Research

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Abscisic acid signalling mediates biomass trade-off and allocation in poplar

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Summary

- Abscisic acid (ABA) is a well known stress hormone regulating drought adaptation of plants. Here, we hypothesised that genetic engineering of genes involved in ABA stress signalling and photoperiodic regulation affected drought resistance by trade-off with biomass production in perennial poplar trees.
- We grew *Populus tremula* × *tremuloides* wild-type (T89) and various transgenic lines (two transformation events of *35S::abi1-1*, *35S::RCAR*, *RCAR:RNAi*, *35S::ABI3*, *35S::AREB3*, *35S::FDL1*, *FDL1:RNAi*, *35S::FDL2* and *FDL2:RNAi*) outdoors and exposed them to drought in the second growth period.
- After the winter, the surviving lines showed a huge variation in stomatal conductance, leaf size, whole-plant leaf area, tree height, stem diameter, and biomass. Whole-plant leaf area was a strong predictor for woody biomass production. The *35S::AREB3* lines were compromised in biomass production under well irrigated conditions compared with wild-type poplars but were resilient to drought. ABA signalling regulated *FDL1* and *FDL2* expression under stress. Poplar lines overexpressing *FDL1* or *FDL2* were drought-sensitive; they shed leaves and lost root biomass, whereas the *FDL* RNAi lines showed higher biomass allocation to roots under drought.
- These results assign a new function in drought acclimation to FDL genes aside from photoperiodic regulation. Our results imply a critical role for ABA-mediated processes in balancing biomass production and climate adaptation.

Introduction

Poplars (*Populus* sp.) are woody biomass crops that can be managed by agronomic techniques in short-rotation plantations (Kauter *et al.*, 2003; Bredemeier *et al.*, 2015). The most important growth limiting factor in poplar plantations is water availability (Linderson *et al.*, 2007). Drought periods are expected to occur more frequently with progressing climate change (Dai, 2013; Schiermeier, 2018). Consequently, improved drought tolerance and the maintenance of growth are important breeding objectives for poplars (McKendry, 2002; Polle *et al.*, 2006). It is therefore important to understand how poplar trees acclimate to dry conditions and to identify traits that enable positive yields under water limitations (Polle *et al.*, 2019).

The phytohormone abscisic acid (ABA) plays a central role in plant acclimation to drought (Raghavendra *et al.*, 2010; de Zelicourt *et al.*, 2016). ABA accumulates in response to a water deficit and causes stomatal closure, therefore enabling plants to balance water supply and consumption (Jones & Mansfield, 1970). The main components involved in ABA signalling are conserved in land plants (for example moss: Tougane *et al.*, 2010; Arabidopsis: Fujita *et al.*, 2009; Ma *et al.*, 2009; Park *et al.*, 2009; poplar: Papacek *et al.*, 2017) and consist of ABA receptor proteins, the RCARs (Regulatory Component of ABA Receptors (RCAR)/proteins pyrabactin resistance 1 (PYR1)/PYR1-Like (PYL)), phosphatases of the PP2C-A type (group A type 2C protein phosphatases), and SnRK2 kinases (sucrose nonfermenting 1-related protein kinase 2) (Fujita *et al.*, 2009; Ma *et al.*, 2009; Park *et al.*, 2009). In the absence of stress, PP2Cs suppress SnRK2 activity by dephosphorylation (Fujita *et al.*, 2009). Under stress, ABA accumulates and binds to RCARs, which then form a complex together with PP2Cs, therefore inactivating them (Ma *et al.*, 2009; Park *et al.*, 2009). Consequently, SnRK2 is kept in its active, phosphorylated form, regulating downstream responses such as ion channels and AREB (ABA-Responsive Element Binding protein) transcription factors (TFs) to induce stomatal closure (Hubbard *et al.*, 2010) and osmotic adjustment (Yoshida *et al.*, 2015).

Functional studies showed that suppression of AREB genes resulted in high drought susceptibility (Fujita *et al.*, 2005; Yoshida *et al.*, 2010, 2015). Overexpression of *RCARs*, *SnRK2s*

and *AREB* genes in a range of species, for example *Arabidopsis* (PYL9), rice, tomato, and poplar, enhanced drought resistance (Fujita *et al.*, 2005; González-Guzmán *et al.*, 2014; Tian *et al.*, 2015; Zhao *et al.*, 2016; Yu *et al.*, 2017a). Genes involved in ABA signalling and downstream responses are members of larger families. For example, the poplar RCAR family (also denoted as PYR/PYL/RCAR or PYRL) has 14 members (Papacek *et al.*, 2017). Overexpression of *PYRL1* and *PYRL5* in poplar (denoted after Yu *et al.*, 2017b) confers drought tolerance (Yu *et al.*, 2017a). In *Arabidopsis*, overexpression of *AtRCAR1/PYL9* results in higher survival after drought than overexpression of other members of the RCAR gene family (Zhao *et al.*, 2016). In poplar, the closest orthologue of AtRCAR1/PYL9 is PYRL10 (denoted according to Yu *et al.*, 2017b), which has also been denoted as PtRCAR1/PYL9 (Singh *et al.*, 2018). PtRCAR1/PYL9 is involved in photoperiod regulation (Singh *et al.*, 2018). Whether PtRCAR1/PYL9 plays a role in drought amelioration is unknown. Previous studies have shown that drought tolerance is compromised when ABA signalling is suppressed by a mutation in the ABI1 gene (*abi1-1*); abi1-1 interrupts ABA signalling by permanently binding to SnRK2 (Umezawa *et al.*, 2010). Poplars (*P.* × *canescens*) strongly overexpressing abi1-1 exhibited impaired stomatal regulation and survived only under high air humidity (Arend *et al.*, 2009).

In addition to stomatal regulation and osmotic adjustment, ABA affects many aspects of plant environmental adaptation and development such as shoot and root growth, leaf size, seed dormancy, senescence and seasonality of perennials (Sharp & LeNoble, 2002; Tylewicz *et al.*, 2015, 2018; Negin & Moshelion, 2016; Zhao *et al.*, 2016). Because the duration of vegetative growth affects the yield of trees, seasonal timing of bud break and dormancy are also traits of interest for productivity. In hybrid poplar, the molecular processes underlying ABA-regulated photoperiodic growth are being intensely studied (Ding & Nilsson, 2016; Maurya & Bhalerao, 2017). Vegetative growth is promoted by the interaction of FT (Flowering Locus T) with FDL1 (bZIP transcription factor FD-like1) (Tylewicz *et al.*, 2015). Under short days, *FT* expression is suppressed and then FDL1 interacts with ABI3 (Abscisic acid insensitive 3) promoting cold acclimation (Tylewicz *et al.*, 2015). Whether FDL1 or its close homologue FDL2 play roles in drought acclimation is unknown.

The goal of this study was to investigate the role of ABA-regulated processes for drought performance and biomass production in hybrid poplars under outdoor conditions. We used wild-type and transgenic lines of hybrid aspen (*P. tremula* × *tremuloides,* T89) with up- or downregulated genes involved in ABA perception and signalling (*35S::RCAR1/PYL9*, *35S::abi1-1*), downstream TFs (*35S::ABI3*, *35S::AREB3*), and seasonal acclimation (*35S::FDL1*, *RNAi FDL1*, *35S::FDL2*, *RNAi FDL2*) (Tylewicz *et al.*, 2015, 2018; Singh *et al.*, 2018). To date, these lines have been used to study photoperiodic regulation under controlled conditions. Here, we tested their drought responses under field conditions, in a seasonal climate and in competition with neighbouring poplars. We also tested their drought responses under glasshouse conditions. Based on previous functional analyses of the above genes in various plant species, we expected enhanced stress tolerance in lines overexpressing *RCAR1/PYL9, ABI3* and AREB3 at the expense of biomass trade-off and poor performance or decline of *abi1-1* overexpressing lines. We further expected that *FDL1* overexpression, which renders the poplars less sensitive to short days (Tylewicz *et al.*, 2015; Parmentier-Line & Coleman, 2016), would enhance biomass production due to an extended phenophase, but might increase the risk of cold damage.

We grew wild-type (WT) and ABA-related transgenic poplar lines (generally two lines per construct representing independent transformation events) for two growth seasons outdoors in a caged area under ambient weather conditions, except for precipitation. The trees were well irrigated, and half of them were subjected to drought stress in the second growth phase. We determined the traits that are important for fitness such as photosynthesis, stomatal conductance, water use efficiency (WUE), leaf size, whole-plant leaf area and biomass production (Bréda & Badeau, 2008; Tardieu *et al.*, 2014). We observed that small transformation event-specific growth modifications were augmented under field conditions, partly superimposing functional analyses of the target genes. Exploiting the variation of growth traits introduced by transformation and drought, we showed that leaf area was the most important predictor for yield. These results were confirmed under glasshouse conditions. Separating event-specific and target gene effects, we found that *AREB3* overexpression resulted in enhanced drought resilience but a massive biomass trade-off. *FDL1* overexpression resulted in high yield and high drought susceptibility. We discovered new functions of FDL1 and FDL2 in the regulation of biomass allocation between roots and shoots.

