Transcriptional changes suggest a major involvement of Gibberellins in *Trifolium pratense* regrowth after mowing

Short title: Gibberellin influences regrowth in red clover

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Abstract

Red clover (*Trifolium pratense*) is used worldwide as a fodder plant due its high nutritional value. In response to mowing, red clover exhibits specific morphological traits to compensate the loss of biomass. The morphological reaction is well described, but knowledge of the underlying molecular mechanisms are still lacking. Here we characterize the molecular genetic response to mowing of red clover by using comparative transcriptomics in greenhouse conditions and agriculturally used field. The analysis of mown and control plants revealed candidate genes possibly regulating crucial steps of the genetic network governing the regrowth reaction. In addition, multiple identified gibberellic

acid (GA) related genes suggest a major role for GA in establishing the regrowth morphology of red clover. Mown red clover plants showing this regrowth morphology were partially "rescued" by exogenous GA application, demonstrating the influence of GA during regrowth. Our findings provide insights into the physiological and genetic processes of mowing red clover, to serve as a base for red clover yield improvement.

Introduction

Trifolium pratense (red clover) is an important worldwide forage crop and thus of great economic interest. This perennial plant offers several advantages like a high protein content and soil improving characteristics, which can reduce the use of artificial nitrogen application and can enhance intake in livestock. Well-known disadvantages of red clover include poor persistence under several land use scenarios, like grazing or cutting [1–3]. *T. pratense* is a member of the Fabaceae (or legumes), which are, due to their economic value, among the most examined families in the plant kingdom with genome sequences available for species like *Medicago truncatula* (barrel clover) [4], *Lotus japonicus* (birdsfoot trefoil) [5], *Glycine max* (soy) [6], *Phaseolus vulgaris* (common bean) [7], *Cicer arietinum* (chickpea) [8], *Vigna unguiculata* (cowpea) [9],*Trifolium subterraneum* (subterranean clover) [10] *Trifolium medium* (zigzag clover) [11], and *T. pratense* (red clover) [12,13].

Facing today's challenges such as an increased demand on food production in an era of global climate change together with the aim to solve these problems in an environmental friendly and sustainable way requires improvement of forage crops like *T. pratense* [14,15]. *T. pratense* breeding aims to offer genotypes with improved key agronomic traits (dry matter yield, high quality, resistance to diseases and abiotic/biotic stress, persistency, [16]), while improving its regrowth ability [2,17]. Unfortunately, the morphological investigations of several *T. pratense* populations showed a correlation of persistency with non-favorable traits, like small plant size and prostrate

44 growth habit [18]. Moreover, most *T. pratense* cultivars or accessions are locally adapted and
45 require their specific local conditions to show the favored traits [19,20], which decreases the
46 stability for individual traits in breeding efforts [21]. *T. pratense* exhibits significant intraspecific
47 variation due to high intrapopulation genetic diversity, thus, persistence and performance in
48 response to mowing or cutting, depends on the variety, as well as developmental stage at the
49 moment of damage [22–25].

50 Persistency can be defined as a sustained forage yield over several growing periods [26] and is a
51 complex trait influenced by a variety of abiotic and biotic factors, and the regrowth ability of a plant
52 [27]. Plants with high regrowth ability can survive more frequent and intense biomass loss and could
53 be therefore more persistent. Decapitation or biomass loss due to herbivory or mowing triggers a
54 complex reaction affected by environmental conditions, plant morphology, architecture,
55 developmental stage and genotype [22]. After decapitation, the first stress response in other
56 legumes like *Medicago sativa* and *Pisum sativum* involves the production of phytohormones:
57 cytokinine, auxin, and strigolactones [28–30]. In addition, the mobilization of energy reserves is
58 activated [31]. Phenotypic plasticity of plant architecture in combination with alterations of
59 hormone concentrations can be observed in *P. sativum (pea)* and *T. pratense* after decapitation
60 [25,30,32]. However, the molecular processes allowing plants to thrive even after an enormous loss
61 of biomass remain still unclear, even in *Arabidopsis thaliana* [33,34].

62 Here, we compare the transcriptomes of mown (cut) vs. unmown (uncut) *T. pratense* plants from
63 two different field locations on the Biodiversity Exploratory “Heinich-Dün” [35] and greenhouse
64 grown plants. Our field samples were subjected to standard agricultural treatment and we can thus
65 discriminate transcriptional changes caused by abiotic factors and biotic interactions in the field
66 from those regulating regrowth. We present the identification and *in silico* characterization of
67 putative developmental regulators differentially expressed in the regrowth phase after mowing in
68 the field and in the greenhouse that may contribute to the regrowth response of *T. pratense* and

69 demonstrate that gibberellic acid (GA) is a major regulator of specific aspects of the regrowth
70 morphology in red clover.

71

72 Material and Methods

73 Plant growth conditions, GA treatment, tissue sampling, and RNA extraction, cDNA
74 library construction and RNA-Seq

75

76 Plant material for RNA-Seq was collected from three locations (fields and greenhouse, Fig. 1 A and
77 table S1, thereby one field location includes two neighboring field sites). Field plant tissue for RNA-
78 Seq was sampled on 11.06.2014 within the area of the Biodiversity Exploratory "Hainich-Dün" [35],
79 located in Thuringia, Germany. Material was sampled on four neighboring sites; two mown pastures
80 and two meadows that were not mown (FaM, FaNM, FbM, FbNM). For the greenhouse samples,
81 seeds of regional *T. pratense* populations (from a region covering mainly Thuringia, Saxony, Saxony-
82 Anhalt, Thuringian Forest and Uckermarck, Germany) were obtained from the Rieger Hofmann seed
83 company (Blaufelden, Germany). Plants were grown in 23 °C with 16 h of light in pots of 12 cm
84 diameter. Plants in the greenhouse were watered daily and compound fertilizer (8'8'6'+) was given
85 every ten days. After 122 days after sowing, half of the plants were cut to 5 cm (GM and GNM).
86 Material from mown plants was sampled approximately 14 days after mowing/cutting, to avoid
87 sequencing of the transcripts related to the first stress response [36]. After collection, the samples
88 were snap frozen in liquid nitrogen. For each site and the greenhouse two biological replicates of
89 four pooled plants (shoot and leaf material) each were collected.

90

Fig. 1: Overview of sampling locations and classification of DEGs. A: Overview of the sampling locations for the plant material. Names of the fields belonging to the Biodiversity Exploratory or greenhouse populations are shown, as well as the conditions (mown/cut and not mown/uncut, HG 15 and HG 42; HG13 and HG 08). Distances between sampling locations in the field have been estimated. B-D: Classification of DEGs with a |log2FoldChange| <2. Percentage share of each class to the corresponding gene list is shown in bar charts B: Classes of DEGs from field a mown vs. unmown. C: Classes of DE field b mown vs. not mown. D: Classes of DE contigs of mown plants grown in the greenhouse vs. unmown plants. E-F: Shared genes between the different treatments and locations. The Venn diagrams show the number of shared upregulated gene within the "mown" samples (E) and the number of shared genes within the "not mown" samples (F), blue circles indicate greenhouse data, green field a and red field b. G: Number of genes belonging to the class "phytohormones" within the DEG list of field (a and b) and greenhouse transcriptomes. The pie chart shows the number of the different plant phytohormones (absicic acid, ABA; auxin, AUX; genes common between the auxin and cytokinin pathway, AUX/CK; cytokinin, CK; ethylene, ET; gibberellins, GA; jasmonic acid; JA; salicylic acid, SA).

RNA was extracted using NucleoSpin® RNA Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions. Preparation of the cDNA libraries and the strand-specific sequencing was conducted by Eurofins Genomics (Ebersberg, Germany). The RNAs of four individuals were pooled for each RNA-Seq library and sequenced on an Illumina Hiseq2000 platform with chemistry v3.0, creating 2x 100 bp paired end reads.

In order to assess the effect of GA during the regrowth reaction of *T. pratense*, 14 red clover plants were mown as described in [25]. Of these plants, seven were used as control plants and seven plants were sprayed with 100 µM GA_3 (Duchefa Biochemie B.V, Haarlem, The Netherlands) once per week as described in [37]. Different morphological characters (leaf number, length/width of leaflets,

116 petiole length, number of inflorescences, and number of main shoots) were measured for four
117 weeks.

