activation for drought response and tolerance in Populus species.	[59]
WATER AVAILABILITY	*Populus trichocarpa*	45 cm tall seedlings, mature leaves	Greenhouse: sufficient irrigation vs. modest dehydration.	sRNA-seq, PARE-seq	Five upregulated and seven downregulated miRNAs were discovered in response to drought stress.	[60]
WATER AVAILABILITY	*Populus trichocarpa*	75 cm tall seedlings, mature leaves from the same position	Greenhouse: well-watered and water-stressed.	BS-seq	DNA methylation in response to stress regulates genes by methylating TEs in promoters and gene body of TFs.	[61]
WATER AVAILABILITY	*Quercus ilex*	Natural populations, fully expanded leaves from the sunny top canopy	Unstressed forest plots vs. plots experimentally exposed to drought for 12 years at levels projected for the coming decades.	MSAP	Hypermethylated loci (as a quantitative variable) percentage increased and fully methylated loci (in opposition to hemi-methylated loci) percentage decreased under drought stress, indicating a rapid	[62]

					acclimation. Although unable to prevent growth decrease and higher mortality, DNA methylation changes occurred together with a dampening in such decreases as the long-term treatment progressed.	
WATER AVAILABILITY - EPIGENETIC MEMORY	*Populus* × *euramericana and Populus trichocarpa*	Winter-dormant shoot apical meristems	Stressful environmental growing conditions during the vegetative period/preceding summer period	HPLC, MeDIP-chip	Global DNA methylation variation between sites was correlated with genotype and biomass production capacity. Differentially methylated regions were identified 6 months after summer, mainly targeting abiotic stress and developmental response genes, which supports the development of and epigenetic memory in Norway spruce shoot apical meristems.	[63]
WATER AVAILABILITY - EPIGENETIC MEMORY	*Populus nigra*	Clones from three populations, shoot apical meristems	Grown under two watering regimes in a common garden.	HPLC	Global DNA methylation is a genetic marker of natural population differentiation under drought in a pedoclimatic context.	[64]
WATER AVAILABILITY - EPIGENETIC MEMORY	*Populus spp.*	Clones of commercial hybrids derived from two different locations, leaves	Drought.	HPLC	Variation in global DNA methylation is dependent on geographic region and history of clone. An epigenomic basis was suggested for the clone	[65]

					history-dependent transcriptome divergence observed.	
WATER AVAILABILITY - EPIGENETIC MEMORY	*Populus x euroamericana*	Shoot apical meristems	Greenhouse: water deficit-rewatering cycle.	HPLC, MeDIP-chip, BS-seq	Shoot apical meristems response to water availability changes involved variations in DNA methylation and gene expression, mainly targeting genes involved in hormone pathways, which may enable phenotypic plasticity. Rewatering conditions showed the highest variation.	[66]
WATER AVAILABILITY - EPIGENETIC MEMORY	*Populus x euroamericana*	Several genotypes, leaves	Greenhouse: well-watered vs. moderate water-deficit conditions. Two contrasting pedoclimatic conditions.	HPLC	Only the first leaves emerging from SAMs displayed genotype- and pedoclimatic site-dependent variations of DNA methylation under changing water conditions.	[67]
			BIOTIC STRESS OR PRIMING			
BIOTIC	*Fraxinus excelsior*	Grafts, leaves	Greenhouse: genotypes with high vs. low susceptibility to ash dieback (ADB).	WGBS	Identification of a set of genes with differential methylation between genotypes with high and low susceptibility to ADB genotypes, providing a valuable basis to study the role of epigenetics in gene dosage compensation and susceptibility to ADB in ash.	[68]
BIOTIC	*Paulownia*	30-day-old tissue-	Plantlets with and	ChIP-seq	Several genes involved in	[69]

	fortunei	cultured plantlets, terminal buds	without Paulownia witches'-broom phytoplasma infection.		metabolic pathways, biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, plant-pathogen interaction and plant hormone signal transduction were differentially modified by the histone marks studied under phytoplasma infection. Differential histone methylation and acetylation affected phytoplasma-responsive genes.	
HORMONES - PRIMING	*Populus euphratica*	1-year-old plants, mature leaves	Greenhouse: control vs. watering with 300 μM ABA solution. Sampling 1 and 4 days after ABA treatment.	sRNA-seq	Changes of miRNAs expression were inversely correlated with the expression profiles of their putative targets and might be involved in some biological process related stress tolerance. The results provide comprehensive view of how P. euphratica miRNA respond to ABA with different temporal dynamics.	[70]
MARKERS, BREEDING AND BIOTECHNOLOGY						
CLONAL DIVERSITY	*Populus alba*	Young leaves	Natural populations vegetatively propagated in different natural environments	MSAP	The limited genetic biodiversity of poplars is counterbalanced by epigenetic inter-population variability. Environmental conditions	[71]

			forming large monoclonal stands (Sardinia, Italy).		strongly influence inner cytosine hemi-methylation and clone ramets were differentially methylated in relation to their geographic position. Plant biodiversity studies should not be restricted to genetic aspects, especially in the case of vegetatively propagated plant species.	
EVOLUTION	*Populus trichocarpa (and other plants and animals)*	Clone, leaves	--------	BS-seq	Although patterns of methylation are very similar in flowering plants, CHG methylation levels in transposons and repeats were much higher in poplar.	[72]
HETEROSIS	*Populus deltoides*	Intraspecific parental (from the inbred seeds of excellent individual plants) and F1 hybrids lines, leaves	After hand pollination, clones showing good performance in height and diameter at breast height were selected and planted in a field study (China).	MeDIP-seq	Parents methylation patterns partially and dynamically passed onto their F1 hybrids, which showed a non-additive (higher) methylation level. Hypermethylated genes in better-parent F1 hybrids were enriched in metabolism and development, which may be highly relevant to heterosis/hybrid vigor (i.e. progeny are superior to their parents (with distinct genetic backgrounds) in many traits).	[73]