Materials and Methods

Plant resources

Hybrid poplars *P. tremula* × *P. tremuloides* (wild-type T89) and transgenic lines were multiplied by micropropagation (half-strength MS (½MS) medium, Murashige & Skoog, 2006). We used transgenic lines with enhanced or suppressed expression of eight different ABA-related genes as listed in Table 1. Details for the production and testing of the following lines have been reported elsewhere: abi1-1 (Tylewicz *et al.*, 2018), *FDLs* and the *ABI3* lines (Tylewicz *et al.*, 2015) and *RCAR1/PYL9* overexpressing lines (Singh *et al.*, 2018). To generate *RCAR1/PYL9* RNAi transgenic plants, a fragment corresponding to the coding sequence was amplified using the primers 5′-GCACAGCCAA ACGCTCTG-3′ and 5′-CACCAGTCATTTGTGGTGGATG TGC-3′ and cloned into the plant transformation vector pK7GWIWG and then mobilised into *Agrobacterium* GV3101pmp90 as described by Tylewicz *et al.* (2015). The *Agrobacterium*-mediated transformation of the hybrid aspen clone T89 was performed as described by Nilsson *et al.* (1992). To generate *35S::AREB3* overexpressing lines, the cDNA sequence of Potri.010G248300 was synthesised (Genescript, Piscataway, USA) and cloned into the plant transformation vector pK7GW2 under the control of the 35S promoter and

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Table 1 Wild-type and transgenic lines of *Populus tremula* × *tremuloides* (T89) with modifications in abscisic acid (ABA) signalling.

Construct number	Line number	Name in figures	Construct	Function	Targeted gene	Numbers of plants	
						Centre	Border
T89		T89	Wild-type		None	8	19
76	1	abi1-1*	35S::abi1-1	PP2C-A		8	0
76	2	–	35S::abi1-1	PP2C-A		8	4
76	3	NA	35S::abi1-1	PP2C-A		0	0†
473	3	RCARa	35S::RCAR	PYL, ABA receptor	Potri.014G097100	8	6
473	10	NA	35S::RCAR1/PYL9	PYL, ABA receptor	Potri.014G097100	0	4
473	18	RCARb	35S::RCAR1/PYL9	PYL, ABA receptor	Potri.014G097100	8	6
474	10	NA	RCAR1/PYL9:RNAi	PYL, ABA receptor	Potri.014G097100	0	8
474	11	NA	RCAR1/PYL9:RNAi	PYL, ABA receptor	Potri.014G097100	0	5
474	15	NA	RCAR1/PYL9:RNAi	PYL, ABA receptor	Potri.014G097100	0	8
165	2	ABI3	35S::ABI3	ABA-responsive TF	Potri.002G252000	8	5
165	6	–	35S::ABI3	ABA-responsive TF	Potri.002G252000	8	0
419	6	AREB3a	35S::AREB3	ABA-responsive TF	Potri.010G248300	8	6
419	11	AREB3b	35S::AREB3	ABA-responsive TF	Potri.010G248300	8	8
2024	3	–	35S::FDL1	FD-like, bZIP TF	Potri.002G018400	8	0
2024	5A	FDL1	35S::FDL1	FD-like, bZIP TF	Potri.002G018400	8	7
434	3	FDL1ia	FDL1:RNAi	FD-like, bZIP TF	Potri.002G018400	8	6
434	11	FDL1ib	FDL1:RNAi	FD-like, bZIP TF	Potri.002G018400	8	4
512	3	NA	35S::FDL2	FD-like, bZIP TF	Potri.005G243400	0	0†
512	17	FDL2	35S::FDL2	FD-like, bZIP TF	Potri.005G243400	8	6
510	12	FDL2ia	FDL2:RNAi	FD-like, bZIP TF	Potri.005G243400	8	7
510	18	FDL2ib	FDL2:RNAi	FD-like, bZIP TF	Potri.005G243400	8	3

*abi1-1 from Arabidopsis (AT4G26080) orthologue to poplar Potri.006G224600.
†Plants were tiny and died before out-planting, – plants died in the first winter. NA, plants not available in the centre; TF, transcription factor.

transformed into the hybrid aspen clone T89 as described by Tylewicz *et al.* (2015). Selection of transgenics was performed on MS plates supplemented with hygromycin (50 mg l^{-1}) or kanamycin (50 mg l^{-1}). Up to 10 lines were initially generated. Following selection, qRT-PCR (quantitative real-time polymerase chain reaction) was performed on cDNA generated from leaf-derived RNA, using wild-type T89 leaves as a control sample (Supporting Information Fig. S1). Two or three lines with the highest upregulation or downregulation were selected for further phenotypic analysis.

For the propagation of the lines, plantlets were cut into *c.* 1 cm long stem segments, each containing one leaf, and were propagated in ½MS medium (Murashige & Skoog, 2006). Therefore, all plantlets had the same starting conditions. After 4 wk, most of the plantlets were rooted.

Experimental design

Rooted plantlets of all transgenic lines (19 lines in total) and the wild-type (Table 1) were transferred from tissue culture directly into soil (Fruhstorfer Erde Type N, Hawite Gruppe GmbH, Vechta, Germany) and kept in pots in a glasshouse (temperature: 22°C, air humidity: 60%, ambient plus additional light) (3071/400 HI-I; Adolf Schuch GmbH, Worms, Germany) to achieve a light period of 16 h and 150 µmol photons m^{-2} s^{-1} photosynthetically active radiation at plant height. All plants were watered regularly and initially kept under transparent covers to avoid drought stress, similar to the methods described by Müller *et al.* (2013). At 7 wk after potting (mid-June 2016), the plants were transferred into 3 l pots and then 3 wk later, moved outdoors for field acclimation (July, 2016). The plants were kept initially under green nets to prevent sun burn for 1 wk. After 1 month acclimation, healthy-growing plants were selected and planted in four large square boxes (3 m × 3 m × 0.5 m, Göttingen, Germany, 51.55739°N, 9.95857°E, 153 m asl) (Fig. S2a) as described previously (Paul *et al.*, 2018). Each box contained 60 plants (6 × 10) planted at a distance of 0.5 m × 0.3 m with 28 plants positioned at the border and 32 plants in the centre (Fig. S2b). Measurements were performed with all plants, but only plants from the centre (four biological replicates per line and treatment) were used for detailed analyses to avoid border effects.

Before planting, the boxes had been filled with a compost-sand mixture (Vogteier Erdenwerk GmbH, Niederdorla, Germany) and were covered with plastic foil to prevent uncontrolled irrigation by rain (Fig. S1c). In each box, five tensiometers (ECH2O Check Soil Moisture Monitors, Decagon Devices, WA, USA) were installed at *c.* 0.09 m depth in the soil, one in the middle and four near the corners. A drip irrigation system with tap water was installed in each box, and irrigation was automatically controlled by an AQUA PRO Irrigation Controller (NETAFIM, Tel Aviv, Israel). The plants were irrigated two times per day (at 10:00 h and 17:00 h, 15 min at each time point), to maintain the soil moisture at field capacity. Air temperature and air humidity data were obtained from the nearby weather station in the Experimental Botanical Garden, University of Göttingen (Göttingen, Germany, 51°33′24″N, 9°57′16″E, 177 m asl). The average air temperatures in summer 2016 (July and August), autumn 2016 (September to November), winter 2016/2017 (December to

February), spring 2017 (March to May), and summer 2017 (June to August) were 18.3, 10.5, 0.8, 9.8 and 17.6°C, respectively, and the average air humidity values for the same time periods were 76%, 85%, 87%, 75%, and 79%, respectively. In the second growth phase, a data logger (Testo SE & Co. KGaA, Lenzkirch, Germany) was installed between box B and box C (Fig. S1a) on 29 March 2017, and air temperatures and humidity were recorded once 1 h until the end of the experiment. Irrigation was stopped during the winter months (November 2016 to March 2017) and restarted on 28 March 2017 when the temperature was constantly above zero.

Irrigation and drought treatment

Plants in two of the four boxes (boxes B and D) were subjected to drought treatments, while the other two boxes (boxes A and C) represented the control treatments (Fig. S2a). Control plants were watered twice a day to maintain soil moisture at field capacity at all times. The irrigation time for the control plants was initially 15 min per time point and was increased to 20 min per time point on 15 June and to 30 min per time point on 19 July to accommodate for larger water consumption by the growing trees. The drought treatment started in the second growth phase after bud burst on 28 April by reducing the water supply initially to 1/3 of that of the control (10 min once per day at 11:00 h). After 1 wk, on 5 May, the irrigation was completely stopped for the drought treatments. The soil moisture was recorded by tensiometers once a week and remained invariant in the control boxes and gradually declined in the drought treatments (Fig. S3).