118

119 ## Assembly of reference transcriptome and annotation

120

121 The raw-read-quality of the RNA-Seq data was analyzed with FastQC (available online at:
122 http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Illumina adapter and low quality
123 regions were trimmed using Trimmomatic [38] with ILLUMINACLIP, SLIDINGWINDOW:5:20 and
124 MINLEN:50 options. Quality trimmed reads were pooled and digitally normalized [39]. Multiple d*e*
125 *novo* assemblies were computed using Trinity [40] and Oases [41] with all odd k-mer parameters
126 between 19 and 85. In addition, a genome guided assembly was performed using Trinity using the
127 draft genome of *T. pratense* 1.0 (GCA_000583005.2) [12,42]. The resulting contigs were screened for
128 potential coding sequences (CDS) using TransDecoder (https://transdecoder.github.io/). The
129 EvidentialGene pipeline (http://arthropods.eugenes.org/EvidentialGene/about/EvidentialGene
130 _trassembly_pipe.html) was used to merge and filter the contigs based on the TransDecoder CDS
131 prediction. Completeness of the final contig was confirmed by computing the mapping-rate of the
132 non-normalized reads to the contigs. The raw sequence reads can be found at NCBI: PRJNA561285.

133 The contigs were uploaded to the “Sequence Analysis and Management System” (SAMS) [43] for
134 functional annotation with the SwissProt [44], TrEMBL [45] and Phytozome [46] (e-value cutoff of
135 1e-5) databases. Additionally, attributes like gene name or functional description were extracted
136 from the blast hits. Contigs were mapped to the *T. pratense* reference genome using gmap [47]. All
137 non-Viridiplantae contigs were discarded. Transcription factors were identified using a blastp search
138 of the protein sequences against the plant transcription factor database Potsdam (PlnTFDB) (48 [48],

version 3.0, http://plntfdb.bio.uni-potsdam.de/v3.0/) protein database with an e-value cutoff of 1e-20. The files contain the functional annotation description of all transcripts e-Appendix (Table S11).

Differential gene expression analysis, enrichment analysis, and classification of differentially expressed genes

Read counts for each contig of the final assembly in each sample were computed using RSEM [49] with bowtie mapping. To identify differentially expressed *T. pratense* genes (DEG) a pairwise comparison of all treatments was preformed using the DESeq2 [50] tool with FDR ≤ 0.01 and |logFoldChange| ≥ 2 between FaM and FaNM, FbM and FbNM; GM and GNM respectively. The top 20 DEG were determined for each comparison based on the expression strength (log2 fold change). Homologues in the next closest species and *A. thaliana* for each *T. pratense* candidate gene were searched based on the *T. pratense* genome sequence deposited in Phytozome [46]. TPM (transcript per million) values were calculated to estimate contig expression level (Wagner et al 2012).

We used the description and gene names obtained from TrEMBLE and SwissProt to search the UniProt [51], NCBI [52] and TAIR [53] databases to obtain further information (Table S8). Raw reads that were assembled to contigs, exhibiting a gene structure (ORF) and attained a putative annotation referred to below as genes.

Blast2Go Analysis of *T. pratense* genomes

Two local BLAST searches [54] with word-size of 3, e-value of 1.0E-3 and HSP length cutoff of 33 were performed against the PlnTFDB using Blast2GO [55]. Only the blast hits with the highest

similarity were used for further comparisons (number of BLAST hits = 1), sequences with similarity below 50% and an e-value higher than 1.0e-4 were omitted. The Blast2GO output was compared with an in-house python3-script utilizing NumPy (https://numpy.org/), Pandas (https://pandas.pydata.org/) and Seaborn (https://seaborn.pydata.org/) applying the list of transcription factors (TF) downloaded from PlnTFDB (http://plntfdb.bio.uni-potsdam.de/v3.0/) to the blast output and furthermore visualizing the generated datasets. We searched Uniprot database hits for development and phytohormone related genes. Subsequently, gene IDs of gibberellin genes we searched for matches within our annotated *T. pratense* transcriptomes. Matches were filtered based on TPM values and classified based on biosynthesis, catabolism activation/repression or signaling/response, corresponding expression patterns within the transcriptome have been identified additionally.

Results

RNA-Seq results, *de novo* assembly, and functional description of contigs

The RNA-Seq produced a total number of short reads between 44.7 and 58.1 million for each library with two exceptions (table S2) totaling 608,041,012 raw reads. The *de novo* assembly of the reference transcriptome of *T. pratense* produced 44,643 contigs, of which 41,505 contigs were annotated and 29,781 contigs were identified as plant specific. The minimum length of the contigs was 124 bp, the maximum length 1171.31 bp (Table S3). After the *de novo* assembly of the *T. pratense* transcriptome, each individual library was mapped back against the reference

184 transcriptome individually, to determine the overall alignment rate, which was between 77.85 % and
185 90.32 % (Table S4).

186 63 % of the 44,643 contigs could be mapped to a known locus of the *T. pratense* genome annotation
187 [12,42]. 32 % could be mapped to an unknown locus of the *T. pratense* genome and 5 % could not be
188 mapped to the *T. pratense* genome (Fig. S1). All plant-specific contigs were annotated with several
189 databases (Table S5). To further verify the quality of our replicates, we identified the transcripts
190 shared by the two replicates. We identified TPM values for each transcript and discarded transcripts
191 with TPM values <1. Then we compared the transcripts of each library with each other and
192 calculated the percentage of this number compared with the total number of transcripts within each
193 library. The percentage of transcripts shared between the two replicates is between 90 % and 94 %
194 for all treatments/localities, suggesting that the RNA-Seq data are reproducible (Table S6).

195 **Specific transcriptional regulator families are differentially expressed during the**
196 **regrowth process**

197 We were firstly interested to identify transcriptional regulators initiating and maintaining the
198 regrowth morphology and mapped the transcriptome to the PlnTFDB to identify these
199 transcriptional regulators. All members of a specific transcriptional regulator family (TRF) were *in*
200 *silico* identified in the transcriptome and their expression was compared between mown and
201 unmown plants (Fig. S2). Only those TRFs are shown for which at least 10% of the members showed
202 significantly differential expression between mown and unmown conditions (Fig. 2).

203

204 Fig. 2: Differentially expressed TRF members in mown and not mown *T. pratense* plants. The y-axis
205 shows the number of expressed contigs (TPM value over 5 TPM) that are members of the specific
206 TRF. Names of the transcriptomes and TRFs are given on the x-axis. Expression of transcription factor
207 members was compared in a pairwise manner (GM vs GNM, FaM vs FaNM, FbM vs FbNM). Shown

208 are only those plant TRFs in which at least one of the comparisons resulted in a difference of more
209 than 10% of the contigs significantly upregulated in either the mown or the unmown condition
210 (orange bars).

211

212 17 TRFs were identified of which at least 10% of the members showed differential expression in the
213 mown versus unmown comparisons (Fig. 2): ABI3VP1, AP2-EREBP, C_2C_2-Dof, C_2C_2-GATA, GRAS, HSF,
214 LOB, MADS, mTERF, MYB, NAC, PHD, SBP, SNF2, TCP, TRAF, WRKY.

215 Two TRFs show expression activation upon mowing: a significant number of WRKY transcripts are
216 up-regulated in mown plants regardless of the provenance. MADS-box transcripts were found
217 upregulated as well, but only in the field-derived transcriptomes. Generally, only five of the 17 TRFs
218 analyzed here showed significant changes in expression towards mowing in the greenhouse-derived
219 plants suggesting that they react less strongly towards mowing than the field-derived plants. Six
220 TRFs (AP2-EREBP, MYB, NAC, PHD, SBP, and TCP) show transcriptional changes in reaction to mowing
221 only in field location a and three TRFs (mTERF, SNF2, TRAF) show this only in field location b
222 suggesting that combination of biotic and abiotic factors with mowing differ between the two field
223 locations.

224 Notably, only the C_2C_2-GATA TRF reacts towards mowing under greenhouse but not under field-
225 conditions suggesting that transcriptional changes in reaction to other biotic and abiotic factors may
226 overlay the regrowth reaction. Taken together, the TRF analysis shows that the reaction towards
227 mowing induces transcriptional changes in only a subset of TRFs, suggesting that those play a major
228 role in relieving the stress biomass loss and regrowth.

229

Differentially expressed genes analysis reveals diverse subsets of genes involved in regrowth influenced by location and environmental conditions

To identify gene expression responses underlying the regrowth response after mowing digital gene expression analysis was performed comparing FaM vs FaNM; FbM vs FbNM; GM vs GNM to identify DETs (Table S12) from mown plants. Interestingly, using the log fold change 2, the number of DEG is rather similar in all comparisons, ranging from 119 (Gm vs. GNM) to 142 (FaM vs. FaNM) (Table 1).

Table 1 Table shows the numbers of differentially expressed transcripts (contigs) between libraries with changes above logfold 2. Up- or down regulation for each comparison is shown.