IN VITRO TECHNIQUES	*Picea abies*	Selected genotype, needles and SEs (proliferation stage)	------	WGBS	Norway spruce genome is heavily methylated due to high transposon content. Somatic embryogenesis cultures used in the industry showed altered DNA methylation patterns.	[74]
IN VITRO TECHNIQUES	*Picea asperata*	Selected genotype, SEs	Partial Dissecation Treatment is applied to increase SEs germination capacity. Mature embryos with well-developed cotyledons transferred onto two layers of dry filter paper for 0, 7, 14, or 21 days.	antibody-enrichment, MS	Lysine acetylation is mainly involved in stress response and central metabolism in desiccated SEs, with the majority of these acetylated interacting proteins highly enriched in ribosome, proteasome, spliceosome, and carbon metabolism clusters.	[75]
IN VITRO TECHNIQUES	*Pinus pinaster*	Two somatic embryogenesis lines - embryonal mass (EM)	Young EM cultures that produced mature SEs vs. Aged EM that stopped producing mature SEs vs. Aged EM treated with the hypomethylating drug 5-AzaC.	HPCE, MSAP	Although global DNA methylation levels were similar in all samples, MSAP analysis unveiled the demethylation events occuring in aged EM. The treatment of aged EM with 5-AzaC affected the type of methylation alterations in the target sequences depending on drug concentration and exposure duration.	[76]
IN VITRO TECHNIQUES	*Populus*	One genotype -	System that mimics	MeDIP-seq	DNA methylation varies in a	[77]

	trichocarpa	internode stem segments from micropropagated explants, dedifferentiated calli, and internodes from regenerated plants	routine in vitro methods for regeneration and transformation in Populus (aiming at the development of methods that avoid the phenotypic variation among plants regenerated through in vitro culture systems).		highly gene- and chromosome-differential manner during in vitro differentiation and regeneration. Hypermethylation of gene bodies may serve a protective role against activation of abundant transposable elements.	
MARKERS	*Pinus pinea*	6-month-old cuttings from 5 natural populations, needles	The natural populations represent the distribution of P. pinea among contrasting climate (Spain). Cuttings were grown in climatic chambers.	MSAP	Variable epigenetic markers discriminate individuals and two populations contrary to genetic variation (based on AFLP analysis). The methylation variability between individuals might explain the significant variation in functional traits observed.	[78]
MARKERS	*Acacia mangium*	Explants from juvenile and mature plant material, microshoots	In vitro-produced microshoots from juvenile and mature explant separated by leaf morphology (a phase change indicator): juvenile-like microshoots(J, compound leaves	HPLC, MSAP	Higher DNA methylation levels were found in J than in M microshoots, irrespective of source material age. However, six age-specific C5mCGG methylated markers were found. Although HPLC quantitative analysis could not distinguish age classes,	[79]

			only) and mature-like microshoots (M, phyllodes exclusively).		qualitative differences were identified by MSAP.	
MARKERS	*Elaeis guineensis*	Adult somaclones (F+1), mature leaves	Clonal populations obtained through somatic embryogenesis from 4 genotypically distinct mother palms (Indonesia): normal vs. "mantled" phenotypes (transformation of male floral organs that may lead to fruit abortion).	MSAP	DNA methylation polymorphism discriminates between the two phenotypes only when they were from the same genetic origin. This result hampers the direct use of MSAP markers for the early detection of variants, even though valuable information on putative target sequences will be obtained from a further characterization of these polymorphic markers.	[80]
PLOIDY LEVELS	*Populus (and other non-forest species)*	Combinations of triploids with their corresponding diploid and/or tetraploid parents, young leaves	Cross of diploid Poplar, and newly synthesized triploid Poplar, which was created by pollen doubling. Comparison of triploid materials relative to their corresponding parents (for all species).	MSAP	DNA methylation is nonlinearly related to the ploidy level and triploid plants displayed a different DNA methylation status (i.e. higher levels of DNA methylation were detected in poplar). The characteristics of DNA methylation are significantly different during the polyploidization of different plant species.	[81]
PLOIDY LEVELS	*Populus pseudo-simonii* ×	F1 hybrid diploid population and	Artificial hybridization with a	MSAP	Both hybridization and polyploidization contributed	[82]

	Populus nigra and Populus beijingensis	allotriploid populations with different heterozygosity, fully expanded leaves	thermic treatment (41°C for 4h). Seeds grown in greenhouse and surviving seedlings transplanted to the field (China).		to cytosine methylation variation (variation in diploid population significantly higher than in the parents; allotriploid populations significantly lower than in the parents). The vast majority of methylated status could be inherited from the parents.	
TRANSGENIC	*Populus tremula x Populus alba*	RNAi-PtDDM1 transgenic poplars, young fully expanded leaves	Construction of poplar DDM1 mutant using RNAi suppression and evaluation of phenotypes during perennial growth and seasonal dormancy. Arabidopsis DDM1 is necessary for the maintenance of DNA methylation and heterochromatin assembly.	HPLC	First report for a DNA methylation modified-tree. The phenotypic consequences of reduced DDM1 activity (mottled leaves) and DNA methylation appears to increase with cumulative plant propagation and growth.	[83]