Field measurements

Tree heights and diameters were recorded regularly during the growing phase for all plants. Tree height after planting into the boxes was measured from the bottom of the tree at the soil surface to the apical leaf once a month using a 2 m folding ruler when the plants were shorter than 2 m or a measuring tool (mEssfix 8 m, Nedo, Kaltenbach, Switzerland) when the trees were higher than 2 m. Stem diameters were measured 4 cm from the bottom in the second growth phase using a digital calliper (Tchibo GmbH, Hamburg, Germany). Stem volume was approximated as taper ($V = 1/3\ \Pi\ r^2\ h$, r = stem radius, h = tree height), and increment during the drought phase (from t_1 = June 12 to t_2 = August 8) was calculated as $V_{t2} - V_{t1}$.

At the end of the first growth season, bud set was observed and scored twice a week from the end of August to October. According to the methods described by Rohde *et al.* (2011), bud set was divided into seven stages using a score from 3 to 0. A score of 3 referred to leaves and 0 to the completely formed bud. A score of 1.5 indicated that the bud form was visible (Rohde *et al.*, 2011). At the beginning of the second growth season, bud burst was scored twice a week from March until the end of April. Bud burst was classified according to five stages (UPOV, 1981), with scores from 1 to 5. A score of 1 indicates that bud burst was initiated, and 5 is a completely opened bud. These measurements were performed and analysed for all plants.

Gas exchange

Photosynthesis, transpiration rate, stomatal conductance, and substomatal CO_2 concentrations were measured on fully developed leaves on the sun-exposed upper part of the main stem of control plants under ambient temperature and humidity (Lcpro+ Portable Photosynthesis System, ADC BioScientific Ltd, Hoddesdon, UK) on 4 August, 10 d before harvest. The average air temperature and air humidity during the measurement between 10:00 h and 16:00 h were 23.3 ± 0.3°C and 58% ± 2%. Photon flux density of the photosynthetically active radiation was set to 870 µmol m^{-2} s^{-1} at leaf level. WUE was calculated as the ratio of photosynthetic rate (A) to transpiration rate (E) (Farquhar *et al.*, 1989). The measurements were conducted on $n = 4$ plants per line growing in the centre.

Stomatal conductance of the fully developed leaves on the top of the control and drought-stressed plants was measured on 22 May, 5 and 19 June and 18 July with a porometer (AP4; Delta-T Devices, Cambridge, UK) under ambient conditions ($n = 4$ per line per treatment, centre plants).

Harvest

The harvest of the aboveground tissues took place from 14 August to 24 August 2017. The stem of each plant was cut at the base *c.* 1 cm above the soil. All leaves were removed, and the number of lateral branches was counted. The fresh weight of each aboveground part (stem, branches and leaves) was determined immediately. Dry weights were determined after oven-drying aliquots of these tissues for 1 wk at 60°C. The dry weight of the whole tissue was calculated as:

$$\frac{\text{Dry weight of the aliquot (g)} \times \text{Total fresh weight of the tissue (g)}}{\text{Fresh weight of the aliquot (g)}}$$

Five fresh leaves collected from the top to the middle of the main stem of each plant were weighed, scanned, dried in the oven at 60°C for 1 wk and weighed again. Examples of upper fully expanded leaves are shown in Fig. S4. Leaf scans were used to determine the specific leaf area (SLA; cm^2 g^{-1}), leaf size (measured area of five leaves/5) and whole-plant leaf area with the following equation:

$$\frac{\text{Leaf area of the 5 leaves (cm}^2\text{)} \times \text{Dry weight of all leaves of the plant (g)}}{\text{Dry weight of the 5 leaves (g)}}$$

The roots were harvested from 25 August to 6 September 2017. To obtain a measure of root mass per plant, a square of 0.16 m × 0.16 m was drawn with the stem stump in the centre and the soil of this square was dug to a depth of 0.20 cm. The stump with roots was washed with tap water, and adhering water was surface dried with paper towels. Fresh weight was determined immediately, and dry weight was determined after 1 wk of oven drying at 60°C.

Glasshouse experiments

Wild-type T89 poplars and transgenic lines (as above) were grown individually in pots under glasshouse conditions (mean air temperature: 22°C, relative air humidity: 60–80%, additional photosynthetic active radiation: 16 h, 100 µmol photons m^{-2} s^{-1}) for 6 wk and then exposed for 4 wk to drought (soil moisture 0.1 m^3 m^{-3}) or were maintained as controls (soil moisture 0.4 m^3 m^{-3}) ($n = 5$ per treatment and line). Stomatal conductance was determined weekly. Leaves, stems, and roots were harvested and used for the determination of leaf size, whole-plant leaf area and biomass as described above. Stomatal frequencies were determined by prints produced with clear nail polish from the lower leaf surface and counted under a compound microscope.

In further experiments, wild-type T89 poplar and abi1-1 plants (lines 76-1 and 76-3) were used as controls or exposed to drought (as above) or supplied with ABA (200 µM twice a week, soil irrigation). After 4 wk of treatments, the developing xylem was used for RNA sequencing as described by Wildhagen *et al.* (2018). The details have been described in Fig. S5. Data are available under the accession number E-MTAB-7588 at the EMBL-EBI database (www.ebi.ac.uk/arrayexpress).

Statistical analyses

The statistical software R 3.4.2 (R Core Team, 2017) and Statgraphics Centurion XVI (Statgraphic Technologies, The Plain, VA, USA) were used for data analyses. Tree height, stem diameter and leaf area of the control plants were analysed by one-way analysis of variance (ANOVA) with plant lines as the main factor. Gas exchange was analysed with multivariate ANOVA using substomatal CO_2 concentration, leaf-level temperature, and ambient partial water vapour pressure as covariates. The relative change in plant biomass under drought stress was analysed by two-way ANOVA with plant lines and treatment as the main factors. The impact of lines and drought on stomatal conductance was tested using general linear models, including all measured time points. When the *P*-values were < 0.05, Fisher's test was used as a post hoc test to determine significant differences between the lines. To test the influence of various parameters on woody biomass production, general linear models were run in which nonsignificant factors were removed stepwise. The model with the lowest Akaike Information Criterion (AIC value) is shown. Data are shown as means (± SE).

Results

Modifications in ABA signalling affect gas exchange

As ABA is involved in the regulation of plant gas exchange (de Zelicourt *et al.*, 2016), we investigated stomatal conductance of WT and transgenic lines with modifications in ABA responses as specified in Table 1. Stomatal conductance of the well irrigated ABA-related transgenic lines was lower than that of the well irrigated WT poplars (Fig. 1). Among the well irrigated transgenic lines, roughly two groups, one with higher (RNAi lines of *FDL1* and *FDL2* and the *ABI3* overexpressing line, letters i, j, k) and one with lower (lines overexpressing *abi1-1*, *RCAR1/PYL9*, *FDL1* and *FDL2*, letters: d, e, f, g) stomatal conductance, could be distinguished (Fig. 1).

WT plants showed the highest photosynthesis (12.6 ± 1.0 µmol CO_2 m^{-2} s^{-1}), whereas net CO_2 fixation rates were generally lower in transgenic lines, ranging from 5.1 to 11 µmol CO_2 m^{-2} s^{-1} (Table S1). Photosynthesis decreased with decreasing stomatal conductance ($R^2 = 0.902$, $P < 0.0001$), showing that stomatal conductance controlled CO_2 assimilation of the transgenic lines. Photosynthesis and transpiration were also tightly correlated ($R^2 = 0.970$, $P < 0.0001$), resulting in stable WUE (amount of water used per fixed CO_2) across all lines (225 ± 4 mol H_2O mol^{-1} CO_2, $P_{(lines)} = 0.410$; Table S1).

Moderate drought was initiated in May of the second growth phase and reached the target of about half of the soil moisture of well irrigated plants in mid-June (Fig. S3). Drought caused strong reductions in stomatal conductance of the WT, whereas the decreases were less pronounced in transgenic lines, especially in the group whose stomatal conductance was already low under

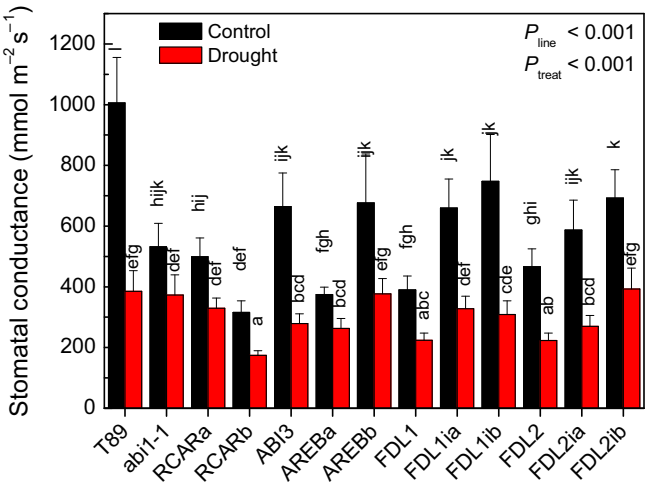

Fig. 1 Stomatal conductance of wild-type (T89) and transgenic poplars (*Populus tremula* × *tremuloides*) in response to drought. Transgenic poplars are named as shown in Table 1. Data are the means (± SE) with $n = 4$ per treatment and time point. Stomatal conductance measurements were conducted in May, early June, mid-June and mid-July (as indicated in Supporting Information Fig. S2). Data were log transformed before statistical analyses (generalised linear models). Interactions between lines and treatment were not significant. Different letters indicate significant differences among the poplar lines at $P \leq 0.05$ (post hoc Fisher's test).

well irrigated conditions (Fig. 1). Therefore, the differences observed between WT and transgenic lines under well irrigated conditions became less distinct under drought conditions (Fig. 1).