Analysis	total DEG	Number of transcripts up regulated (library)	Number of transcripts down regulated (library)
GM vs. GNM	119	54 (greenhouse mown)	65 (greenhouse not mown)
FaM vs. FaNM	142	49 (mown)	93 (not mown)
FbM vs. FbNM	122	59 (mown)	63 (not mown)

We were then interested to identify developmental processes in greater detail that are required for the regrowth process. Thus, the results of the DEG analysis were restructured such that the DEG were grouped in 16 descriptive classes by database and literature mining (Table S7 and Table S8). Those classes describe major functional groups and serve to identify the potential role of a gene.

The results of the top 20 DEG showed that the greenhouse plants displayed more DEG involved in regrowth processes and less genes related to environmental conditions when compared with field plants. Most likely, the greenhouse grown plants displayed the regrowth reaction more prominently,

248 as they grew under less stressful conditions than the field grown plants, for which more stress

249 related DEG were observed (Fig. 1 B-D /Table 2-4).

Table 2: Top twenty differentially expressed genes of GM vs. GNM analysis. The table shows the transcript name, log2 fold change of the corresponding transcript, the library in which the transcript is upregulated (pattern), gene name based on *T. pratense* genome annotation, corresponding Phytozome description, gene name and species name of the next homologues and *A. thaliana* gene name and locus name based on information available on Tair.

ID	Pattern	Contig ID	log2 Fold Change	Class (basis of classifiation)	Gene name *T. Pratense*	Next homologue gene name	Next homologue species name	*A. thaliana* gene name	*A. thaliana* locus name
1	GHNM	tdn_99733	-9.5	Growth (*M. truncatula*)	Tp57577_TGAC_v2_mRNA4544.v2	Medtr4g029550.1	*M. truncatula*	-	-
2	GHNM	k41_54584	-6.3	Biotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA28349.v2	Medtr5g073620.1	*M. truncatula*	*ATEXO70B1*	AT5G58430
3	GHNM	tdn_92791	-5.5	Abiotic/biotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA20498.v2	Medtr1g041150.1	*M. truncatula*	*ATCPK1*	AT5G04870
4	GHNM	k41_130218	-5.5	-	-	-	-	-	-
5	GHNM	tdn_53091	-4.8	Phytohormone (*M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA39912.v2	Medtr4g010250.1	*M. truncatula*	-	AT5G20190
6	GHNM	tgg_43136	-4.4	Transcription (*M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA29629.v2	Medtr4g098630.1	*M. truncatula*	*ANAC071*	AT4G17980
7	GHN	tdn_141837	-4.3	Abiotic stress (*T.pratense, M. truncatula, A.*	Tp57577_TGAC_v2_mRNA760.v2	Medtr2g022700.1	*M. truncatula*	*ATGPT2*	AT1G61800

	M			*thaliana*)					
8	GHNM	tdn_40997	-4.2	Abiotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA25718.v2	Medtr4g130540.1	*M. truncatula*	*HSP70B*	AT1G16030
9	GHNM	k71_5292	-4.1	Biotic stress (*T.pratense, M. truncatula*)	Tp57577_TGAC_v2_mRNA23166.v2	Medtr0163s0020.1	*M. truncatula*	*LECRK-IX.1*	AT5G10530
10	GHNM	k59_6358	-3.9	Growth (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA12337.v2	Medtr3g435430.1	*M. truncatula*	*ATEXP15*	AT2G03090
11	GHM	tdn_86219	8.0	Biotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA29036.v2	Medtr4g066210.1	*M. truncatula*	*BGLU12*	AT5G42260
12	GHM	k23_115785	8.0	Abiotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA22071.v2	Glyma.01G001000.1	*G. max*	-	AT5G58110
13	GHM	tdn_91159	8.1	Biotic stress (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA7745.v2	Medtr4g035870.1	*M. truncatula*	-	AT5G62360
14	GHM	k65_43517	8.3	Phytohormone (*T.pratense, A. thaliana*)	Tp57577_TGAC_v2_mRNA6281.v2	Medtr1g082750.1	*M. truncatula*	*ATAMI1*	AT1G08980
15	GHM	tgg_18067	8.4	-	Tp57577_TGAC_v2_mRNA32019.v2	-	-	-	-
16	GHM	k61_38813	9.0	-	-	-	-	-	-
17	GHM	k49_82496	9.0	Abiotic/biotic stress (*G. max, A. thaliana*)	Tp57577_TGAC_v2_mRNA37976.v2	Glyma.06G268800.1	*G. max*	-	AT4G04790

18	GHM	k67_38815	9.1	Biotic stress (*T.pratense*)	Tp57577_TGAC_v2_mRNA41666.v2	Medtr0062s0020.1	*M. truncatula*	-	-
19	GHM	k45_11164	9.6	Transcription (*T.pratense*)	Tp57577_TGAC_v2_mRNA29953.v2	Medtr3g092510.1	*M. truncatula*	*ATRBP37*	AT4G10610
20	GHM	tdn_25484	9.6	Growth (*Phaseolus vulgaris*)	Tp57577_TGAC_v2_mRNA13093.v2	Phvul.006G033800.1	*Phaseolus vulgaris*	-	-

Table 3: Top twenty differentially expressed genes of FaM vs. FaNM analysis. The table shows the transcript name, log2 fold change of the corresponding transcript, the library in which the transcript is upregulated (pattern), gene name based on *T. pratense* genome annotation, corresponding Phytozome description, gene name and species name of the next homologues and *A. thaliana* gene name and locus name based on information available on Tair.

ID	Pattern	Contig ID	log2FoldChange	**Class (basis of classifiation)**	Gene name *T. pratense*	Next homologue gen name	Next homologue species name	*A. thaliana* gene name	*A. thaliana* locus name
1	TPNM2	k33_17052	-9,0	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA21474.v2	Medtr4g079440.1	*M. truncatula*	na	AT1G06260
2	TPNM2	k43_111792	-8,8	Biotic stress (*M. truncatula*)	Tp57577_TGAC_v2_mRNA26333.v2	Medtr8g101900.1	*M. truncatula*	CCOAOMT7	AT4G26220
3	TPNM2	tdn_34568	-8,6	-	Tp57577_TGAC_v2_mRNA9104.v2	Glyma.13G061800.1	*G. max*	-	AT5G39530
4	TPNM2	tdn_49640	-8,6	-	-	-	-	-	-

5	TPNM2	tdn_58745	-8,5	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA20190.v2	Medtr8g075200.1	*M. truncatula*	-	AT1G75900
6	TPNM2	tdn_47209	-8,5	Growth (*M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA10703.v2	Medtr1g053315.1	*M. truncatula*	-	AT1G03390
7	TPNM2	tdn_48478	-8,4	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA19516.v2	Medtr2g099020.1	*M. truncatula*	-	AT3G59510
8	TPNM2	k41_17597	-8,4	Growth stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA8526.v2	Medtr1g036490.1	*M. truncatula*	ATCOMT, ATOMT1	AT5G54160
9	TPNM2	k51_82581	-8,2	Growth (*T. pratense*)	Tp57577_TGAC_v2_mRNA23127.v2	Medtr2g436480.1	*M. truncatula*	KCS21	AT5G49070
10	TPNM2	tdn_82424	-8,1	Growth (*T. pratense*)	Tp57577_TGAC_v2_mRNA17103.v2	Medtr2g013740.1	*M. truncatula*	KCS10	AT2G26250
11	TPM2	k49_380	7,5	Development (*A. thaliana*)	Tp57577_TGAC_v2_mRNA37185.v2	SapurV1A.0885s0040.1	*Salix purpurea*	DAYSLEEPER	AT3G42170
12	TPM2	tdn_49869	7,6	-	-	-	-	-	-
13	TPM2	tdn_54983	7,7	-	-	-	-	-	-
14	TPM2	k37_9029	7,8	-	-	-	-	-	-
15	TPM2	k45_6120	8,4	-	Tp57577_TGAC_v2_mRNA2166.v2	Medtr2g007510.1	*M. truncatula*	-	-
16	TPM2	k71_23808	8,4	Development (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA14131.v2	Medtr1g021320.1	*M. truncatula*	-	AT4G33280
17	TPM2	k59_3541	8,4	Development (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA34193.v2	Medtr4g089030.1	*M. truncatula*	CYP71A26	AT3G48270
18	TPM2	k59_360	8,6	Metabolism (*Linum usitatissimum, T. pratense*)	Tp57577_TGAC_v2_mRNA21875.v2	Lus10012445	*Linum usitatissimum*	-	AT1G50020
19	TPM2	k53_38903	9,0	Abiotic stress (*A. thaliana*)	Tp57577_TGAC_v2_mRNA37328.v2	Medtr8g063190.1	*M. truncatula*	PRIN2	AT1G10522

20	TPM2	tdn_129978	9,6	-	Tp57577_TGAC_v2_mRNA9318.v2	Medtr7g062280.1	*M. truncatula*	-	AT5G01140

Table 4: Top twenty differentially expressed genes of FbM vs. FbNM analysis. The table shows the transcript name, log2 fold change of the corresponding transcript, the library in which the transcript is upregulated (pattern), gene name based on *T. pratense* genome annotation, corresponding Phytozome description, gene name and species name of the next homologues and *A. thaliana* gene name and locus name based on information available on Tair.

ID	Pattern	Contig ID	log2 Fold	Class (basis of classifiation)	Gene name *T. pratense*	Next homologue gen name	next homologue species name	*A. thaliana* gene name	*A. thaliana* locus name
1	TPNM3	tdn_100726	-9,4	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA24659.v2	Medtr4g094772.1	*M. truncatula*	*CYP81D*	AT4G37340
2	TPNM3	tgg_49631	-8,0	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA37846.v2	Medtr6g034470.1	*M. truncatula*	-	AT2G34930
3	TPNM3	tdn_152262	-7,9	-	-	-	-	-	-
4	TPNM3	tdn_56712	-7,9	Biotic stress (*T. pratense, M. truncatula*)	Tp57577_TGAC_v2_mRNA30556.v2	Medtr8g027540.1	*M. truncatula*	-	-
5	TPNM3	tdn_87762	-7,9	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA10533.v2	Medtr7g451400.1	*M. truncatula*	*ATMCP1B, ATMCPB1*	AT1G02170

6	TPNM 3	tdn_86129	-7,1	General cell functions (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA10207.v2	Glyma.11G154500.1	*G. max*	*RPB5E*	AT3G54490
7	TPNM 3	k55_46241	-6,9	Growth (*T.pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA31452.v2	Medtr4g128150.1	*M. truncatula*	*histone 4*	AT2G28740
8	TPNM 3	tdn_55533	-6,2	Abiotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA39263.v2	Medtr5g007790.1	*M. truncatula*	*ATCRM1, ATXPO1*	AT5G17020
9	TPNM 3	tgg_51443	-4,7	Growth (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA37076.v2	Medtr5g019580.2	*M. truncatula*	*UGT72E2*	AT5G66690
10	TPNM 3	tdn_136706	-4,7	-	-	-	-	-	-
11	TPM1	tdn_140636	8,8	General cell functions (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA28209.v2	Medtr8g005980.1	*M. truncatula*	*C-NAD-MDH2*	AT5G43330
12	TPM1	tdn_154158	8,9	General cell functions (*T. pratense, M. truncatula*)	Tp57577_TGAC_v2_mRNA39482.v2	Medtr3g114970.2	*M. truncatula*	-	AT5G55150
13	TPM1	tdn_65187	9,1	Transposon (*T. pratense, Prunus persica A. thaliana*)	Tp57577_TGAC_v2_mRNA30115.v2	Prupe.4G011200.1	*Prunus persica*	-	AT4G29090
14	TPM1	tdn_100956	9,2	Metabolism (*T. pratense, Capsella rubella, A. thaliana*)	Tp57577_TGAC_v2_mRNA9542.v2	Carubv10008027m	*Capsella rubella*	*AHA2*	AT4G30190
15	TPM1	k63_21505	9,3	Biotic stress (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA19467.v2	Medtr3g022400.1	*M. truncatula*	-	AT3G14470

16	TPM1	tdn_142681	9,3	Secondary metabolite biosynthesis (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA15473.v2	Medtr8g074550.1	*M. truncatula*	-	AT2G18570
17	TPM1	k45_6120	9,6	-	Tp57577_TGAC_v2_mRNA2166.v2	Medtr2g007510.1	*M. truncatula*	-	-
18	TPM1	tdn_52922	10,1	-	Tp57577_TGAC_v2_mRNA41271.v2	mrna20290.1-v1.0-hybrid	*Fragaria vesca*	-	AT1G21280
19	TPM1	tdn_65185	10,9	-	-	-	-	-	-
20	TPM1	tdn_109277	11,7	Transcription (*T. pratense, M. truncatula, A. thaliana*)	Tp57577_TGAC_v2_mRNA29560.v2	Medtr5g028610.1	*M. truncatula*	-	AT3G14460

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Several functional groups show a similar pattern in the mown vs. unmown plants of all three locations: more genes related to biotic stress processes and metabolism were upregulated in the unmown locations (Fig. 1 B-D). In mown plants, more genes related to signaling and transposons were upregulated. Only a single functional group (growth) shows similar patterns in only the field locations suggesting that plants in the two field locations cope with very different habitat conditions and stress factors.

The photosynthesis- and phytohormone-related genes of field a show a similar pattern to the greenhouse plants as do the development- and signaling related genes. Genes related to development, general cell functions and transcription have similar patterns between field b and the greenhouse grown plants, such that more transcription - and development-related genes are upregulated in mown plants. DEG related to symbiosis were found upregulated in unmown plants grown in the greenhouse, even though these plants were fertilized. And unexpectedly, senescence-related genes are upregulated in mown plants of field A. However, because our analysis cannot discriminate between activating and repressing factors of senescence, we cannot conclude from our data if the mown plants have activated or repressed their senescence program.

The largest group of differentially expressed genes is the one related to biotic stress with up to 38% differentially expressed genes in one location (field b, Fig. 1 C). This suggests that different biotic stresses act upon the mown vs. unmown plants. A similar phenomenon can be observed for growth related processes, where up to 24% genes were upregulated in the mown and unmown plants indicating that different growth programs are active in mown vs. unmown plants.

Taken together we can state that mown plants in all locations change their regulatory programs upon mowing to cope with different biotic factors suggesting that they massively change their metabolism and signaling processes. Further, transposons are more active in mown plants. Apart

289 from these conclusions, the molecular answer to substantial biomass loss differs between all three
290 locations.

291 To find similarly regulated genes between the treatments and/or locations, a Venn diagram was
292 generated to compare the number of shared significantly DEG within the “mown” samples and the
293 “not mown” samples (Fig. 1 E-F, Table S9). Within the “mown” samples we detected no overlap
294 between the groups with the exception of four genes that are differentially expressed and
295 upregulated in “mown” condition and are shared between the two field transcriptomes (FbM and
296 FaM (Fig. 1 E). Within the “not mown” samples also four genes are shared between the field
297 transcriptomes (FbNM and FaNM)) and one is shared between the field b and the greenhouse (Fig. 1
298 F). No genes are shared between all three samples, neither in the “mown” treatment, nor in the “not
299 mown” treatment. The genes that were shared between the transcriptomes belong to the main
300 classes “growth”, “phytohormone”, “general cell functions”, “biotic stress”, “development” and
301 “transcription” (Table S9).

302 Two of the genes could not be annotated. The annotated genes include for example genes
303 tdn_60472 (shared between FaM/FbM, class: phytohormone), that was found to be the homolog of
304 the *A. thaliana* locus AT1G75750, describing a GA-responsive GASA1 protein homolog. Another *A.*
305 *thaliana* homolog was identified, Chitinase A (ATCHIA), shared between FaNM/FbNM (tdn_129843,
306 class: biotic stress). In addition one gene was found, with a *T. pratense* annotation but no further
307 description or homologs to *A. thaliana* (k45_6120, shared between FaM/FbM). This suggests that
308 the molecular mechanisms directing regrowth overlaid by other processes, such as stress response
309 which have a more dramatic impact on the number of DEG than growth processes have. The shared
310 genes between the field conditions and the almost complete absence of shared genes between field
311 and greenhouse indicates that the growth conditions in the field are more like each other, even
312 when the fields are far apart from each other than any field to a greenhouse.

313

Gibberellins are major players in the regrowth reaction

As phytohormones play a major role in the regulation of development and stress response, we identified DEGs related to phytohormone synthesis, homeostasis, transport, and signaling within all transcriptome comparisons (Table 1). DEG links for all major classes of phytohormones were identified, except for strigolactone. DEGs association to four phytohormones was most abundant: abscic acid (ABA, 8 DEGs), gibberellins (GA, 8 DEGs), salicylic acid (SA, 6 DEGs), and auxin (AUX, 5 DEGs) (Fig. 1 G). While ABA and SA are mainly involved in response to biotic and abiotic stresses, and AUX is known to play a major role in growth and development, we identified GA as a novel candidate phytohormone for regrowth response.

To learn more about the role of GA in the regrowth response, we identified 32 GA-related genes out of 151 within the transcriptomes of the greenhouse and the field grown plants, matching our selection criteria (TPM <5, involved in GA biosynthesis, signaling, GA responsive genes or catabolism, displaying certain expression patterns Fig. 3 A) and classified them according to their function in the GA biosynthesis and signaling processes (Table S13). Ranges of expression strength were calculated and color coded to compare expression patterns (Fig. 3 A).