Modifications in ABA signalling have indirect effects on yield via their impact on leaf area production

Leaf traits such as leaf size, SLA, and whole-plant leaf area are useful indicators of yield, that is aboveground woody biomass (Weih & Nordh, 2005). We used general linear mixed (GLM) models to dissect the effects of line, drought treatment, stomatal conductance, and leaf traits (Table S2) on woody yield. The best model contained the factors: 'drought', 'SLA ($cm^2\ g^{-1}$ leaf mass)', and 'whole-plant leaf area (cm^2 plant^{-1})' and explained 83% of the variation of woody biomass (Table 2). Although highly significant, the factors 'drought' and 'SLA' contributed little to the overall variation (4–5%). Whole-plant leaf area was the dominant factor explaining woody biomass yield across all lines and conditions (Fig. 2). The best performing lines with regard to stem biomass production were overexpressing lines of *FDL1*, *RCAR1*/*PYL9* and *ABI3*, and the worst were the *AREB3* overexpressing lines (Fig. 2).

Notably, our GLM model did not contain 'line', 'stomatal conductance' or 'leaf size' (Table 2), indicating that woody yield was not directly driven by these factors. Stomatal conductance, leaf size and whole-plant leaf area, however, showed significant variation among transgenic lines and wild-type poplars (Table S2; Fig. 1). Furthermore, these traits were significantly correlated among each other (Table 3). Taken together, these results support the idea that distinct transgenic events and constructs influenced stomatal conductance and leaf size and, therefore, affected leaf area production as the main driver of woody biomass (Table 2; Fig. 2). These results were further corroborated by glasshouse experiments with the wild-type and subsets of the transgenic lines (Fig. S6). Woody biomass was strongly correlated with whole-plant leaf area (Fig. S6a) but not with stomatal frequency (Fig. S6b). Leaf size, stomatal conductance, and biomass were also correlated with each other (Fig. S6c).

Modifications in ABA signalling influence biomass allocation under drought

At the end of the second growth season under field conditions, whole-plant biomass (sum of the root, stem, branches and leaves) showed almost 10-fold differences among the poplar lines (Fig. 3a). Lines resulting from different transformation events but expressing the same gene construct also showed differences in biomass production (Fig. 3a). The reason for these drastic differences in some lines was that small growth differences, which existed among transgenic events and different constructs (Fig. S7), were augmented during the two vegetation periods studied here. Initially, we planted two lines per gene construct but lines 76-2 (*35S::abi1-1*), 165-2 (*35S::ABI3*), and 2024-3 (*35S::FDL1*), which were much shorter than the other lines (Table S2), did not survive the first winter. Among these lines, the *FDL1* overexpressing

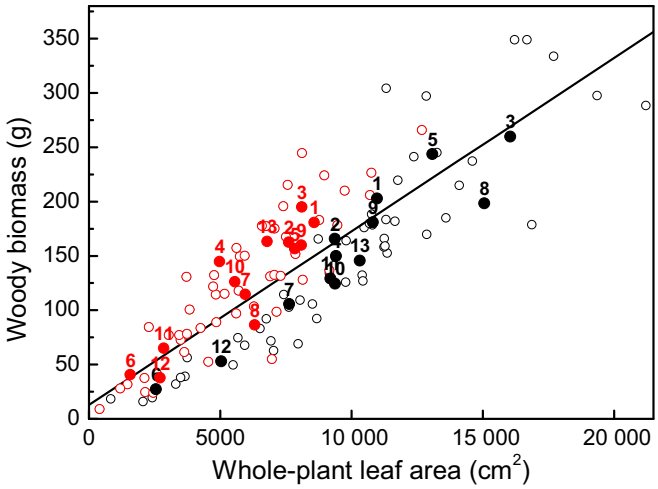

Fig. 2 Relationship between whole-plant leaf area and aboveground woody biomass of wild-type (T89) and transgenic poplar (*Populus tremula* × *tremuloides*) lines. Regression line: $y = 0.016x + 12$, $R^2 = 0.853$, $P < 0.001$. Black open circles refer to well irrigated and red open circles to drought-exposed plants. Each symbol indicates one plant. Black dots (irrigated) and red dots (drought) indicate means per line. Numbers refer to: 1, T89; 2, abi1-1; 3, RCAR10a; 4, RCAR10b; 5, ABI3; 6, AREBa; 7, AREBb; 8, FDL1; 9, FDL1ia; 10, FDL1ib; 11, FDL2; 12, FDL2ia; 13, FDL2ib. Further information on line numbers is shown in Table 1.

Table 2 Analysis of variance of aboveground woody biomass by General Linear Mixed Models.

Source	Sum of squares	df	Mean square	*F*-ratio	*P*-value
Model	840 128	3	280 043	189.31	0.0000
Treatment	34 788	1	34 788	23.52	0.0000
SLA	25 325	1	25 325	17.12	0.0001
WPLA	553 086	1	553 086	373.88	0.0000
Residual	159 764	108	1479		
Total$_{(corrected)}$	999 893	111			

Quantitative factors (stomatal conductance (mmol $m^{-2}\ s^{-1}$), specific leaf area (SLA, $cm^2\ g^{-1}$ leaf mass), leaf size (cm^2 per leaf), and whole-plant leaf area (WPLA, cm^2 per plant)) were stepwise removed, and the model with the lowest AIC was selected. Treatment (drought and well irrigated) and line (poplar lines according to Table 1) were included as fixed factors and removed if $P > 0.05$. Data are shown in Table S2.

Table 3 Correlations between stomatal conductance (mmol $m^{-2}\ s^{-1}$), leaf size (cm^2 per leaf), and whole-plant leaf area (cm^2 per plant) of poplar lines.

	Whole-plant leaf area	Leaf size	Stomatal conductance
Whole-plant leaf area	–	< 0.0001	< 0.0001
Leaf size	0.8174	–	< 0.0001
Stomatal conductance	0.3972	0.4633	–

Data are shown in Supporting Information Table S2. Upper diagonal indicates *P*-values, and lower diagonal indicates Spearman correlation coefficients (R^2).

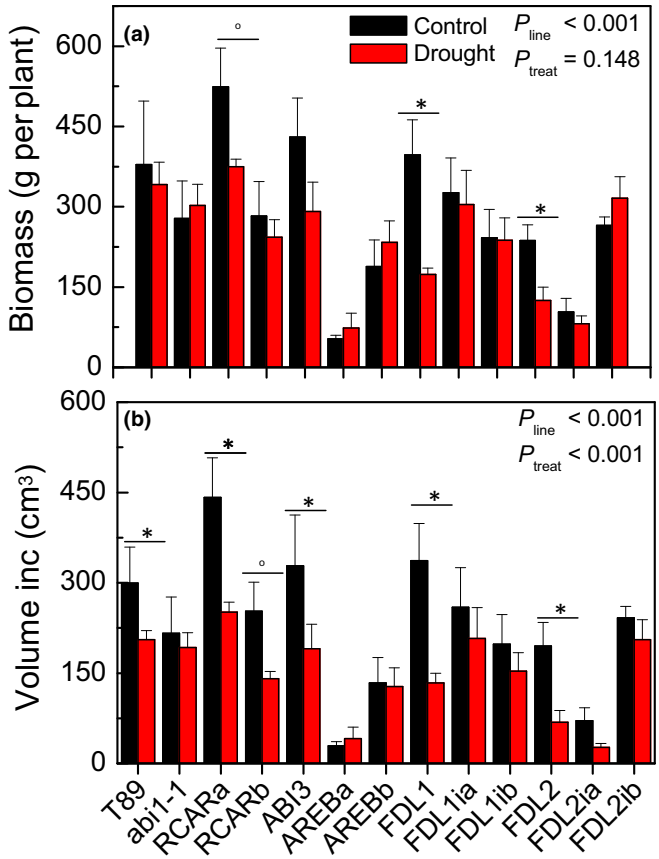

Fig. 3 Biomass (a) and volume increment (b) of well irrigated and drought-exposed wild-type (T89) and transgenic poplars (*Populus tremula* × *tremuloides*). Transgenic poplars are named by their construct as shown in Table 1. Volume increment was calculated on the basis of diameter and height growth during the period of drought stress (June 12 to August 8). Data are the means (± SE) with $n = 4$ per treatment. Data were log transformed for two-way ANOVA (biomass). Interactions between lines and treatment were not significant. Differences between the treatment at $P \leq 0.05$ (Fisher's test) are indicated by a star and trends at $P < 0.1$ with an open circle.