Fig. 3: Analysis of GA related contigs and regrowth processes. A) Differentially expressed GA-related contigs within the *T. pratense* transcriptomes. Ranges of expression were calculated (0-39.99% blue (low expression), 40-59.99% grey (neutral expression), 60-100% yellow (high expression)) according to their TPM values. On the left, the gene names of the *T. pratense's* closest *A. thaliana* homologs are given if available. B) and C) show morphological changes in leaves after GA treatment. B) leaflet area in cm^2, C) length of petioles in cm. The graphs show average values for each sampling date and 95% confidence interval. GA treated plants, blue; control plants, orange.

Five genes predicted to be involved in GA biosynthesis and signaling show similar expression differences between mown and unmown plants from at least two of the three locations: tdn_142825 (AT2G46590) and the homologs of *ZHD1, GID1B, MPT1*, and *MPT3*. Further, homologs of three GA responsive genes (tdn_75969 (*MYB 44*, AT5G67300), tdn_157683 (Os04g0670200, AT1G47128), and *XERICO* and homologs of two GA catabolism genes (*GA2OX1*, *GA2OX8*) react towards mowing. Interestingly, we also find many differences in expression between the field sites in the GA related genes suggesting fundamental differences in the living conditions between the two field sites that also impact regrowth after biomass loss.

When considering only the greenhouse grown plants, homologs of GA biosynthesis genes were not differentially regulated, but genes most likely involved in GA signaling such as *MPT1*, *MPT3*, and *SOC1* are down regulated in mown plants. Five homologs of GA responsive genes are upregulated in the mown plants, while three, among them *XERICO*, are down regulated. Further, of the three *GA2OXIDASE8* homologs encoded by the *T. pratense* genome, two are differentially expressed, one up- and the other down regulated upon mowing. Also, one *GA2OXIDASE1* homolog is down regulated in mown plants.

In summary, this suggests a highly dynamic response of several GA-related genes to mowing *or T. pratense*. Interestingly, we were unable to identify larger changes in the GA biosynthesis pathway of the greenhouse plants, but in GA catabolism genes, suggesting that GA availability in response to mowing is regulated by catabolism and signaling rather than by biosynthesis. Further, we identified two contrastingly regulated sets of genes acting in the mowing response.

GA treatment after mowing induces specific changes to the regrowth response

Identification of several GA related genes that changed expression suggested an involvement of GA in the regulation of development after mowing. We were interested to corroborate this hypothesis experimentally and treated *T. pratense* plants with GA after mowing. Weekly GA application during the regrowth process led to significant and specific changes in morphology (Fig. 3 B, C). Previous work suggested that regrowing plants produce smaller and rounder leaflets with shorter petioles than uncut plants [25]. Number of leaves, shoots and inflorescences, leaf area and the roundness of leaflets were measured (Fig. 3 B, C, Suppl. Fig. 3). The first visible effects of GA treatment were recognized after 1.5 weeks, showing a significant difference in leaflet area between GA treated and control plants. Later it was observed that the petioles of treated plants were in average twice as long as petioles of untreated plants (16.7 ± 1.9 cm and 8 ± 1.2 cm, respectively). GA leaflets were with 4.7 ± 0.9 cm^2 almost double the size than those of untreated plants (2.4 ± 0.6 cm^2). However, GA treated plants grew only 30% more total leaf area than control plants, because the control plants had more leaves than GA treated plants (Fig. S3 A, B, F, and G). Other morphological traits such as number of inflorescences, leaves, and shoots remained unaffected by the GA treatment. In summary, mown plants normally produce leaves with shorter petioles, restrict their leaflet area and their leaves become rounder. GA treatment partially alleviated these developmental changes such that the mown, GA treated plants produced larger leaves with longer petioles while the leaf shape was unaffected by GA treatment.

Discussion

RNA-Seq and assembly

383 The *de novo* assembly in combination with a reference-based approach for the annotation led to
384 44643 contigs of which 29781 could be annotated as plant-specific (Fig. S1). With the prior *de novo*
385 assembly it was possible to attain 4051 additional contigs that could be not found within the
386 genome of *T. pratense* 1.0 (GCA_000583005.2) [12,42]. The estimated genome size of *T. pratense* is
387 ~440 Mbp [28]. The *T. pratense* transcriptome data in study was ~55 Mbp in size, corresponding to
388 ~12.5% transcribed regions in the *T. pratense* genome, which is within the range of previously
389 published transcriptomes (~10% (42 Mbp) [56]). Interestingly, we found plant-specific, previously
390 unreported contigs suggesting that the *T. pratense* genome might need improvement in terms of
391 sequencing coverage and protein coding sequence annotation.

392 **Cell walls are remodeled after mowing**

393

394 Our data analysis shows that several plant TRFs are predominantly involved in the regrowth reaction
395 (Fig. 2 and S2). After massive biomass loss like mowing inflicts on *T. pratense*, plants firstly need to
396 seal wounded tissues. Several transcriptional regulators are known to play a role in the tissue
397 reunion processes were identified in *Solanum lycopersicum*, *Cucumis sativus*, and *A. thaliana*
398 (reviewed in [36]). Homologs of these genes were also identified to be differentially regulated in the
399 *T. pratense* transcriptome after mowing, such as several members of the Auxin Response Factor
400 (ARF) family or the No Apical Meristem (NAM) family member *ANAC071.* [57] suggested that high
401 levels of AUX induce the expression of *ANAC071* via ARF6 and ARF8 (in the upper part of incised
402 stems), at the same time reduced AUX level directly after the cutting activate the expression of
403 *RAP2.6L*. In addition auxin signaling via ARF6 and ARF8 influences JA synthesis, via the activation of
404 *DAD1*, thus together with LOX2 increases *RAP2.6L* expression during tissue reunion in *A. thaliana*
405 [57]. Further it was demonstrated that ANAC071 can as a transcription factor initiate the expression
406 of members of xyloglucan endotransglucosylase/hydrolases family (XTH20 and XTH19) which
407 recombine hemicellulose chains to drives the cell proliferation during tissue reunion [58].

Interestingly, we were able to identify all members of the cell wall remodeling pathway mentioned above, displaying distinct expression pattern with some of them upregulated in mown plants including for example *XTH32* (k69_7012, upregulated in FbM, tdn_94651, upregulated in GM, FaM and FbM), *XTH6* (tdn_91763, upregulated in GM), *XTH8* (k71_5058, upregulated in GM, FbM), *XTH9* (tdn_113578, upregulated in GM), *XTHA* (tdn_87930, upregulated in GM), *LOX2* (tdn_156279, upregulated FbM), and *ARF8* (tdn_156886 upregulated in GM, tdn_156890 upregulated in GM) (Table S10, S12 and S13). This is suggesting that the early steps in the regrowth reaction are conserved in core eudicots and that the cell wall remodeling processes continue at least two weeks after mowing.

Biotic and abiotic stresses contribute to differential gene expression

RNA-Seq experiments create a large amount of raw data which requires significant downstream analysis to provide a biologically meaningful dataset. We thus compared those 20 genes, that differed most strongly in their expression between the different treatments and locations (see table 4-6 and Fig. 1 B-D). These comparisons revealed that the mown greenhouse plants show the highest percentage of genes possibly involved in regrowth processes. Contrasting, the field transcriptomes display patterns of abiotic and biotic stress reactions. Comparisons of the top 20 DEG of the unmown field transcriptomes showed that plants grown on field a and b face biotic stress more than abiotic stress. One of the upregulated genes in field a is a chitinase homolog suggesting that those plants are under attack of fungi and/or insects. Follow-up analyses to correlate environmental conditions, biotic and abiotic stresses monitored within the Biodiversity Exploratories with differential gene expression at the two field locations would be an interesting project but are beyond the scope of this work. In contrast, the top 20 DE transcripts of the greenhouse plants include phytohormone- and transcription-related genes, but also a high proportion of biotic and abiotic stress-related genes. This suggests that also these plants have to cope with stresses, but to a

lesser extent. Thus, their regrowth reaction is more visible within the top 20 DEG. Generally, the non-mown plants show a much higher number of upregulated biotic stress-related genes during a phase in their life when senescence commences and they become more susceptible to pathogen attacks. The mown plants during their regrowth phase are not senescing and their younger organs seem be less affected by pathogens.