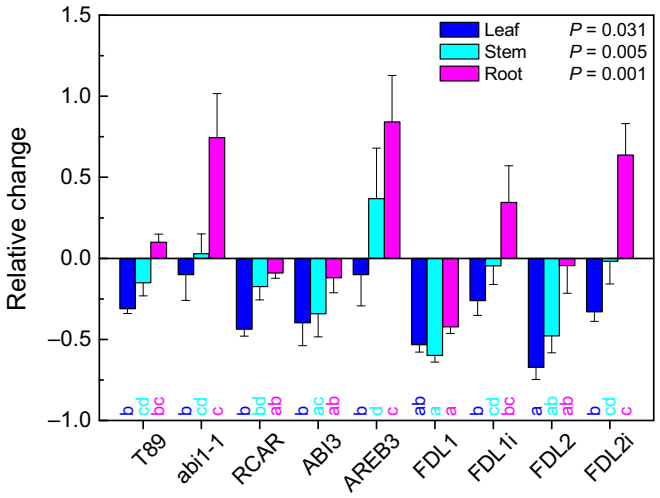

Fig. 4 Relative change in leaf, aboveground woody (Stem + Branches), and root biomass of wild-type (T89) and transgenic poplars (*Populus tremula* × *tremuloides*) in response to drought. The relative biomass change in each tissue was calculated as (Biomass$_{(drought)}$ − mean biomass$_{(control)}$)/mean biomass$_{(control)}$. Data shown are the means ($n = 4$ or 8, ± SE). Lines with the same gene construct were analysed together. To achieve variance homogeneity, data were transformed (log + 1) before ANOVA. Different letters indicate significant differences for each tissue among the poplar lines ($P \leq 0.05$, Fisher's test).

line 2024-3 did not reach bud set at all (Fig. S8). The second *FDL1* overexpressing line (2024-5A) included in our study exhibited delayed bud set and continued to grow significantly during a time period when the WT had already stopped shoot elongation (Fig. S7). This line survived the winter and was among the largest in the second year (Fig. 3a).

Most lines showed reductions in height and diameter increments during the 2-month drought period (Fig. S7), resulting in significant reductions in the production of stem volume (Fig. 3b). The associated losses in stem biomass and leaf production were obviously too small to result in significant effects on whole-plant biomass under the present conditions, with the exception of the *FDL1* and *FDL2* overexpressing lines (Fig. 3a).

Normalisation of event-specific effects and calculation of the relative biomass changes per gene construct revealed shifts in poplar biomass allocation under drought conditions (Fig. 4). Drought caused relative losses in leaf biomass, which were generally stronger in the *FDL1* and *FDL2* overexpressing lines than in other transgenic lines or the WT (Fig. 4). With the exception of the *AREB3* overexpressing lines, the woody biomass was also reduced under drought conditions (Fig. 4). The *FDL1* and *FDL2* overexpressing lines showed stronger woody biomass loss than the WT or the *FDL1* RNAi and *FDL2* RNAi lines (Fig. 4). Root biomass was usually increased under drought conditions, particularly in the RNAi lines of *FDL1* and *FDL2*, in *AREB3*, and in *abi1-1* (Fig. 4). The *FDL* overexpressing lines, especially *35S::FDL1*, showed a relative loss in root mass compared with well irrigated plants. Overall, drought-induced relative biomass losses were strongest in the *FDL1* and *FDL2* overexpressing lines and lowest in *AREB3*, while *RCAR1/PYL9* and *ABI3* overexpression did not influence the biomass allocation pattern under drought conditions compared to the WT (Fig. 4).

Drought and ABA-feeding of wild-type and abi1-1 poplars link ABA signalling and FDL regulation

To obtain insights into the molecular regulation of ABI1, RCAR1/PHYL9, ABI3, AREB3, and FDLs in response to ABA, we compared the transcriptional responses of these genes in drought-stressed or ABA-fed wild-type and abi1-1 poplar lines. ABA and drought caused enhanced transcript abundances of *ABI1* and *RCAR1/PYL9* (Fig. 5). The expression of *AREB3* was unaffected by the treatments (Fig. 5). *FDL1* exhibited very low expression levels and moderate increases in response to drought (Fig. 5). *FDL2* showed a strong increase in response to ABA (Fig. 5). None of these responses was found in the abi1-1 line (Fig. 5).

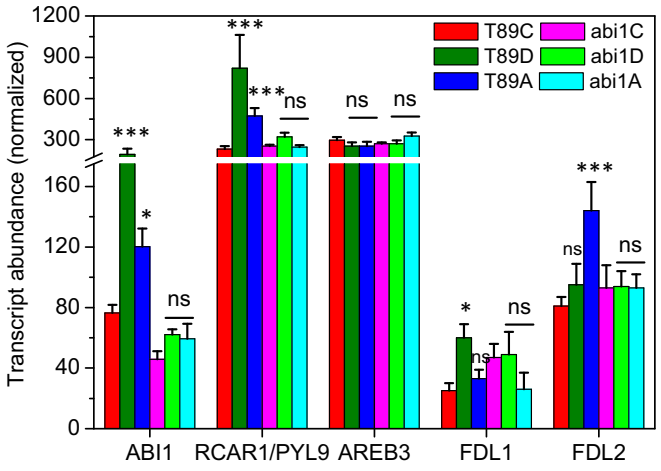

Fig. 5 Transcript abundances of *ABI1, RCAR1/PYL9, AREB3, FDL1* and *FDL2* in wild-type poplar (T89, *Populus tremula* × *tremuloides*) and *abi1-1* overexpressing poplars after exposure to drought (D), abscisic acid (ABA) treatment (A) or well irrigated control conditions (C). Data are the means of normalised transcript abundances ($n = 4$ or 5 biological replicates per treatment and line). Stars refer to $P_{adjusted} < 0.001$*** and 0.05*; adjusted *P*-values (after Bonferroni correction) are reported. ns, not significant.

Discussion

ABA-responsive genes mediate biomass trade-off in the absence of drought stress

Biotechnological tree improvement requires field testing of the traits of interest. Here, we evaluated the performance of poplars with transgenic modifications in ABA-related growth processes and stress compensation. We found significant phenotypic effects imposed by the gene construct but also variation among transformation events. As the implementation of transgene technologies, variability among transformation events is well known and has been ascribed to differences in gene expression intensity, the insertion site of the construct, etc. (Peach & Velten, 1991; Busov *et al.*, 2010). Although the transformation events generated with the same construct had been selected for similar phenotypes under laboratory conditions, our results show that moderate differences became pronounced during our long-term field study because of the exponential nature of growth and interaction with the natural environment. Lines with stronger growth impairment were generally those with higher levels of overexpression (FDL1 lines: 35-fold vs 15-fold (Tylewicz *et al.*, 2015), FDL2 lines: 250-fold vs 60-fold (Tylewicz *et al.*, 2015), RCAR1/PYL9 lines: 100-fold vs 60-fold (Singh *et al.*, 2018), abi1-1 lines: 850-fold vs 500-fold, this study), except the AREB3 lines, which exhibited moderate overexpression (three-fold to four-fold, this study). Poplar lines in which the transformation event caused strong growth impairment under field conditions were outcompeted by neighbouring plants and did not survive one full growth cycle.

A common response to interference with ABA signalling and response in the transgenic lines used in our study was reduced stomatal conductance, which correlated with leaf size. Plant leaf area is a highly plastic trait and determined by multiple physiological variables, among which hydraulic limitation is the main factor (Zwieniecki *et al.*, 2004; Brodribb *et al.*, 2010). Leaf size is linked with petiole hydraulic conductance and stomatal conductance (Juhrbrandt *et al.*, 2004; Carins Murphy *et al.*, 2014; Brocious & Hacke, 2016). Therefore, it is likely that stomatal conductance affected leaf size by affecting leaf expansion. In line with many ecophysiological studies on different poplar genotypes and crosses (Pellis *et al.*, 2004; Marron *et al.*, 2005; Monclus *et al.*, 2009), variation in leaf size of our transgenic poplar lines was correlated with whole-plant leaf area. In fast-growing tree species, whole-plant leaf area is known as a major determinant of aboveground biomass production (Pellis *et al.*, 2004; Weih & Nordh, 2005) and is related to water availability in their natural habitat (Viger *et al.*, 2016). Our results are in agreement with those findings and, moreover, demonstrate that ABA signalling and correct downstream responses exert a strong control on leaf traits with massive consequences for yield. These effects were particularly strong in the lines constitutively overexpressing *AREB3*. These poplars produced the lowest whole-plant leaf area and lowest biomass among all of the lines under well irrigated conditions. The consequences of *AREB3* overexpression in poplar resemble those found for a constitutively activated form of *AREB1* (*35S::ABREB1ΔQT*), which caused enhanced drought tolerance and reduced rosette growth in *Arabidopsis* (Fujita *et al.*, 2005).

Poplar FD-like genes have not previously been studied under field conditions. Poplar FDL1 is required to initiate ABA-mediated dormancy under short day conditions, whereas the function of its close homologue FDL2 remained elusive (Tylewicz *et al.*, 2015, 2018). The *35S::FDL1* lines studied here showed delayed bud set, whereas no clear effect of *35S::FDL2* on bud set was observed. These findings confirm the specific role of FDL1 in photoperiodic growth regulation (Parmentier-Line & Coleman, 2016; Tylewicz *et al.*, 2018). The *35S::FDL1* line 2024-3 did not attain complete bud closure and died during the winter, underscoring the importance of FDL1 in mediating adaptedness to phenology. The surviving FDL1 overexpressing line with a mild photoperiodic phenotype benefited from a prolonged growth season. Similarly, one of the ABI3 lines also showed strongly delayed bud set and was not viable during the winter, while the surviving line was among the genotypes with the highest biomass. ABI3 is known to interact with FDL1 (Tylewicz *et al.*, 2015), affecting bud maturation (Rohde *et al.*, 2002). Overall, our results suggested that ABI3 and FDL1 constitute the basis for important biomass traits.

It is notable that hybrid aspen (*P. tremula* × *tremuloides*) are among the most productive species in northern Europe (Tullus *et al.*, 2012). Their enhanced productivity has been ascribed to a longer growth phase compared to local aspen (*P. tremula*), which are adapted to shorter growing seasons (Yu *et al.*, 2001). It would be very interesting to test if FDL1 and its interacting factors such as ABI3 (Tylewicz *et al.*, 2015) were responsible for differences in seasonality between northern aspen and their hybrids with American aspen genotypes. Collectively, the empirical results and our molecular insights underpin that FDL1-mediated and ABI3-

mediated seasonal acclimation are critical traits for the balance between biomass production and climate adaptation.

Drought stress uncovers novel functions for ABA-related genes in biomass allocation

A central question of this study was how poplars with modifications in the expression of ABA-related genes performed under moderate drought stress. In general, poplars exhibit a drought-avoidance strategy by maximising water uptake and minimising water loss; the acclimation to low water availability is achieved by production of an extensive root system for water uptake and by reducing stomatal conductance, shoot growth, and leaf area by a decreased leaf size and leaf shedding (Wang *et al.*, 2003; Fischer & Polle, 2010; Popko *et al.*, 2010; Hamanishi & Campbell, 2011; Harfouche *et al.*, 2012; Polle *et al.*, 2019). Consequently, drought-responsive root and shoot development led to an increased root-to-shoot ratio (Wilson, 1988). In other words, to cope with drought stress, biomass allocation patterns are changed in favour of roots compared with shoot production (Poorter *et al.*, 2012). Taking all genotypes together, our results confirmed these allocation patterns for hybrid poplar because we found reductions in stem growth, leaf size and leaf area but hardly any effects on total biomass under drought conditions compared to well irrigated poplars. However, comparing the responses of poplars carrying distinct gene constructs to drought uncovered important gene-specific differences and unexpected behaviours among transgenic lines modified in ABA signalling on the one hand and downstream responses on the other hand.

First, among the genes important for ABA signalling, poplars overexpressing *Arabidopsis abi1-1* were expected to succumb under environmental stress because previous studies showed high vulnerability under glasshouse conditions (Arend *et al.*, 2009). This assumption was met by two of the three abi1-1 lines tested here: one line died before out-planting (not shown), one line died in the first winter, but the other line behaved similar to the wild-type, despite a growth phenotype under glasshouse conditions. We can only speculate that exposure to environmental stress outdoors might have outcompeted the negative regulation by abi1-1 in the lower overexpressing line.

In our study, the *ABI3* overexpressing poplars also behaved similar to the wild-type under drought conditions. This result might have been expected since previous studies showed that unlike in *Arabidopsis*, ABI3 is not responsive to ABA in poplar, instead playing a role in seasonal cold acclimation (Maurya & Bhalerao, 2017).

In poplar, the ABA receptors (RCARs) form a family with 14 members (Papacek *et al.*, 2017). The expression of distinct *RCARs* decreased under drought conditions (Cohen *et al.*, 2010) and recovered upon re-watering (Bizet *et al.*, 2015). Overexpression of *RCAR1* in *Arabidopsis* as well as in poplar resulted in more drought tolerant plants (Ma *et al.*, 2009; Yu *et al.*, 2016, 2017a, b), while only multiple *RCAR* knock-out mutants exhibited enhanced stress sensitivity (Park *et al.*, 2009; González-Guzmán *et al.*, 2012). In our study, we used poplar lines overexpressing the ABA receptor *RCAR1/PYL9* (Singh *et al.*, 2018). Poplar RCAR1/PYL9 is induced by ABA and interacts with HAIs (highly ABA-induced, which are members of the PP2C-A clade), demonstrating widely conserved functions in both *Arabidopsis* and poplar (Papacek *et al.*, 2017; Tischer *et al.*, 2017). Yu *et al.* (2017a,b) observed higher biomass production in *RCAR1* overexpressing poplar lines and ascribed this behaviour to hypersensitive stomatal regulation and activation of oxidative stress responses. In our study, overexpression of *RCAR1/PYL9* resulted in enhanced biomass production under well irrigated conditions, but the drought responses of both lines tested here were similar to that of the wild-type; stomatal conductance showed reductions similar to those of other transgenic poplar lines in our study. Clearly, enhanced drought resistance under field conditions was not achieved by our approach.

A second group of transgenic poplars included in our study were overexpressing or underexpressing TFs acting downstream of ABA signalling. Overexpression of an *AtAREB3* orthologue (here termed AREB3 for simplicity and relatedness to *Arabidopsis* AREB TFs, but *PtAREB12* according to the denotation suggested by Ji *et al.*, 2013) resulted in growth reduction in both tested lines along with low stomatal conductance but high resilience to drought. Drought even caused a relative promotion of stem biomass compared to well irrigated *35S::AREB3* lines. Furthermore, these genotypes showed the largest relative increase in root mass under drought among all lines studied. These findings assign a central role to AREB3 in biomass allocation between leaves and other tissues, which has not been reported before. In *Arabidopsis*, a related TF, AREB1, has been functionally characterised (Fujita *et al.*, 2005). Overexpression of *AREB1* improved drought tolerance only when it was constitutively activated (*35S::AREB1ΔQ*; Fujita *et al.*, 2005), while this modification was obviously not required for *AREB3* functioning in poplar. In *Arabidopsis*, *AREB3* members are mainly expressed in seeds and play roles in seed maturation (Fujita *et al.*, 2011). Poplar homologues to AtAREB3 show variable expression patterns. For example, *PtAREB12* showed decreased expression levels in response to exogenous ABA (Ji *et al.*, 2013). Searching the POPGenie Expression browser (Sundell *et al.*, 2015), we found that *PtAREB12* expression was also suppressed under drought. However, in our drought and ABA-feeding experiment, we did not observe changes in gene expression in *AREB3*, which may point to differences in stress susceptibility of different poplar species. Enhanced expression levels of *AREB3* in overexpressing lines had negative effects on productivity under well irrigated conditions but can obviously enhance drought resilience. Therefore, *AREB3* overexpressing poplar lines would not be preferred for wood production but might be useful for other purposes such as prevention of erosion in drought-prone areas. Furthermore, it would be worthwhile to test the stress resistance of AREB3 poplars under more harsh conditions and under a drought-inducible promoter.

A notable, unexpected finding in our study was the involvement of FDL genes in drought acclimation. Poplars constitutively overexpressing *FDL1* or *FDL2* were the only genotypes with highly significant strong reductions in biomass under our relatively moderate drought conditions. Biomass reduction was

due to the largest loss of leaves among all tested genotypes and consequently a strong reduction in stem biomass, indicating that FDL overexpression rendered poplars more drought susceptible. Furthermore, while all other genotypes at least tended towards an increased root biomass under drought, the *FDL* overexpressing lines showed a massive loss of root mass compared with well watered treatments. By contrast with the *FDL* overexpressing lines, the *FDL* RNAi lines tested here showed no significant decrease in stem volume growth under drought and an allocation pattern similar to that of wild-type with a trend towards higher biomass allocation to roots. Our study therefore assigned for the first time a functional role to FDL2 in regulating biomass allocation and fitness under drought. These functions are shared between FDL1 and FDL2 but obviously cannot compensate for each other. We found that ABA signalling is involved in the regulation of these genes. Further studies are required to clarify the details.

In conclusion, this study underscores that poplar stem biomass production is a highly plastic process integrating endogenous and environmental signals. The ability of transgenic poplars to produce leaf area was of paramount importance for yield. This study highlights that ABA is an important factor in regulating the woody plant biomass, most likely by affecting leaf area. We identified ABI3 and FDL1 as important biomass traits because poplars overexpressing these genes benefited from an extended growth season. It is conceivable that a higher yield may be realised in a future warmer climate by such transgenic lines but obviously at the expense of reduced stress tolerance. Both *FDL* overexpressing lines (FDL1 and FDL2) were highly sensitive to moderate drought, resulting in drastic biomass reduction, while the RNAi lines showed no reduction in stem volume growth by contrast with the wild-type and the overexpressing lines. These results assigned a central role to FDLs in poplar drought acclimation. The molecular events integrating drought responses and the action of FDLs need to be elucidated in future studies. *AREB3* overexpressing lines exhibited massive biomass trade-off under well irrigated conditions but were the most drought-resilient trees among all of the studied poplar lines. AREB3 may therefore be a suitable target for poplar improvement using biotechnological approaches and molecular screening of AREB genes in natural populations.