GA related genes influence regrowth of *T. pratense*

GAs are involved in multiple aspects of plant development like cell elongation, flowering time regulation, and seed germination. Consequently, genes encoding for proteins involved in the synthesis, perception, and catabolism of the various GAs influence plant form. The RNA-Seq data presented showed a high abundance of GA associated genes (Fig. 1 G and 3) which might explain the morphological changes to mowing, such as rounder leaves, temporary dwarf-like appearance, and higher cumulative biomass production in mown plants [25]. Two of the genes, expressed higher in mown plants than in control clover plants are *GA20OX1* and *GA20OX2*. They are key enzymes of GA synthesis by producing precursors of the active GA forms (reviewed in [59]) and a deficiency in their activity is correlated with a dwarfed growth phenotype in *A. thaliana* and *O. sativa* [60,61]. These mutants show also slow down cell division and expansion rate [62]. The upregulation of *GA20OX2* in mown plants may meet the enhanced demand of active GA to promote and sustain the regrowth to increase cell division and elongation. Similar to an increased GA synthesis, the expression of genes involved in reception of GA, such as the ortholog of AtGID1B is enhanced in mown plants. In *A. thaliana*, loss-of-function mutants of this GA receptor show a dwarfed phenotype [63] and over-expression of GID1 in *Medicago sativa* promotes biomass accumulation and leaf roundness [64].

In contrast to these GA responsive genes, GA2OX2 and GA2OX8 are involved in GA catabolism [65] and high levels of GA are known to activate the expression of degrading enzymes [66]. Both genes are expressed higher in not mown control plants than in mown plants and they inactivate the bioactive GAs GA2OX over-expression results in stunted plants and delayed flowering time [67,68]. While mown clover plants show a higher expression of genes associated with growth activation, the ABA biosynthesis regulator XERICO is higher expressed in unmown plants (Table S13). It is a direct target of the DELLA protein RGL2 and in addition negatively regulated by GID1B [69,70]. Thus, XERICO might restrict GA mediated growth to confer the drought adaptation of the not mown plants as more water is lost through the high leaf biomass.

In summary, mowing seems to trigger differential gene expression of GA activating enzymes and catabolic enzymes suggesting a dynamic GA response, but the gene expression patterns were not informative in respect to the consequences for the phenotype. When analyzing the morphological effects of GA application to mown plants (Fig. 3 A-C) we could show that external GA application lead to the disappearance of specific traits typical of the mowing response. Mown plants develop shorter petioles and produce a smaller leaf size area [25], but when treated with GA, leaves and petioles grow up the size seen in unmown plants.

The growth promoting abilities of GAs by cell expansion and proliferation via stimulating the degradation of growth-repressing DELLA proteins are well established [62]. The length increase of petioles in GA treated mown plants is in line with reported data from non-mown *Pisum sativum* (pea) plants, but in those, leaf sizes remained unchanged after GA treatment [71], suggesting a more specific role for GA in the regrowth reaction after biomass loss. Moreover, it was shown in *A. thaliana* previously, that elevated GA concentrations enhance cell division rates in the distal end of leaves (reviewed in [72]). If these results are transferred to *T. pratense* GA treatment should result in longer leaflets after GA treatment of mown plants. However, the leaf shape did not change, only the

480 size increased suggesting a regrowth-specific shift of growth pattern which is unaffected by GA but
481 similar to leaf shape of juvenile plants [25].

482 Interestingly, GA treatment of mown *T. pratense* plants does not generally lead to stronger
483 longitudinal growth as leaves retained the round shape characteristic for untreated mown plants.
484 These regrowth-specific characteristics can also be found in other species, for example in *A.*
485 *thaliana*, *Fragaria ananassa*, *Duchesnea indica* and *G. max* GA treatment causes elongated petioles
486 and increased leaf sizes and a more erect growth habit [73–76]. This proposes a new method to
487 increase the accumulation of biomass, suitable for animal fodder. Previous experiments with the
488 grasses *Leymus chinensis* and *Lolium perenne* showed GA action to be limited by N fertilization
489 [77,78]. Red clover, living in symbiosis with nitrogen fixing bacteria, is not dependent on additional N
490 fertilization and can produce high-protein content biomass without fertilizer on poor soils.

491

492

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497

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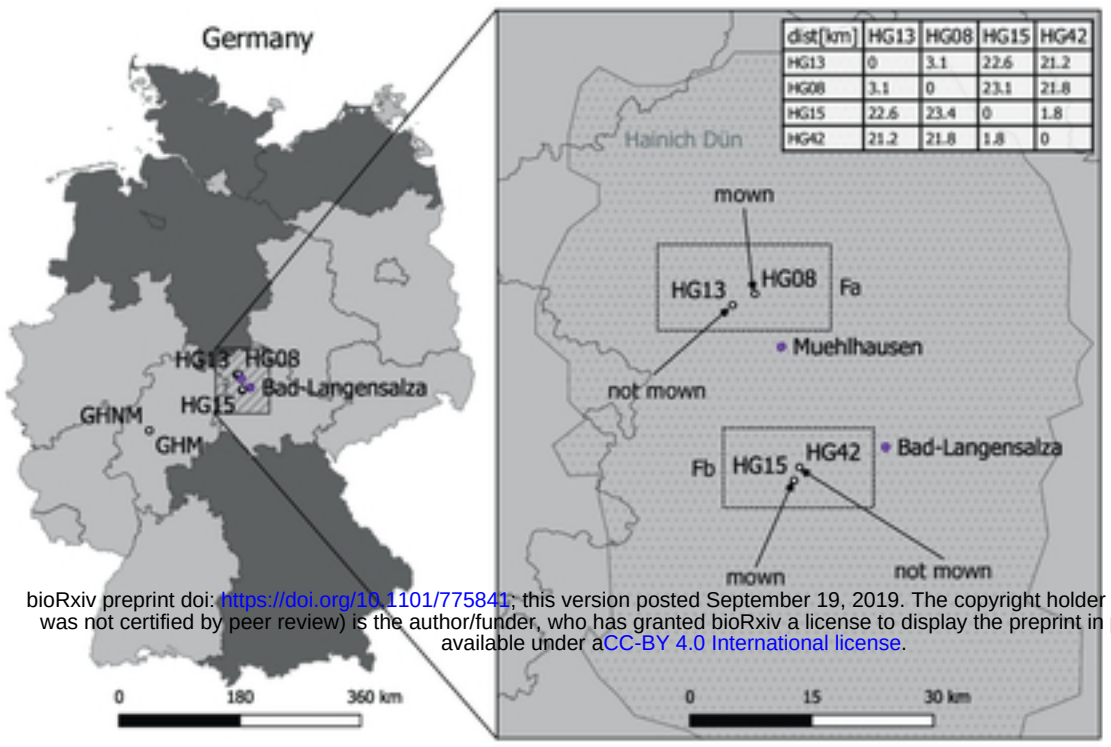
Supplement Figures

Figure S1: Annotation Overview: A: Distribution of transcripts that could be mapped to the *T. pratense* genome, to a known locus and were annotated with *T. pratense* genome identifier. B: Distribution of transcripts that could be mapped to an unknown T. *pratense* gene locus. C: Distribution of transcripts that could not be mapped o the *T. pratense* genome. D: Distribution of transcripts of whole transcriptome representing all 12 libraries.

Figure S2 Differential expression of putative transcription factors of *T. pratense.* The Y axis denotes the number of expressed TF family members, the x axis shows the treatments and TF families. Orange bars indicate that >10% of the TF members are differentially expressed between the treatments, the red bars indicates that >5% are differentially expressed.

Figure S3: Plant architectural characteristics and growth habit of GA treated plants. A-E Measured, counted or calculated plant characteristics during phenotypic monitoring experiments. GA treated plants, blue; control plants, orange. Graphs show average values and 95% confidence intervals. Time is shown in weeks. Growth habit of control plants (left side) vs GA treated plants (right side), after approximately 2 weeks of GA treatment and regrowth (F), and after 4 weeks (G).