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Author contributions

DY conducted the experiments, analyzed the data, and wrote the draft manuscript. HW helped with the experimental design, supervised research and commented on the manuscript. RPB, ST and PCM provided the transgenic lines, discussed the data and commented on the manuscript. AP conceived and supervised the study, contributed to data analyses and manuscript writing. All authors approved the final version of this paper.

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References

Arend M, Schnitzler JP, Ehlting B, Hansch R, Lange T, Rennenberg H, Himmelbach A, Grill E, Fromm J. 2009. Expression of the *Arabidopsis* mutant *abi1* gene alters abscisic acid sensitivity, stomatal development, and growth morphology in gray poplars. *Plant Physiology* **151**: 2110–2119.

Bizet F, Bogeat-Triboulot MB, Montpied P, Christophe A, Ningre N, Cohen D, Hummel I. 2015. Phenotypic plasticity toward water regime: response of leaf growth and underlying candidate genes in *Populus*. *Physiologia Plantarum* **154**: 39–53.

Bréda N, Badeau V. 2008. Forest tree responses to extreme drought and some biotic events: towards a selection according to hazard tolerance? *Comptes Rendus Geoscience* **340**: 651–662.

Bredemeier M, Busch G, Hartmann L, Jansen M, Richter F, Lamersdorf NP. 2015. Fast growing plantations for wood production – integration of ecological effects and economic perspectives. *Frontiers in Bioengineering and Biotechnology* **3**: 72.

Brocious CA, Hacke UG. 2016. Stomatal conductance scales with petiole xylem traits in *Populus* genotypes. *Functional Plant Biology* **43**: 553–562.

Brodribb TJ, Feild TS, Sack L. 2010. Viewing leaf structure and evolution from a hydraulic perspective. *Functional Plant Biology* **37**: 488–498.

Busov VB, Strauss SH, Pilate G. 2010. Transformation as a tool for genetic analysis in *Populus*. In: Jansson S, Bhalerao R, Groover A, eds. *Plant genetics and genomics: crops and models. Genetics and genomics of Populus*. New York, NY, USA: Springer New York, 113–133.

Carins Murphy MR, Jordan GJ, Brodribb TJ. 2014. Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. *Plant, Cell & Environment* **37**: 124–131.

Cohen D, Bogeat-Triboulot MB, Tisserant E, Balzergue S, Martin-Magniette ML, Lelandais G, Ningre N, Renou JP, Tamby JP, Le Thiec D *et al.* 2010. Comparative transcriptomics of drought responses in *Populus*: a meta-analysis of genome-wide expression profiling in mature leaves and root apices across two genotypes. *BMC Genomics* **11**: 21.

Dai A. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change* **3**: 52–58.

Ding J, Nilsson O. 2016. Molecular regulation of phenology in trees-because the seasons they are a-changin'. *Current Opinion in Plant Biology* **29**: 73–79.

Farquhar G, Ehleringer J, Hubick K. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503–537.

Fischer U, Polle A. 2010. *Populus* responses to abiotic stress. In: Jansson S, Bhalerao R, Groover A, eds. *Genetics and genomics of Populus*. Berlin, Germany: Springer Verlag, 225–247.

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K. 2005. AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell* **17**: 3470–3488.

Fujita Y, Fujita M, Shinozaki K, Yamaguchi-Shinozaki K. 2011. ABA-mediated transcriptional regulation in response to osmotic stress in plants. *Journal of Plant Research* **124**: 509–525.

Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, Umezawa T, Fujita M, Maruyama K, Ishiyama K *et al.* 2009. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in *Arabidopsis*. *Plant and Cell Physiology* **50**: 2123–2132.

González-Guzmán M, Pizzio GA, Antoni R, Vera-Sirera F, Merilo E, Bassel GW, Fernández MA, Holdsworth MJ, Perez-Amador MA, Kollist H *et al.* 2012. *Arabidopsis* PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. *Plant Cell* **24**: 2483–2496.

González-Guzmán M, Rodríguez L, Lorenzo-Orts L, Pons C, Sarrión-Perdigones A, Fernández MA, Peirats-Llobet M, Forment J, Moreno-Alvero M, Cutler SR *et al.* 2014. Tomato PYR/PYL/RCAR abscisic acid receptors show high expression in root, differential sensitivity to the abscisic acid agonist quinabactin, and the capability to enhance plant drought resistance. *Journal of Experimental Botany* **65**: 4451–4464.

Hamanishi ET, Campbell MM. 2011. Genome-wide responses to drought in forest trees. *Forestry: An International Journal of Forest Research* **84**: 273–283.

Harfouche A, Meilan R, Kirst M, Morgante M, Boerjan W, Sabatti M, Scarascia Mugnozza G. 2012. Accelerating the domestication of forest trees in a changing world. *Trends in Plant Science* **17**: 64–72.

Hubbard KE, Nishimura N, Hitomi K, Getzoff ED, Schroeder JI. 2010. Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. *Genes & Development* **24**: 1695–1708.

Ji L, Wang J, Ye M, Li Y, Guo B, Chen Z, Li H, An X. 2013. Identification and characterization of the *Populus* AREB/ABF subfamily. *Journal of Integrative Plant Biology* **55**: 177–186.

Jones RJ, Mansfield TA. 1970. Suppression of stomatal opening in leaves treated with abscisic acid. *Journal of Experimental Botany* **21**: 714–719.

Juhrbrandt J, Leuschner C, Hölscher D. 2004. The relationship between maximal stomatal conductance and leaf traits in eight Southeast Asian early successional tree species. *Forest Ecology and Management* **202**: 245–256.

Kauter D, Lewandowski I, Claupein W. 2003. Quantity and quality of harvestable biomass from *Populus* short rotation coppice for solid fuel use—a review of the physiological basis and management influences. *Biomass and Bioenergy* **24**: 411–427.

Linderson M-L, Iritz Z, Lindroth A. 2007. The effect of water availability on stand-level productivity, transpiration, water use efficiency and radiation use efficiency of field-grown willow clones. *Biomass and Bioenergy* **31**: 460–468.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**: 1064–1068.

Marron N, Villar M, Dreyer E, Delay D, Boudouresque E, Petit J-M, Delmotte FM, Guehl J-M, Brignolas F. 2005. Diversity of leaf traits related to productivity in 31 *Populus deltoides* × *Populus nigra* clones. *Tree Physiology* **25**: 425–435.

Maurya JP, Bhalerao RP. 2017. Photoperiod- and temperature-mediated control of growth cessation and dormancy in trees: a molecular perspective. *Annals of Botany* **120**: 351–360.

McKendry P. 2002. Energy production from biomass (Part 1): overview of biomass. *Bioresource Technology* **83**: 37–46.

Monclus R, Villar M, Barbaroux C, Bastien C, Fichot R, Delmotte FM, Delay D, Petit J-M, Bréchet C, Dreyer E *et al.* 2009. Productivity, water-use efficiency and tolerance to moderate water deficit correlate in 33 poplar genotypes from a *Populus deltoides* × *Populus trichocarpa* F1 progeny. *Tree Physiology* **29**: 1329–1339.

Müller A, Volmer K, Mishra-Knyrim M, Polle A. 2013. Growing poplars for research with and without mycorrhizas. *Frontiers in Plant Science* **4**: 332.

Murashige T, Skoog F. 2006. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473–497.

Negin B, Moshelion M. 2016. The evolution of the role of ABA in the regulation of water-use efficiency: from biochemical mechanisms to stomatal conductance. *Plant Science: An International Journal of Experimental Plant Biology* **251**: 82–89.

Nilsson O, Alden T, Sitbon F, Little CHA, Chalupa V, Sandberg G, Olsson O. 1992. Spatial pattern of cauliflower mosaic virus 35S promoter-luciferase expression in transgenic hybrid aspen trees monitored by enzymatic assay and non-destructive imaging. *Transgenic Research* **1**: 209–220.

Papacek M, Christmann A, Grill E. 2017. Interaction network of ABA receptors in grey poplar. *The Plant Journal: For Cell and Molecular Biology* **92**: 199–210.

Park S-Y, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow T-FF *et al.* 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**: 1068–1071.

Parmentier-Line CM, Coleman GD. 2016. Constitutive expression of the Poplar FD-like basic leucine zipper transcription factor alters growth and bud development. *Plant Biotechnology Journal* **14**: 260–270.

Paul S, Wildhagen H, Janz D, Polle A. 2018. Drought effects on the tissue- and cell-specific cytokinin activity in poplar. *AoB PLANTS* **10**: plx067.