A



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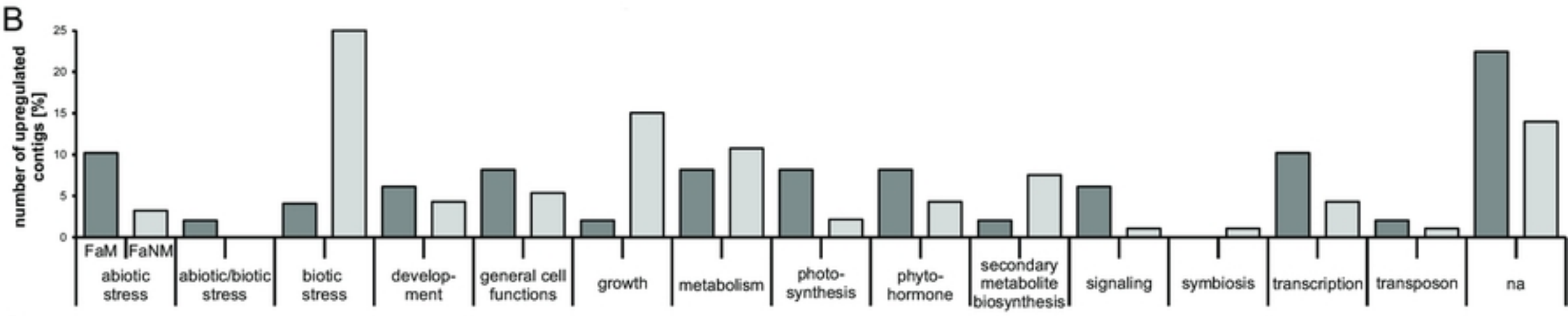

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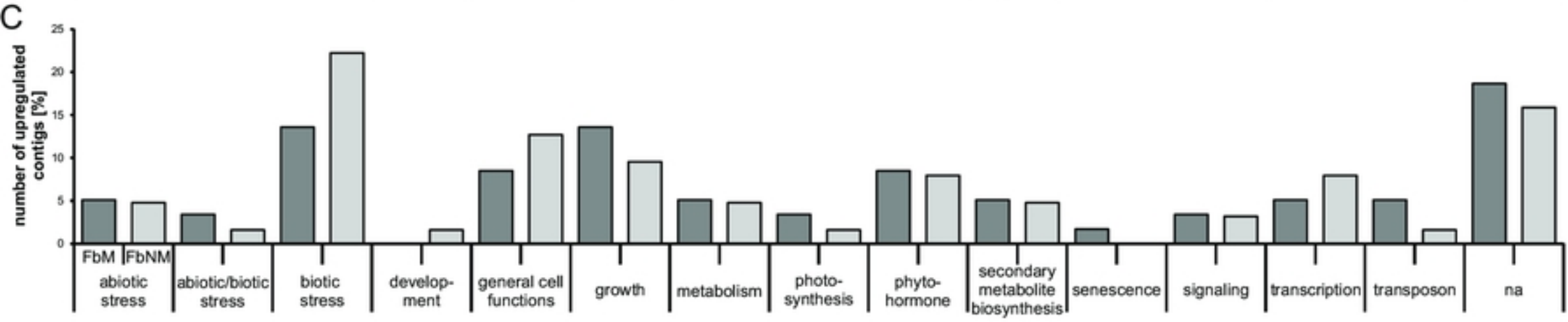

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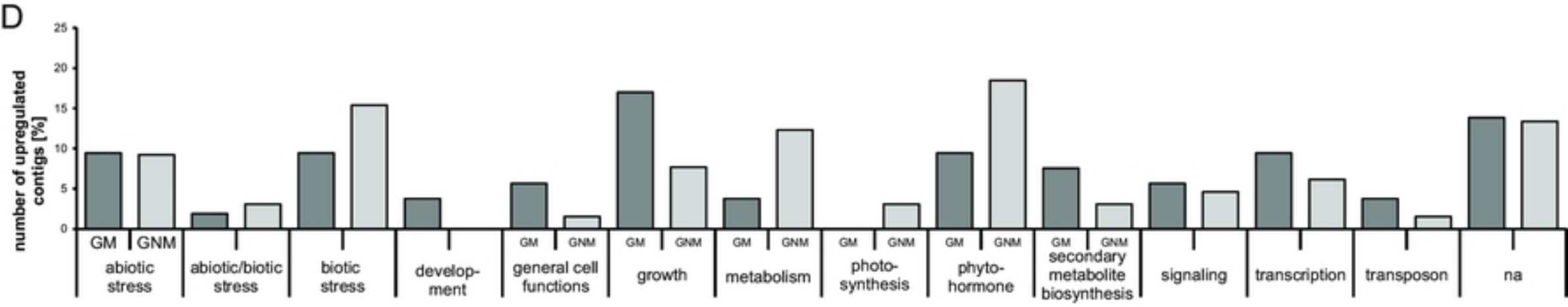

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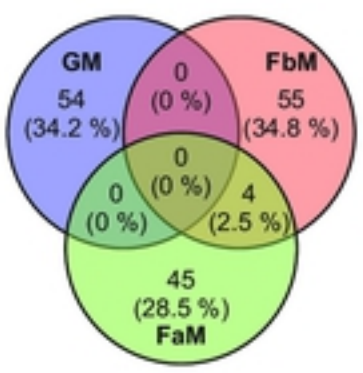

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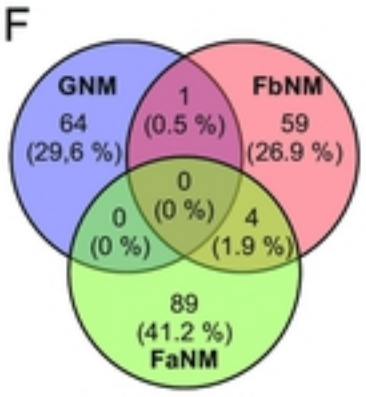

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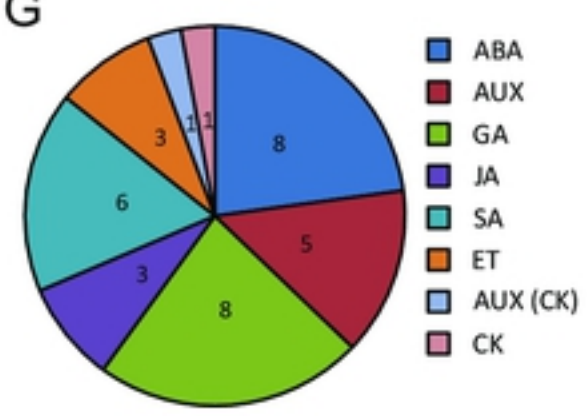
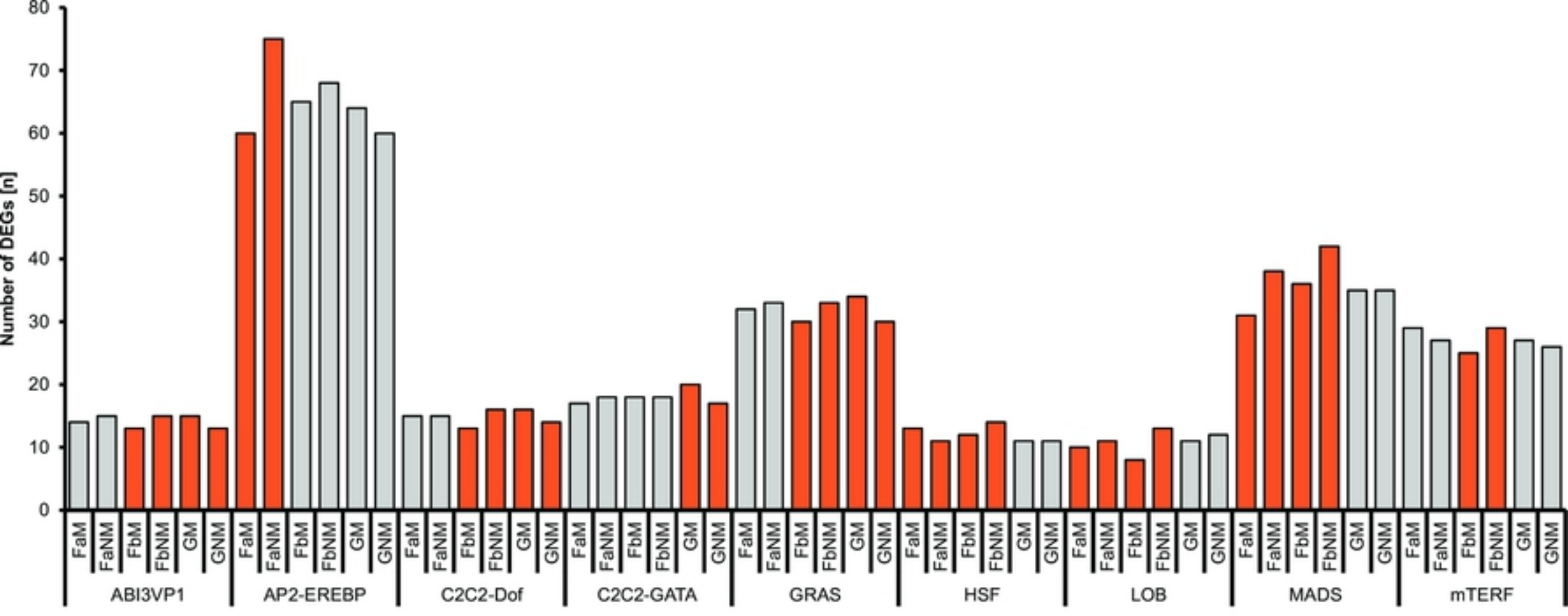