Peach C, Velten J. 1991. Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters. *Plant Molecular Biology* **17**: 49–60.

Pellis A, Laureysens I, Ceulemans R. 2004. Growth and production of a short rotation coppice culture of poplar I. Clonal differences in leaf characteristics in relation to biomass production. *Biomass and Bioenergy* **27**: 9–19.

Polle A, Altman A, Jiang X. 2006. Towards genetic engineering for drought tolerance in trees. In: Fladung M, Ewald D, eds. *Tree transgenesis: recent developments*. Berlin, Germany: Springer Berlin Heidelberg, 275–297.

Polle A, Chen S, Eckert C, Harfouche A. 2019. Engineering drought resistance in forest trees. *Frontiers in Plant Science* **9**: 1875.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.

Popko J, Haensch R, Mendel RR, Polle A, Teichmann T. 2010. The role of abscisic acid and auxin in the response of poplar to abiotic stress. *Plant Biology* **12**: 242–258.

R Core Team. 2017. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raghavendra AS, Gonugunta VK, Christmann A, Grill E. 2010. ABA perception and signalling. *Trends in Plant Science* **15**: 395–401.

Rohde A, Bastien C, Boerjan W. 2011. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. *Tree Physiology* **31**: 472–482.

Rohde A, Prinsen E, Rycke RD, Engler G, Montagu MV, Boerjan W. 2002. PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. *Plant Cell* **14**: 1885–1901.

Schiermeier Q. 2018. Droughts, heatwaves and floods: how to tell when climate change is to blame. *Nature* **560**: 20.

Sharp RE, LeNoble ME. 2002. ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany* **53**: 33–37.

Singh RK, Maurya JP, Azeez A, Miskolczi P, Tylewicz S, Stojkovič K, Delhomme N, Busov V, Bhalerao RP. 2018. A genetic network mediating the control of bud break in hybrid aspen. *Nature Communications* **9**: 4173.

Sundell D, Mannapperuma C, Netotea S, Delhomme N, Lin Y-C, Sjödin A, Van de Peer Y, Jansson S, Hvidsten TR, Street NR. 2015. The plant genome integrative explorer resource: PlantGenIE.org. *New Phytologist* **208**: 1149–1156.

Tardieu F, Parent B, Caldeira CF, Welcker C. 2014. Genetic and physiological controls of growth under water deficit. *Plant Physiology* **164**: 1628–1635.

Tian X, Wang Z, Li X, Lv T, Liu H, Wang L, Niu H, Bu Q. 2015. Characterization and functional analysis of pyrabactin resistance-like abscisic acid receptor family in rice. *Rice* **8**: 28.

Tischer SV, Wunschel C, Papacek M, Kleigrewe K, Hofmann T, Christmann A, Grill E. 2017. Combinatorial interaction network of abscisic acid receptors and coreceptors from *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **114**: 10280–10285.

Tougane K, Komatsu K, Bhyan SB, Sakata Y, Ishizaki K, Yamato KT, Kohchi T, Takezawa D. 2010. Evolutionarily conserved regulatory mechanisms of

abscisic acid signaling in land plants: characterization of *ABSCISIC ACID INSENSITIVE1*-Like Type 2C Protein Phosphatase in the liverwort *Marchantia polymorpha*. *Plant Physiology* **152**: 1529–1543.

Tullus A, Rytter L, Tullus T, Weih M, Tullus H. 2012. Short-rotation forestry with hybrid aspen (*Populus tremula* L.× *P. tremuloides* Michx.) in Northern Europe. *Scandinavian Journal of Forest Research* **27**: 10–29.

Tylewicz S, Petterle A, Marttila S, Miskolczi P, Azeez A, Singh RK, Immanen J, Mähler N, Hvidsten TR, Eklund DM *et al.* 2018. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. *Science* **360**: 212–215.

Tylewicz S, Tsuji H, Miskolczi P, Petterle A, Azeez A, Jonsson K, Shimamoto K, Bhalerao RP. 2015. Dual role of tree florigen activation complex component *FD* in photoperiodic growth control and adaptive response pathways. *Proceedings of the National Academy of Sciences, USA* **112**: 3140–3145.

Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K. 2010. Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. *Plant & Cell Physiology* **51**: 1821–1839.

UPOV. 1981. *Test guidelines TG/21/7 Poplar*. [WWW document] URL https://www.upov.int/edocs/tgdocs/en/tg021.pdf [accessed 25 April 2019].

Viger M, Smith HK, Cohen D, Dewoody J, Trewin H, Steenackers M, Bastien C, Taylor G. 2016. Adaptive mechanisms and genomic plasticity for drought tolerance identified in European black poplar (*Populus nigra* L.). *Tree Physiology* **36**: 909–928.

Wang W, Vinocur B, Altman A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**: 1–14.

Weih M, Nordh N-E. 2005. Determinants of biomass production in hybrid willows and prediction of field performance from pot studies. *Tree Physiology* **25**: 1197–1206.

Wildhagen H, Paul S, Allwright M, Smith HK, Malinowska M, Schnabel S, Paulo J, Cattonaro F, Vendramin V, Scalabrin S *et al.* 2018. Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content, and wood anatomical traits in *Populus nigra*. *Tree Physiology* **38**: 340–361.

Wilson JB. 1988. A review of evidence on the control of shoot: root ratio, in relation to models. *Annals of Botany* **61**: 433–449.

Yoshida T, Fujita Y, Maruyama K, Mogami J, Todaka D, Shinozaki K, Yamaguchi-Shinozaki K. 2015. Four *Arabidopsis* AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. *Plant, Cell & Environment* **38**: 35–49.

Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K. 2010. AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *The Plant Journal* **61**: 672–685.

Yu JL, Ge H, Wang X, Tang R, Wang Y, Zhao F, Lan W, Luan S, Yang L. 2017a. Overexpression of pyrabactin resistance-like abscisic acid receptors enhances drought, osmotic, and cold tolerance in transgenic poplars. *Frontiers in Plant Science* **8**: 1752.

Yu JL, Li H, Peng Y, Yang L, Zhao F, Luan S, Lan W. 2017b. A survey of the pyrabactin resistance-like abscisic acid receptor gene family in poplar. *Plant Signaling & Behavior* **12**: e1356966.

Yu Q, Tigerstedt P, Haapanen M. 2001. Growth and phenology of hybrid aspen clones (*Populus tremula* L. x *Populus tremuloides* Michx.). *Silva Fennica* **35**: 15–25.

Yu J, Yang L, Liu X, Tang R, Wang Y, Ge H, Wu M, Zhang J, Zhao F, Luan S *et al.* 2016. Overexpression of poplar pyrabactin resistance-like abscisic acid receptors promotes abscisic acid sensitivity and drought resistance in transgenic *Arabidopsis*. *PLoS ONE* **11**: e0168040.

de Zelicourt A, Colcombet J, Hirt H. 2016. The role of MAPK modules and ABA during abiotic stress signaling. *Trends in Plant Science* **21**: 677–685.

Zhao Y, Chan Z, Gao J, Xing L, Cao M, Yu C, Hu Y, You J, Shi H, Zhu Y *et al.* 2016. ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proceedings of the National Academy of Sciences, USA* **113**: 1949–1954.

Zwieniecki MA, Boyce CK, Holbrook NM. 2004. Hydraulic limitations imposed by crown placement determine final size and shape of *Quercus rubra* L. leaves. *Plant, Cell & Environment* **27**: 357–365.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 *Populus tremula* × *tremuloides* lines overexpressing AREB3 under the 35S promotor (a) and RNAi lines of RCAR1/PYL9 (b).

Fig. S2 Wild-type (T89) and transgenic poplars (*P. tremula* × *tremuloides*) grown outdoors in a caged area.

Fig. S3 Soil moisture during long-term exposure poplars to well irrigated control conditions (circles) and to moderate drought (triangles) grown under field conditions.

Fig. S4 Overview of individual leaf sizes of wild-type (T89) poplar (*P. tremula* × t*remuloides*) and ABA-related transgenic lines grown under field conditions.

Fig. S5 Glasshouse grown *P. tremula* × *tremuloides* transgenic lines abi1-1 76-1 (orange tags), 76-3 (pink tags) and wild-type T89 (white tags) and experimental conditions.

Fig. S6 Performance of wild-type (T89) and transgenic abi1-1 poplars (*P. tremula* × *tremuloides*) grown under glasshouse conditions.

Fig. S7 Daily growth (cm d^{-1}) of wild-type (T89) and transgenic poplars (*P. tremula* × *tremuloides*) grown under field conditions.

Fig. S8 Bud set (a) and bud break (b) of wild-type (T89) and transgenic poplars (*P. tremula* × *tremuloides*) grown under field conditions.

Table S1 Gas exchange in wild-type (T89) and transgenic poplars (*P. tremula* × *tremuloides*).

Table S2 Tree height, stem diameter, leaf size, specific leaf area (SLA) and whole-plant leaf area (WPLA) of control plants of wild-type (T89) and transgenic poplars (*P. tremula* × *tremuloides*) in the second growth phase (August).

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