Figure 1


Number of DEGs [n]
0
10
20
30
40
50
60
70
80
FaM
FaNM
FbM
FbNM
GM
GNM
ABI3VP1
AP2-EREBP
C2C2-Dof
C2C2-GATA
GRAS
HSF
LOB
MADS
mTERF

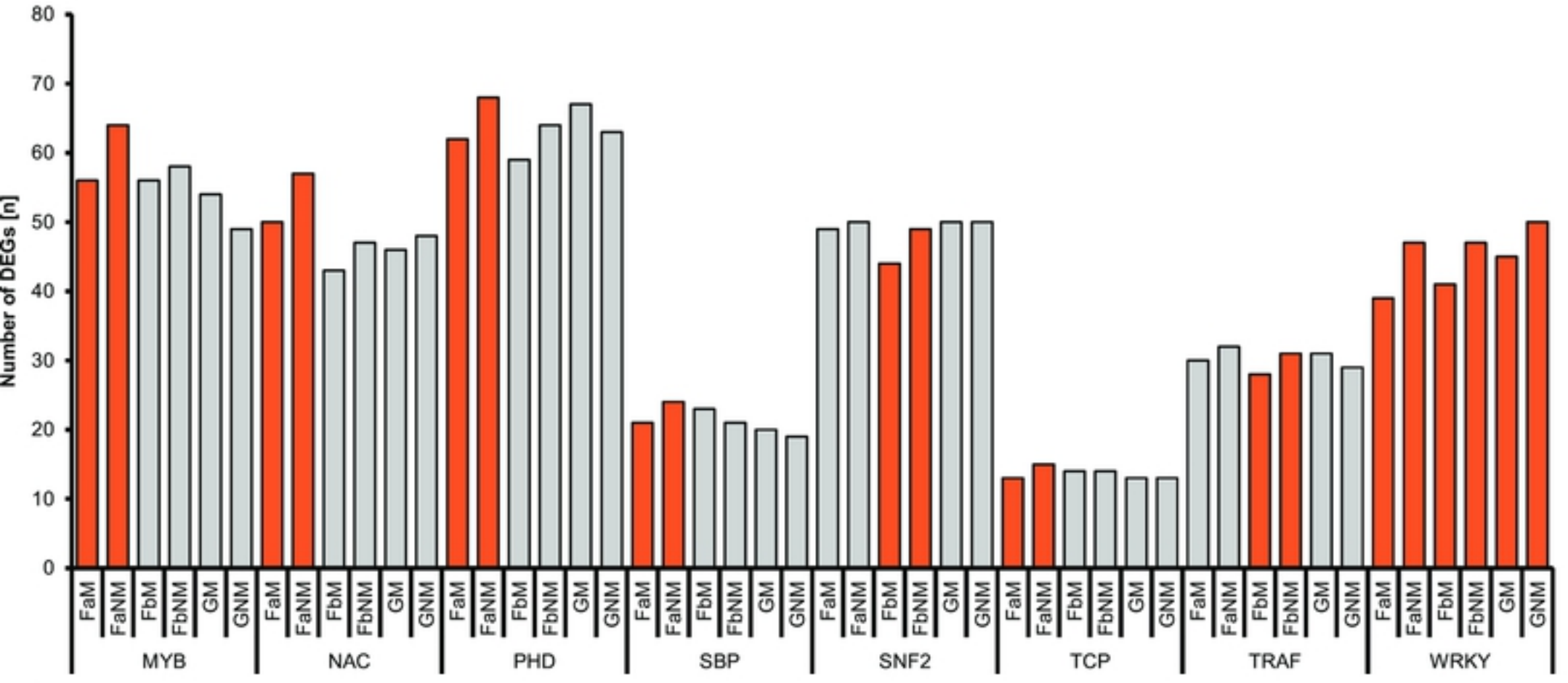
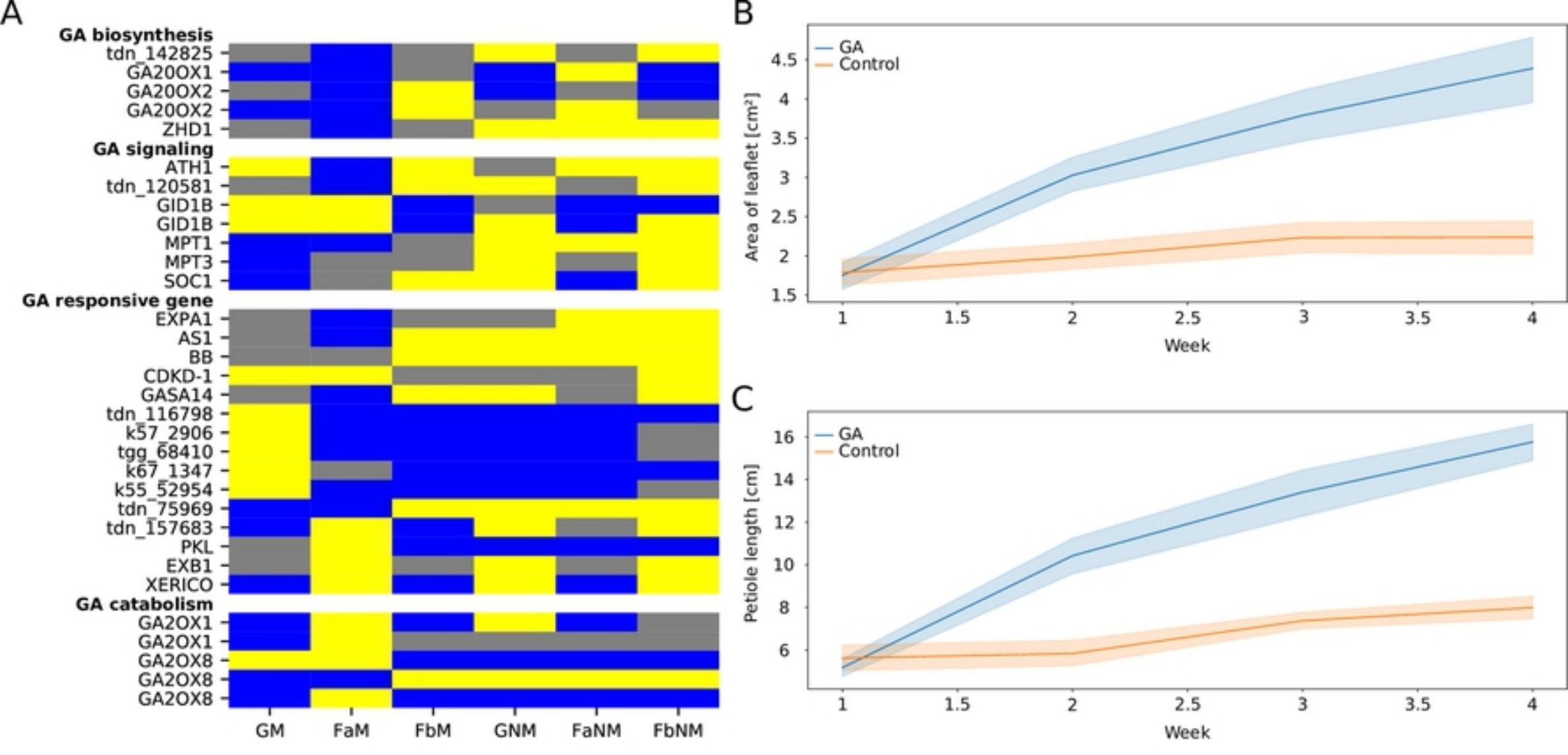

Number of DEGs [n]
0
10
20
30
40
50
60
70
80
FaM
FaNM
FbM
FbNM
GM
GNM
MYB
NAC
PHD
SBP
SNF2
TCP
TRAF
WRKY

Figure 2


A
GA biosynthesis
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GA20OX2
GA20OX2
ZHD1
GA signaling
ATH1
tdn_120581
GID1B
GID1B
MPT1
MPT3
SOC1
GA responsive gene
EXPA1
AS1
BB
CDKD-1
GASA14
tdn_116798
k57_2906
tgg_68410
k67_1347
k55_52954
tdn_75969
tdn_157683
PKL
EXB1
XERICO
GA catabolism
GA2OX1
GA2OX1
GA2OX8
GA2OX8
GA2OX8
GM
FaM
FbM
GNM
FaNM
FbNM
B
GA
Control
Area of leaflet [cm²]
Week
C
GA
Control
Petiole length [cm]
Week

Figure 3